Future Pharmacists Guide

Microbiology for Pharmacy Students

Exploring how microbiology is crucial in pharmaceutical practice.

Dr. Mazin Saleem Salman

Estabraq Abdulkareem Qahtan

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First Edition

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MICROBIOLOGY FOR PHARMACY STUDENTS

Mazin Saleem Salman

Estabraq Abdulkareem Qahtan

Muna Abdulsattar Faisal

Dedication

To the memory and spirit of my dear mother and father. My beloved, my dear wife Fatima and my children Ahmed, Jannat and Mustafa.

Mazin

To my dear family, whose endless support and encouragement have been my foundation. May this book serve as a beacon of knowledge and inspiration to every student and researcher seeking to uncover the unseen world of microorganisms.

Estabraq

To my beloved husband Maan and my daughters Ayaa, Maryam and Zainab.

Muna

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Preface

Since the field of pharmacy, like other scientific disciplines, has been penetrated by microbiology, The study of life and activity of microorganisms. The stem word of "Microbiology" is microbi, which is used to refer to small objects or living things. The organisms that are examined in the science of microbiology are known as microorganisms. They may be of prokaryotic (bacteria and archaea) or eukaryotic origin, and include viruses, as well as some microorganisms (fungi, some protozoa and some algae). Microbiology is an inter-disciplinary research area and its principles are used to design of various disciplines of other sciences (like: immunology, genetics, biochemistry, molecular biology, molecular genetics, virology, pharmacology, biotechnology). Microbiologists are also routinely in collaboration with and contribute to other fields of science, including botany, pharmacology, medicine, veterinary science, agriculture, geology, and marine biology. Microorganisms can be used with the purpose of microbiology such as manufacturing and applied microbiology. Fundamental microbiology includes extensive research on bacteriology, virology, mycology, nematology, biotechnology and other such subjects and its ultimate objective is better understanding of these organisms as their fundamental work. It is equally important for physicians and dentists to have knowledge of microbiology. In the context of pharmacy, microbiology is applied to pharmaceuticals and biologics. Microbiology, in a sense, is the basis for pharmacology. Insights into microbial structures and physiology can be gained by analyzing the activity of small biological molecules directed toward microorganisms. In this text we will take a pharmacist's approach to microbiology. This is the purpose of this book, a brief outline of microbiology to help you along this way. The microorganisms are closely related to the process of drug development, drug effectiveness and safety. Modifications within the microbiota in the human body may have a direct impact on the drug metabolism and thus lead to great changes in its efficacy. Because of the growing development of more complex biological molecules---like monoclonal antibodies---that are manufactured and administered via a microbiological pathway, there is now a necessity for a specialist pharmacist in this area of science. Given that microorganisms are ubiquitous at the majority of stages in pharmaceutical manufacturing, they can also be an added microbiological risk which could compromise the safety and quality of the final product. In this instance, intermediate controls which have to be implemented into the decisive stages of the preparation of the medicament are necessary. Moreover, pharmacists need to have some knowledge of the various methods of sterilization of the materials.

Pharmacists should also be knowledgeable in the area of antibiotic and biocide resistance, and should be actively participating in initiatives to reduce antibiotic use, both in pharmacy and in other medical areas. The patient should be alerted to the significance of compliance before the completion of antibiotic therapy. Pharmaceutical microbiology programs typically cover some microbiology subject

matter, instructors from pharmaceutical microbiology departments explain. Accordingly, they must be familiar with all microbiological activities. Finally the microbiology component of pharmaceuticals needs to be integrated as a core component of pharmacy education. Any one who professes to be a practical pharmacist of the future must in no wise overlook a thorough comprehension of the evils placed before him. Pharmacists play a key role in the rational use of antimicrobials across settings. A drug utilisation study began in 1990.

Mazin Saleem Salman

PhD Medical Microbiology

Basra, January, 2025.

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Chapter One

Characteristics of Microorganisms

1. Introduction to Microorganisms

Microorganisms are microscopic organisms too small to be seen clearly with the unaided human eye. The group includes a wide spectrum of simple life-forms like bacteria, yeasts, molds, protozoans, algae, and viruses. These organisms play an indispensable role in sustaining the ecosystems of the earth. All these simple organisms are more or less ubiquitous in the natural environment. Microbes pose significant health hazards, causing fatal diseases in humans, animals, and plants. In contrast, there are microbes that are beneficial to humans, plants, and animals. In the biological cycles happening in nature, they play an indispensable role. There are other specific fields wherein the activities of microorganisms become beneficial to humans. (Volland et al.2022)

Microorganisms are adapted to exist in almost every environment of the globe, ranging from areas of permanently frozen ice on the polar caps to areas of permanent warmth and humidity such as hot springs. Microorganisms are present in large numbers in the air, in soil, and water, on rooftops, on the bodies of plants and animals, and on the surface of all objects including humans. There are at least twice as many microbial cells as human cells present in the normal healthy human body. Microorganisms exist both as single cells and as complex, multicellular communities.

Usually, a microbe is a situation that is not visible to the naked eye. The first person to have a thought on microorganisms was Fransesco Redi. After Redi, it was Louis Pasteur who disclosed the nature of microorganisms. An important discovery in microbiology was that of the microscope in the 17th century. With the invention of the microscope, it was revealed a whole world of microorganisms.

2. Classification of Microorganisms

There is a pressing need to classify microorganisms or microbes (a term that in a microbiological context can also include multicellular organisms like fungal spores) for convenient and productive scientific study. That systems of classification exist reflects the significance of the microbial segment (including both cellular and acellular forms). There are probably subrogation arrays to the “taxonomic hierarchy”

which are briefly detailed here. Starting at the most inclusive level, some are biological, whilst others are based on microscopic ultrastructure and/or metabolic characteristics. With respect to the biological approach, while the classical "5-kingdom system" is commonly used in bio-texts, most microbiologists currently prefer to employ either the 2- or 3-domain schema where Prokaryota is a domain dominated by bacteria augmented by Archaea and to which all plants, animals, and fungi (the higher "meta-iotic" organisms) are grouped in the second domain (Eukaryota). In addition, consideration of Fungi as a separate kingdom is often discarded, with these organisms being treated together with Algae in a solitary phylum (Chromista in the multi- or cellular version or "Phyco-Fungi" when referring to a "semi-cotraploid" multi-cellular filament containing a commensal green alga) within the plant kingdom. Microbiologists favoring a broader definition of microbes (which can include metazoan larvae as well as subcellular entities like viroids and plasmids) often endorse a 7-"kingdom" system.

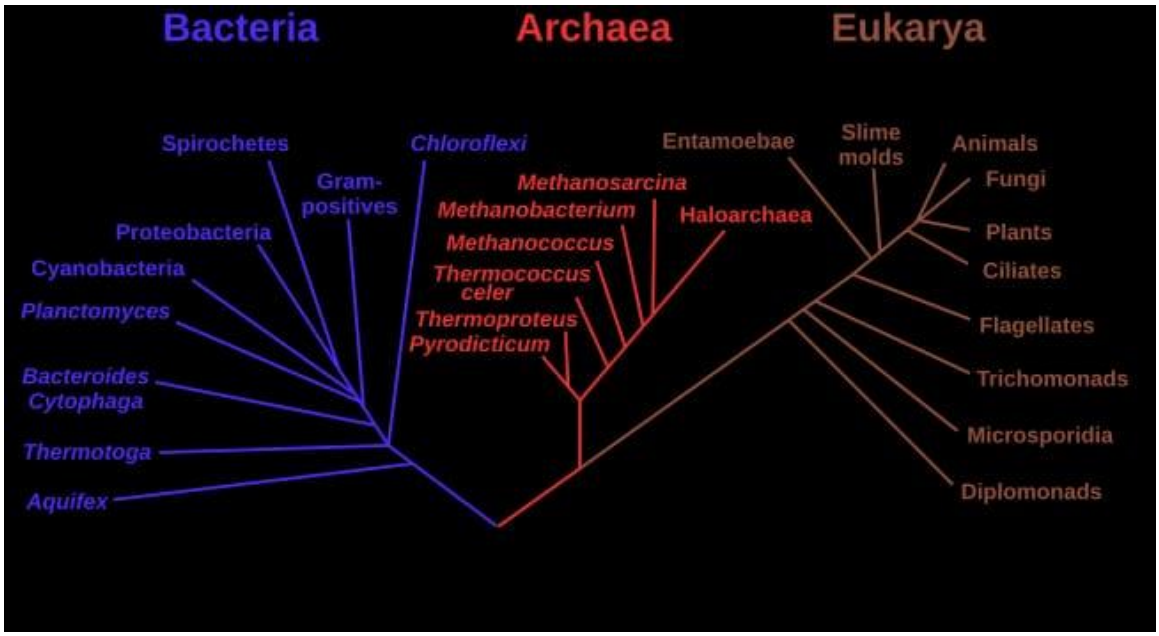


Figure 1: Carl Woese 's 1990 phylogenetic tree based on rRNA data shows the domains of Bacteria, Archaea, and Eukaryota. All are microorganisms except some eukaryote groups. (source: reference (Microorganism, 2025)

2.1. Bacteria

Bacteria (singular: bacterium) are the most diverse group of microorganisms and occupy a wide range of niches on earth. They are unicellular organisms and have a prokaryotic cell structure with nuclear material not enclosed within a nuclear membrane. Since the discovery of bacteria in 1676, this biological group has been the center of attention for numerous scientists. By 1875, Ferdinand Cohn established the

concept of bacteria as a kingdom, and subsequently, classification into families, genera, and species was tenably done.

The metabolic capabilities of bacteria are quite diverse; some are phototrophic, deriving energy from light and fixing CO_2 through photosynthesis. Bacteria serve critical roles in various ecological cycles, such as the nitrogen, carbon, and sulfur cycles. In the mineralization of organic substances released into nature, bacteria have a vital role in the decomposition process. Here, the organic materials are oxidized by several bacteria, and finally, carbon dioxide is released back to the atmosphere. Over 10,000 billion tonnes of carbon are moved through this route each year. Nitrogen is fixed from the atmosphere by various bacteria, and due to these strains, nitrogen becomes available as a nutrient for other organisms. Bacteria take part in every pathway of the sulfur cycle. There are two major groups of bacteria: gram-positive and gram-negative. The most obvious differences between them are revealed through gram-staining procedures. Among these differences, a major distinction is that the peptidoglycan layer of gram-positive bacteria is much thicker than that of the gram-negative bacteria. The outer membrane of gram-negative bacteria contains phospholipids and lipopolysaccharides, but gram-positive bacteria lack this structural feature (Soni et al., 2024).

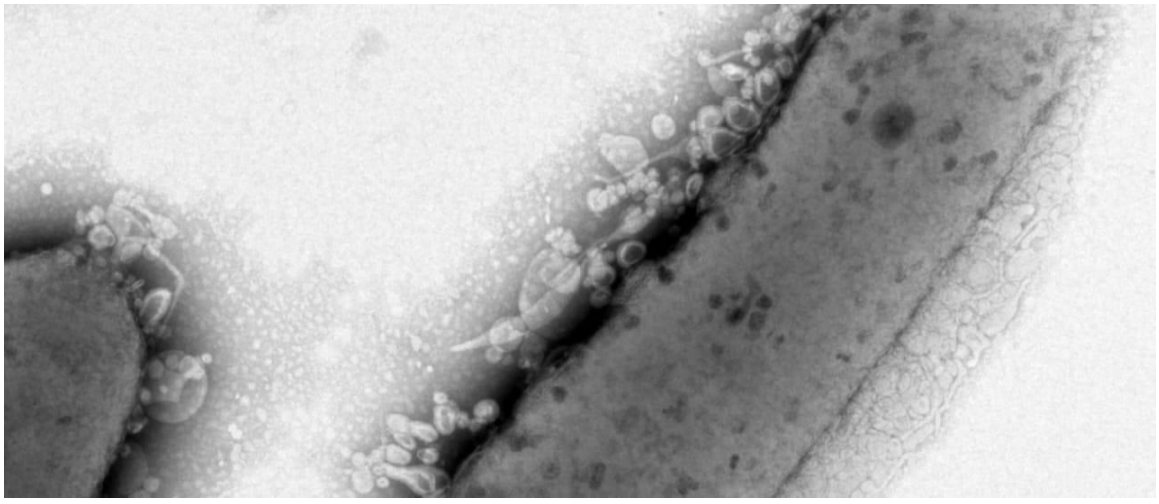


Figure 2: This image of a bacterial cell shows lipid droplets on the exterior of the cell wall. The lipids have escaped through a cell envelope that has been modified to increase lipid production. Image courtesy of University of Wisconsin-Madison and DOE Great Lakes Bioenergy Research Center (GLBRC) (source: reference (DOE ExplainsMicrobiology | Department of Energy, 2025)

Pathogenic bacteria are responsible for causing numerous infectious diseases in humans; healthcare practitioners still encounter difficulties treating diseases caused by newly emerged bacterial strains with antibiotic resistance. Taxonomy of bacteria

was first established by Carl Woese in 1978, who classified them into three groups (Okafor, 2011).

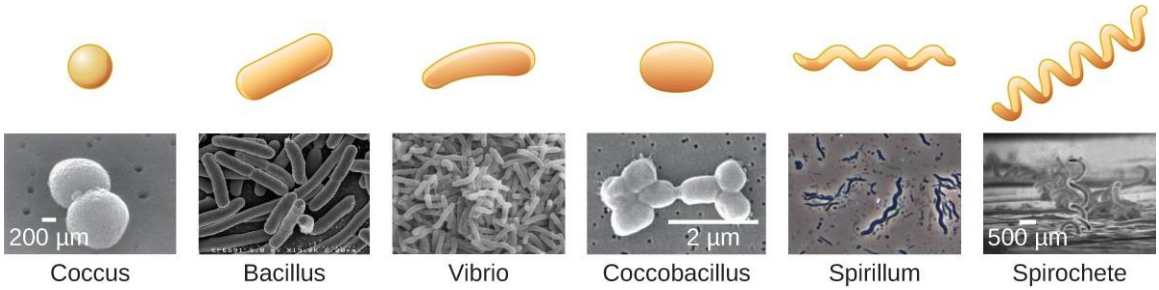


figure 3:Common bacterial shapes. Note how coccobacillus is a combination of spherical (coccus) and rod-shaped (bacillus). (credit "Coccus": modification of work by Janice Haney Carr, Centers for Disease Control and Prevention; credit "Coccobacillus": modification of work by Janice Carr, Centers for Disease Control and Prevention; credit "Spirochete": Centers for Disease Control and Prevention)

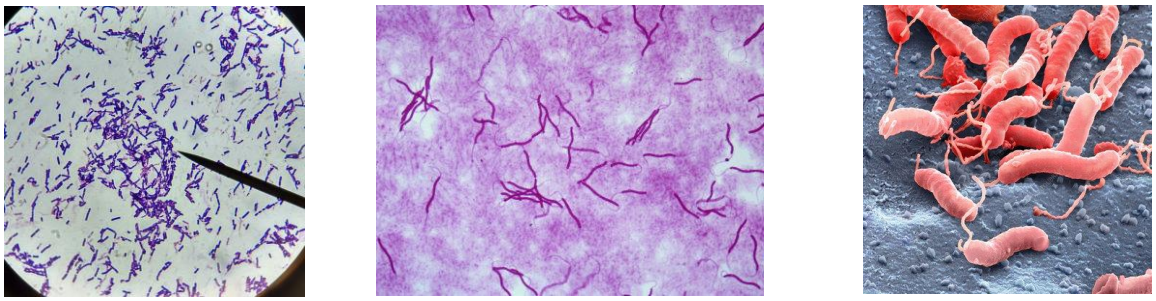


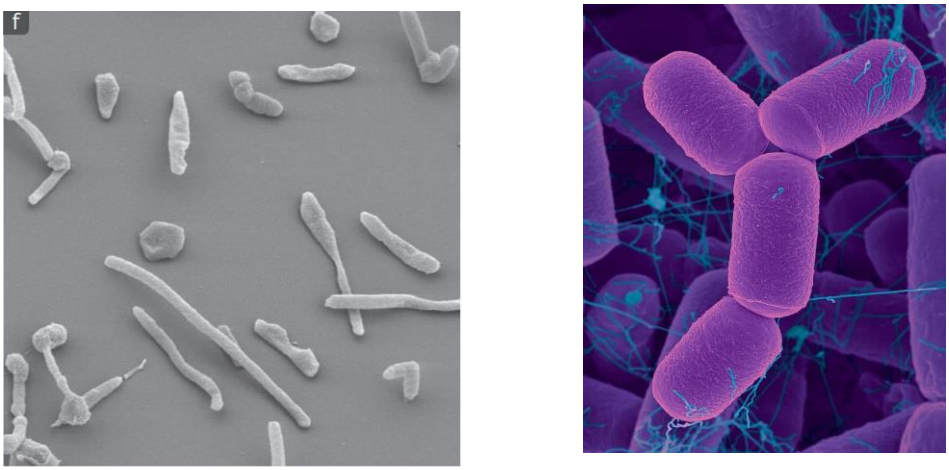
Figure4: some forms of bacteria.

2.2. Archaea

Archaea constitute a domain of life that is distinct from bacteria and eukaryotes (Allers & Mevarech, 2005). While archaea are prokaryotic microorganisms and are superficially similar to bacteria, they are considerably different from those microorganisms on a fundamental level. Some characteristics of archaea that are not found in either bacteria or eukaryotes include their unique rRNA based phylogeny, biochemistry and distinctive RNA polymerase, transcription factors, and ribosomal proteins. Archaea are found throughout the biosphere and a semi-symbiotic relationship exists for some species. Archaea classically have been studied using extreme environments over the past two decades and the isolation of more novel species has driven interest in the research community. Methanogens colonise the gastrointestinal tract in humans and other animals, and are responsible for the methane production in the bowel. The scientific interest in archaea has rapidly developed since the completion of the first genome sequence, due to the novel genes of archaea are found in the highly compact genome as well as the unique metabolic

pathways of archaea. Although similar to bacteria on an anatomical level, archaea possess biochemistry and genetics that are closely related to eukaryotes due to an independent evolutionary origin for these features. Three distinct groups of archaea have been recognised: the methanogens, the extreme halophiles, and the thermophiles. The latest classification models are now based on conserved rRNA trees, and this suggests that about 12 major groups of archaea are found. Biochemical pathways that are unique to certain organisms have motivated the taxonomy of those organisms. Taxonomic groups of archaea have also been formed based on the amino acid structure of the braun lipoprotein and this has resulted in a five kingdom model that includes the Euryarchaeotina, Crenarchaeotina, Thermoprotei, “Caldilobales”, and “Thermoplasmacrelales”. The two different groups of proteins found in the flagella of bacterial bacillus subtilis and the archaea halobacterium have driven research on the evolution of flagella. Recent research investigating signal sequence characteristics has shown that the archaellins of archaea are more similar to the signal sequence of TAT system substrates in E. coli than to the corresponding proteins of FEA system. To survive in extreme environments, archaea have evolved stable enzymes. RNA polymerase from the archaea T. acidophilum has been crystallised recently so the possibility exists for a crystallographic approach to the determination of the enzyme's structure. Additionally, the structure of the ether lipid bilayer of thermoplasma has also been determined. Just as with the bacterial domain the vast majority of archaea have not yet been isolated and cultured, the known species represent but a small fraction the vast diversity that is believed to exist. The rapidly expanding field of molecular techniques has opened up many of the uncultured, and more interesting archaea to investigation. This is of considerable ecological significance; many archaea play a key role in nutrient cycling, with specific groups of archaea breaking down complex chemicals that cannot otherwise be digested (methanogens in the rumen of cows, termites, and other animals, converting plant material into a form that is usable by the host animal). The NH3 not produced by the small fraction of methanogenic archaea are produced by a vast number of cheap genomic archaea. The majority of that NOT leads to exhalation of the flammable gasses contained in the NGAS. The research in this area has rapidly developed with the understanding of the broad global significance of this NH3 cycling. Another area of potential significance, however, that is only just beginning to be explored is the production of CH4, other gaseous hydrocarbons, and gaseous oxidation products of CH4. It is estimated that atmospheric CH4 produced from the Earth's surface is exclusively biogenic in origin and a significant proportion of global CH4 is produced by microorganisms. The majority of this is produced by methanogenic archaea, and the understanding of methane metabolism in these organisms is developing due to the rapidly increasing number of completed and ongoing genome projects. A pressing concern is understanding the potential changes that may follow changes in global

climate, as the methane hydrates currently present in permafrost have the potential to bring about a “runaway greenhouse effect”. Since the discovery of unique rRNA-based phylogeny, archaea have been studied in a geological context and many of these organisms are now known to live at or near one of the first three A Universal energy-producing biochemical pathways are found in archaea, as is the capacity for magnesium chelation of ATP. Potential constraints on life in these environments make tempeand ho!ophllic archaea superficially good candidates for Earth's first life forms. Recent advances in the field of microbiology have led to the discovery of a third form of cellular life, the domain archaea. As such, since the mid-1970s it has been understood that the “procaryotic” cellular forms of life, which lack a membrane-bounded nucleus, endomembrane system, typically contain a singular circular chromosome, and have a cytoplasm bounded by a plasma membrane are a fundamentally distinct group of organisms from those with an “eucaryotic” form of cells. Despite what are fundamentally different cellular architectures for the two prokaryotic domains, Bacteria and Archaea are superficially similar in anatomical sense. Indeed, for the longest while it was supposed that there exist but two cellular forms of life. All the presently increasing tallies of life’s diversity fit into these two gross features of cell morphology. Purported examples of taxa that bridged the gap between the putatively two gross forms of life were not accepted by the majority of biologists. That notion was forever shattered when Carl R. Woese presented his molecular phylogeny of cellular organisms based on small subunit ribosomal RNA, and it became apparent that the domain Archaea is a third form of cellular life, which is quite distinct from the commonly well-studied, cultured model organisms, which fall into the domain Bacteria and Eukarya.



a b

Figure 5: a-Scanning electron micrograph of *H. turkmenica* strain 4kT. B-Intestine archaea Methanobrevibacter smithii, SEM.

2.3. Fungi

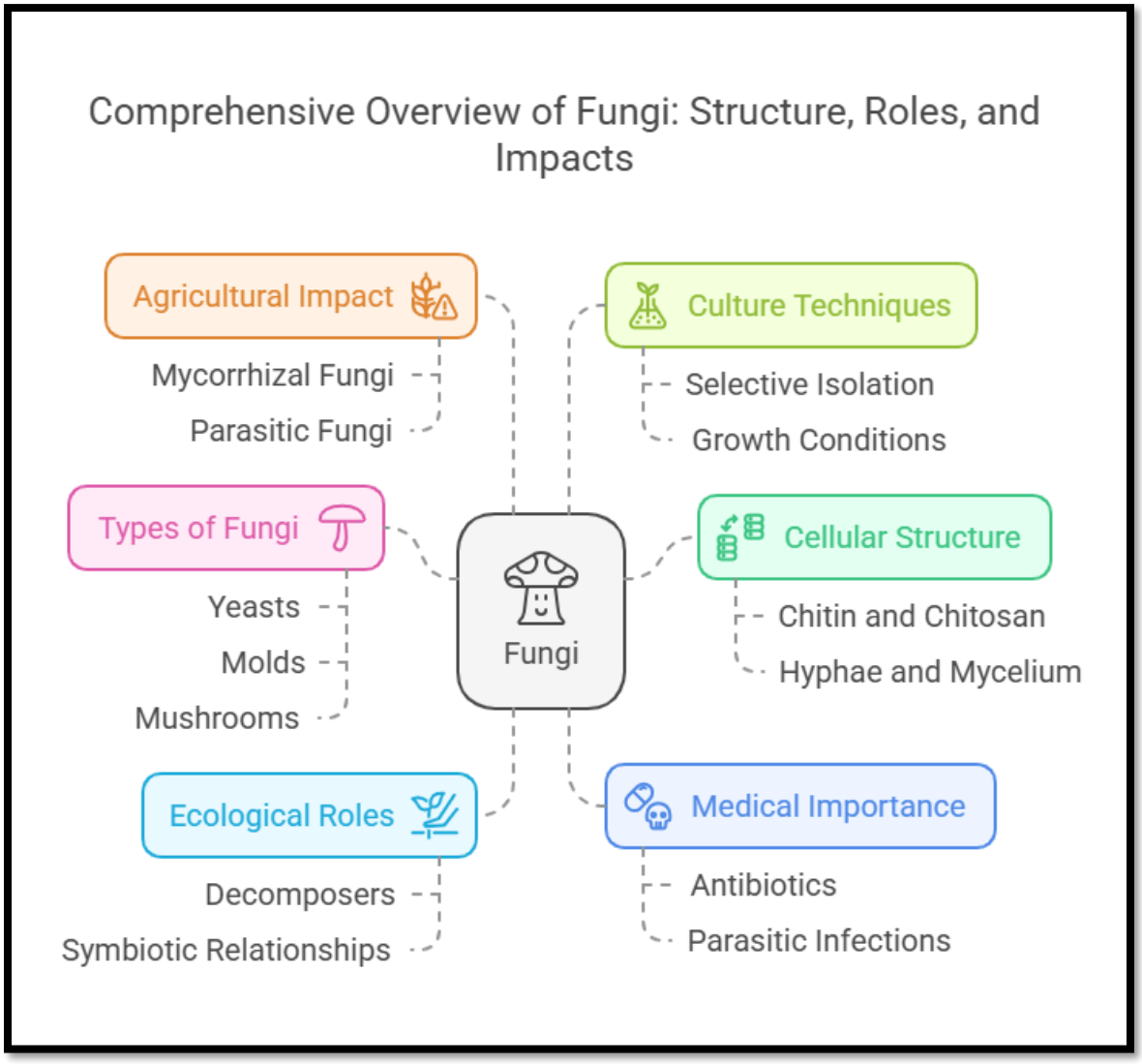
Fungi are distinct from plants and animals in their cellular structure. While the cell walls of plant cells are primarily composed of cellulose and those of algae are formed from cellulose and/or alginates, the cell walls of fungi are mainly made up of chitin and chitosan (Bahram & Netherway, 2021). The fungal body exists as a network of distinct, separated thread-like tubes called hyphae. A dense mat of hyphae makes up the thallus (or mycelium) of a fungus, upon which spore-producing structures can develop. The bodies of most fungi grow as mycelium (a mass of entangled hyphae) that penetrate the substrate. Cellular replication can only occur at the tips of the hyphae (Bahram & Netherway, 2021).

There are many classes of fungi, including the microscopic yeasts, filamentous molds, and the macroscopic mushrooms. Yeasts are unicellular fungi that reproduce asexually through budding or division, though sexual reproduction can also occur. They play an important role in the production of bread, wine, beer, and SCPs. Molds are similar in structure to yeasts, but are multicellular and form long hyphae called mycelia. Some molds are important in the production of cheese, while others cause various diseases in plants and animals. The functions of the macroscopic mushrooms can be compared to above-ground “fruits”, acting as reproduction structures.

Fungi have an array of ecological and biological uses. As decomposers, fungi break down dead organisms, excreting nutrient-rich enzymes back into the substrate. In this way, they play a central role in the cycling of elements such as carbon and nitrogen. Fungi can also form associations with other organisms. For example, mycorrhizal associations are formed between plant roots and certain fungi. In return for carbon resources from the plant, the fungus increases the surface area from which the plant can uptake nutrients. In this way, fungi assist in phosphorous and nitrogen uptake. Overall, fungi are ubiquitous and found in diverse ecosystems, forming complex relationships with other species from almost all eukaryotic groups.

Fungi are also important in medicine. Alexander Fleming’s discovery of the antibiotic penicillin was an accidental result of his petri dish cultures contaminated with the fungus Penicillium notatum. This discovery revolutionized medicine, dramatically increasing the effectiveness of surgeries and reducing death due to bacterial infectious diseases. Since the discovery of penicillin, over a hundred other species of mold have been found to produce antibiotics. Fungi can also produce a variety of other bioactive compounds, some of which have been developed as pharmaceuticals. Taxol, the anti-cancer drug, was first isolated from the yew tree, and statins, a class of drugs used to treat high cholesterol, are derived from fungi. However, some species of fungi can also have negative impacts in medicine. For example, the infectious fungal disease ringworm is caused by several species of the genus Trichophyton, and fungi

of the Candida genus can cause life-threatening systemic infections. Fungi are an especially important group in agriculture. Mycorrhizal soil fungi have a mutualistic relationship with plants, forming associations with the plant root tissue and enhancing plant growth. However, this mutualism comes at a cost, for example, mycorrhizal fungi are also in competition with the plant for resources. Some other species of fungi form parasitic relationships with plants, examples include the genus Ustilago, which causes smut disease in crops, and Ophiostoma, which causes Dutch elm disease. Other species of fungi are also commonly found on crops, such as Aspergillus flavus and A. parasiticus, which form aflatoxins, a family of chemically similar compounds that are both toxic and carcinogenic.



Due to the complexity and diversity of fungi, a number of techniques have been developed for their culture and identification. As their cell walls are composed of different materials, fungi can be selectively isolated from other microorganisms by

changes in pH or chemical treatment. This allows for selective growth in culture mediums containing chitin, such as colloidal chitin, or a combination of chitin and glucans. Several selective mediums have been developed for this purpose, one example is Martin's Rose Bengal Agar, which is commonly used for the isolation of saprobic fungi. Due to their unique growth requirements, fungi can be grown under a wide range of conditions. Light is a key parameter in the growth of mushrooms and related netted spore groups as most of these species need light for spore germination. The pH also influences fungal growth, in commercial coffee-grinding units this is particularly useful, controlling the growth of mycotoxigenic and contaminator molds, whilst promoting the growth of filamentous fungi with beneficial, temperature, and pressure-promoting properties. Sucrose is a good growth stimulator for both ring appoints and filamentous fungi. At 20 and 37°C, higher fungal growth is obvious in the presence of sucrose, promoting the production of biomass and enzyme activity. Reducing growth at 37°C for contaminant molds colonized with phycosporous filaments isolated from spoiled coffee beans through storage at 18% relative humidity.

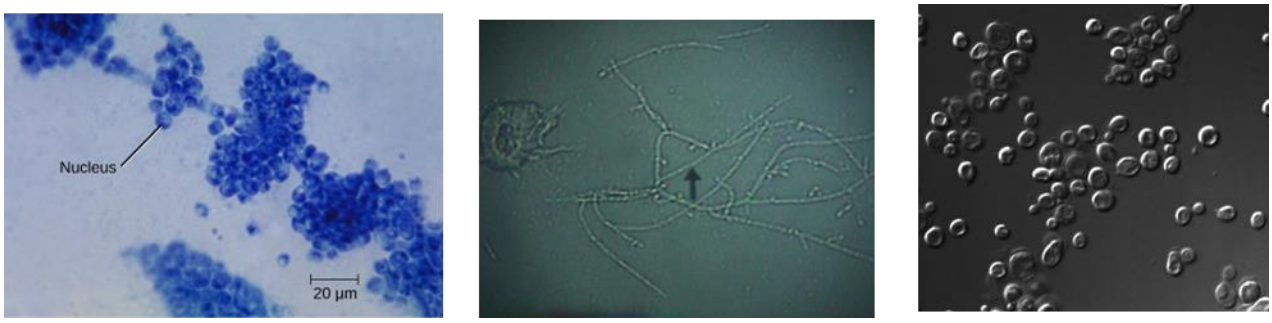


Figure 6: some forms of fungi.

2.4. Protozoa

60,000 species, diverse, amoeboid, flagellates, ciliates, growth, aquatic, predators, prey, animalcules, protozoona, stramenopiles, cellular waste materials, cycles, defferential, gravity, nutrient environment (T Larson, 2023), one-celled, cytostome, zooflagellates, nutrients, ambulate, monophyletic groups, multicellularity, osmotic, 11,000 species, helc stomach, nonphotosynthetic, two-progeny, fission, direct, conjugation, Plasmodium vivax, P. falciparum, P. ovale, P. malariae, four, sporulation, merozoites, haustorium, erythrocyte, blood, merozoites, recurrent, blood cell, blood cell breakdown, fever, shortage, blood cell, hemolytic, toxicity, uremic, asexual, microscopy, apparatus, marine environment, metagenomic, sediment, culture, buccal, cutaneous, fecal, influenza, pathogenic, influenza A, characteristically, cilliated, peristomial, noctiluca, eukarya, much research, lagunas, plamitz (Van Etten et al., 2022)

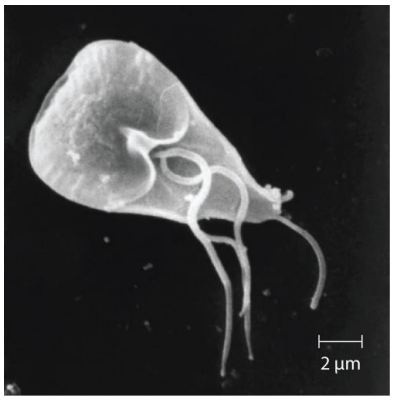


Figure 7: Giardia lamblia, an intestinal protozoan parasite that infects humans and other mammals, causing severe diarrhea. (credit: modification of work by Centers for Disease Control and Prevention) (source: reference (Types of Microorganisms, 2025)

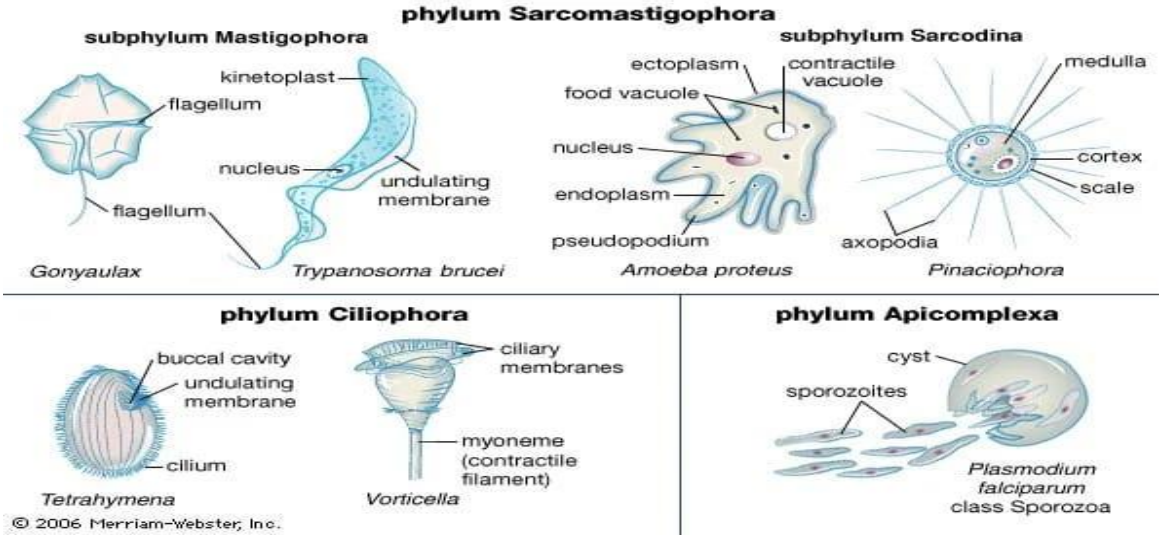


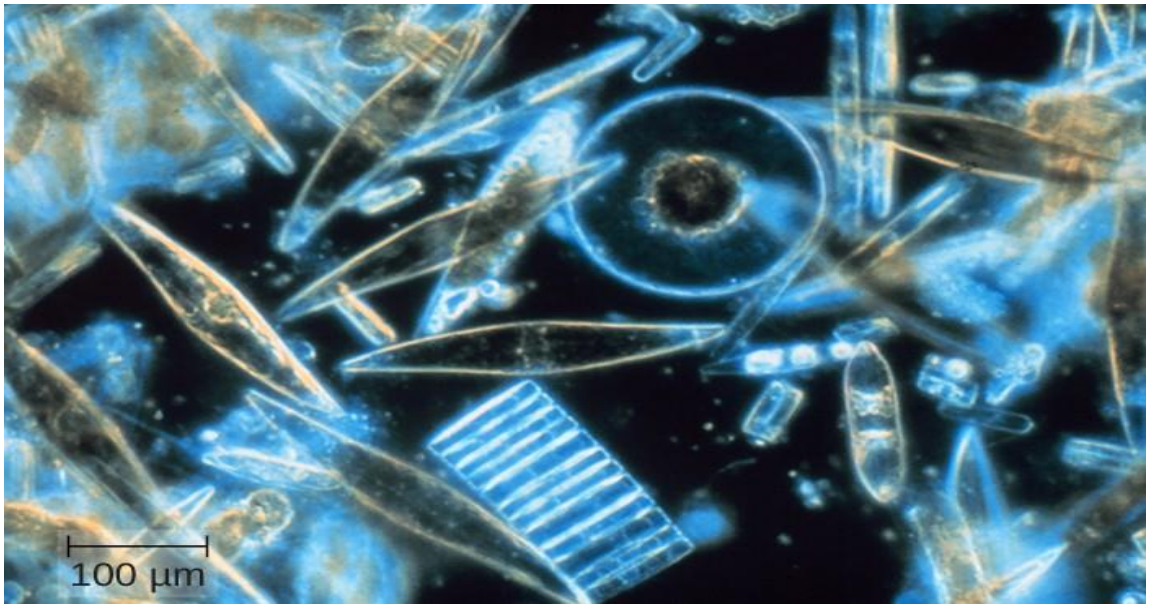
Figure 8:Representative some phylum that belong to the prtozoa.

The phytoflagellate Gonyaulax is one of the dinoflagellates responsible for the occurrence of red tides. The zooflagellate Trypanosoma brucei is the causative agent of African sleeping sickness. The amoeba is one of the most common sarcodines. Other members of the subphylum Sarcodina, such as the radiolarians, heliozoans, and foraminiferans, usually possess protective coverings. The heliozoan Pinaciophora is shown covered with scales. The phylum Ciliophora, which includes the ciliated Tetrahymena and Vorticella, contains the greatest number of protozoan species but is the most homogeneous group. The malaria-causing Plasmodium is spread by the bite of a mosquito that injects infective spores (sporozoites) into the bloodstream.

2.5. Algae

Algae are a diverse group of photosynthetic microorganisms that are important primary producers in aquatic environments. Algae share some common features such as habitat, pigmentation, manner of nutrition, and protection; they are not homologous monophyletic groups. They are currently classified according to their habitat (terrestrial and aquatic) and macroscopic and microscopic structure as well as their pigmentation, such as green, brown, and red algae. The chlorophylls and accessory pigments define the classification into these main groups. This is about the only classification that fits both macroscopic macroalgae and microscopic phytoplanktonic algae. The former include macroscopic, multicellular sea herbs, whereas the latter consist mainly of unicellular cells in the aquatic medium. Microscopic unicellular and coenocytic freshwater green and blue–green "filiform" algae are held in limbo-classification. However, this also separates most of them from photosynthetic cyanobacteria (Blue–green algae) (Lee & Ryu, 2021).

Algae include almost all kinds of photosynthetic organisms in aquatic environments such as diatoms, dinoflagellates, chlorophyte green algae, and certain other noncoarser plants. These microorganisms are considered major primary producers in aquatic environments and involve many special and interesting evolutional adaptations of algal cells and mats. Some of them, such as planktonic groups of algae, are particularly interesting because of their role as significant components of communities and as prevailing primary producers in many aquatecological zones, and they are currently under extensive ecological scrutiny. Despite the polyphyletic origin of the different groups of algae, their photosynthesis apparatus is quite similar. Different pigments adjust the light absorption maximum at several wavelengths, though red light seems to have evolved only in a few ocean algae. For this reason,



most of the other algae still use mostly blue light waves. As for other plants, the range of wavelengths absorbed by the main pigments (chlorophylls c and a) is between 400 and 680 nm, with peaks at 430 and 660 nm. The accessory pigments are bacteriochlorophylls, fucoxanthin, peridinin, phycoerythrin, and phycocyanin.

Figure 9 : Assorted diatoms, a kind of algae, live in annual sea ice in McMurdo Sound, Antarctica. Diatoms range in size from 2 µm to 200 µm and are visualized here using light microscopy. (credit: modification of work by National Oceanic and Atmospheric Administration) (source: reference (Types of Microorganisms, 2025)

These pigments evolved special antenna systems that played a major role in the success of algae and higher plants in creating major and heterogeneous biomasses in aquatic environments. The photosynthetic quantum yield of most phytoplanktonic groups is much higher than higher plants (0.15), and it is strongly attenuated by high irradiance or fast fluctuations (Salami et al., 2021).

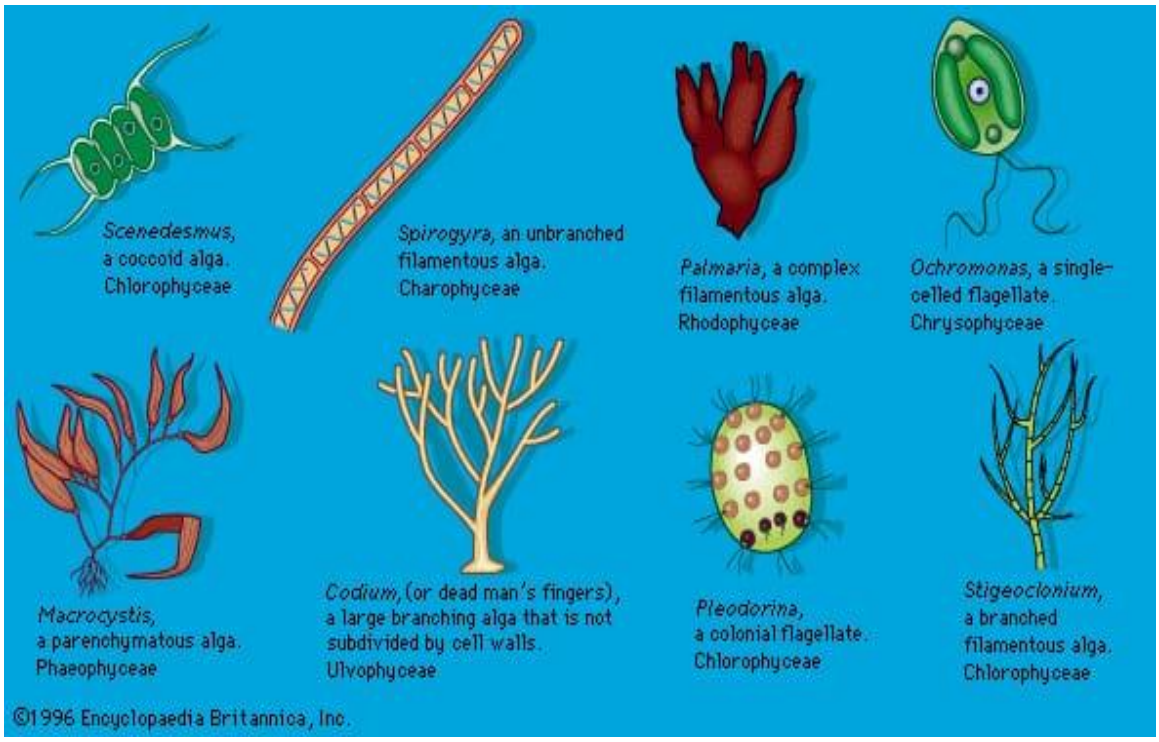


Figure 10: Different types 0f algae

2.6. Viruses

Newspapers will duplicate the text of one another. Once a news event is reported widely in the news media, subsequent reports will often be written verbatim according to the original account. Faunal assemblages are also susceptible to this kind

of cascading—an especially serious problem in areas where the archaeological record is poorly known. In areas of New Guinea where archaeological work is in its early phases, this problem results in fictive taphonomies that draw undue attention to "standard" or emblematic sets of environmentally sensitive artifacts. A different danger—an omniscient authority for microscopic analyses—threatens archaeobotanical replication studies (D. Manuel & C. Snyder, 2024). The success or failure of a micro-study can depend on the behavior of individual phytoliths or starch grains and the precise conditions in which they are preserved. In the wrong hands and under unfavorable preservation conditions, the same specimens can "disappear"; in the right hands and under favorable conditions, "unmistakable" results can be produced. Archaeobotanical replication depends on a chance series of events that is beyond the archaeologist's control. Basanite is a salt that is found at the base of bogs and is lipid-binding. The lipid-preserving nature of basanite, evidenced by the complete preservation of lipids in stalk-scale layers, offers the potential for the replication of phytolith or starch analyses. This replication can be critical in view of the dramatic ways that fragile residues can be transformed by either natural or archaeological processes.

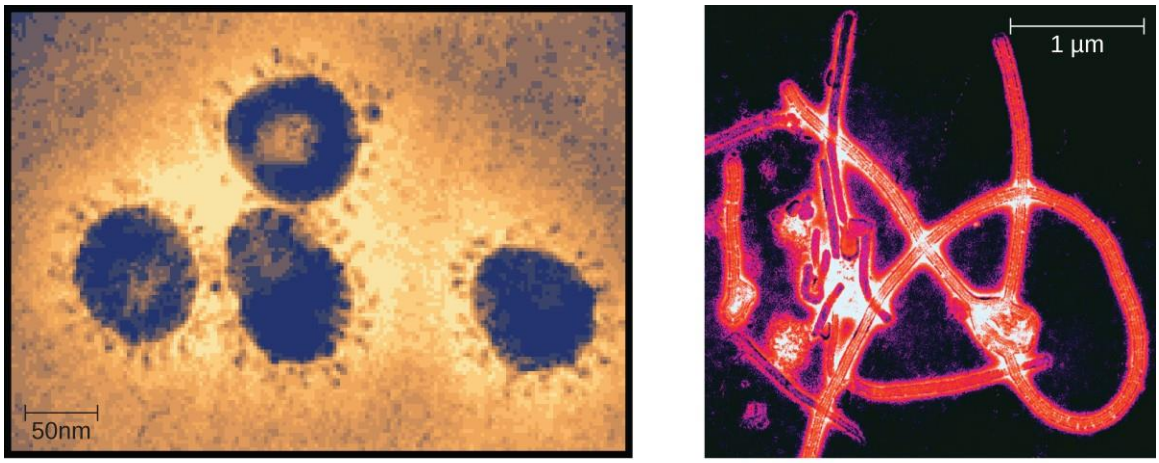


Figure 11: (a) Members of the Coronavirus family can cause respiratory infections like the common cold, severe acute respiratory syndrome (SARS), and Middle East respiratory syndrome (MERS). Here they are viewed under a transmission electron microscope (TEM). (b) Ebolavirus, a member of the Filovirus family, as visualized using a TEM. (credit a: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by Thomas W. Geisbert)

3. Structure and Function of Microorganisms

Most microorganisms are unicellular (single-celled) but some are multicellular. The former is very ancient, having evolved roughly 3.5 billion years ago. The majority of

infections in humans are caused by single-celled microorganisms rather than multicellular forms. It follows then, that understanding cellular organization, structure and function helps understand how microorganisms operate and cause disease. In the 20th century, with great advances in protein and genetic analysis, microbial cellular anatomy has been analyzed at the molecular level (KRELL & BEVERIDGE, 2014). Thus a rediscovery of the essentials put forward in the 19th century now supplemented with a sophisticated understanding of intracellular biochemistry is possible. Therefore, in this context the essentials such as cell organization, basic chemical activities, and cellular growth and reproduction will be discussed. In prokaryotic cells, structural organization depends on cell size, and if within the size range of 0.25-5μm, biophysical considerations prevail. Eukaryotic cells are typically 10-20μm in diameter, and functional organelles contribute to the overall cell design. What prokaryotic and eukaryotic cells have in common are DNA, cytosol, ribosomes and cell membrane. Eukaryotic cells are more complex with membrane enclosed organelles, most notably the nucleus that houses the DNA, endoplasmic reticulum, Golgi apparatus, mitochondria and lysosomes. Although transcription may be spatially separated from translation in some instances, eukaryotic cells use RNA as an intermediary between the two processes. In contrast, prokaryotic cells embody a more homogenous mixture of DNA and ribosomes, within 1μm of the cell envelope. The simplicity of prokaryotic cell structure reflects efficiency. This is seen in energy generation, DNA replication, and protein synthesis. Prokaryotic cells, having focused on a pathway because enzymes of that pathway are clustered together, can thus produce a particular molecule quickly. The more generalized eukaryote cell has a reduced capacity in this regard and each compartment must therefore be larger to maintain a probability of encounters. Eukaryotic cells use compartmentalization to generate complexity and diversity, so cellular location of enzymes, substrates and metabolites then creates a population control mechanism that sequesters specific metabolic pathways thus enhancing cellular flexibility when challenged with differing substrates. Random, metabolite diffusive encounters in prokaryotic cells can create problems with interactions. Substrates not normally found in a cell can poison it: the cell can take in foreign antibiotics and compounds imported to kill the cell and poison it instead. An extreme example of this adaptability is seen in the genus Serratia, whose natural defense mechanisms predates the 20th century's progressive antimicrobial onslaught. As for eukaryotic vegetative cells, microbial resistance mechanisms often mimic those employed in the 10-fold larger mammalian counterparts.

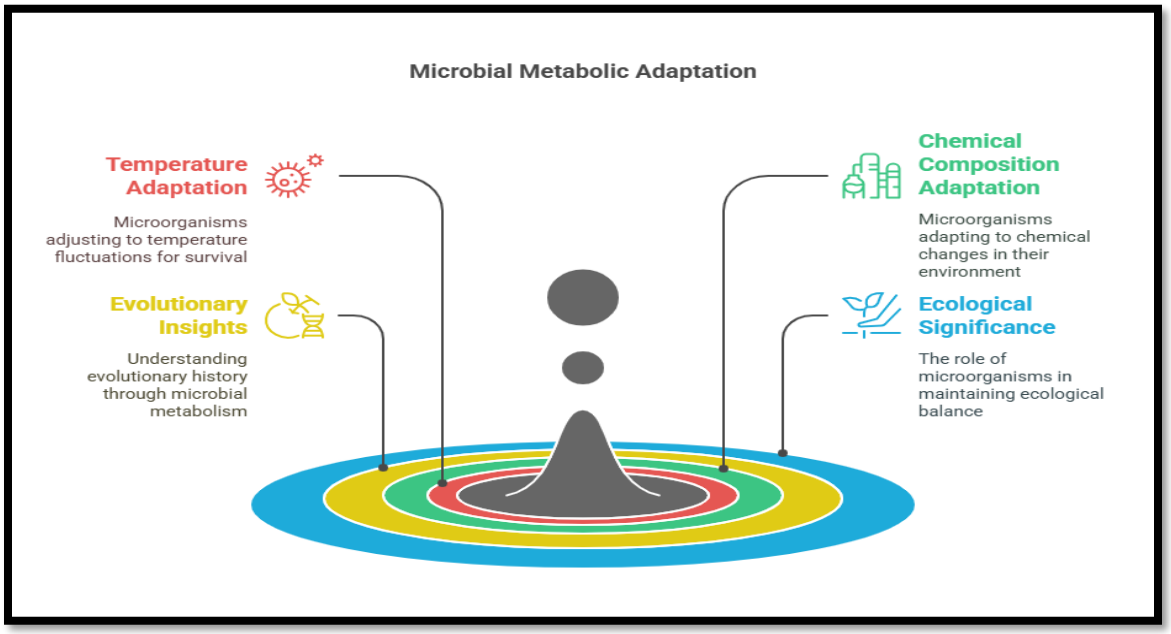
3.1. Cellular Structure

Cells are the smallest structures capable of self-maintenance and growth that exist on their own. Every organism is composed of one or more cells. There are two types of

cell: eukaryotic and prokaryotic. The fundamental difference between these is concerned with the way in which the genetic information is stored — in eukaryotic cells, the information is stored in an isolated compartment, a nucleus; in prokaryotic cells, the genetic information is stored directly in the cellular cytoplasm. Cells fall into two divisions: eukaryotic organisms encompass a large variety of organisms — animals, plants, fungi, and protists. Each cell in eukaryotic organisms has a nucleus. It is a central compartment in which the genetic information is stored along with other proteins. Prokaryotes are relatively simple types of cells having no true nucleus (Widłak, 2013). Included within the classification of prokaryotic organisms are bacteria. Single cells compose the vast majority of eukaryotic organisms, thus in practice the cell names also apply to the whole organism. The classification of prokaryotic organisms has been augmented with the discovery of a new group distinct from bacteria. This new classification encompasses three groups: archaea and bacteria. Both archaea and bacteria are single-cell organisms; by contrast eukaryotic organisms are composed of many cells. Archaea in a sense are prokaryotic organisms as they lack a true membrane enclosed nucleus in which the genetic material is stored. Archaea live mostly in extreme environments and have been divided into a few groups based mostly on the environment they live in. In contrast with eukaryotes for which a precise classification of organisms is possible based on the structure of the organism (e.g. the presence of the nucleus), the uniform nature of the cellular structure in prokaryotes makes it impossible to divide them into additional groups based on the same principles. On the other hand archaea are classified as prokaryotic organisms. However, some groups of bacteria and archaea are distinguished mainly by metabolic characteristics.

3.2. Metabolism and Energy Production

Metabolism consists of a series of chemical reactions that occur within cells to sustain life. Metabolic processes involve the utilization of carbohydrates or fatty acids, the assembly of macromolecules, and the breakdown of macromolecules to generate ATP. Microorganisms, due to their metabolic diversity, have the ability to exploit many different habitats and energy sources ranging from simple organic compounds to complex polymers and even inorganic matter. What differentiates the three domains of life is their evolution of different metabolisms over billions of years. Metabolism involves the conversion of simple precursors (substrates) into more complex compounds (products) and the breakdown of complex compounds into



simpler precursors. Metabolism is based on a large number of complex enzymatically catalyzed reactions that form specific pathways and networks (Judge & Dodd, 2020).

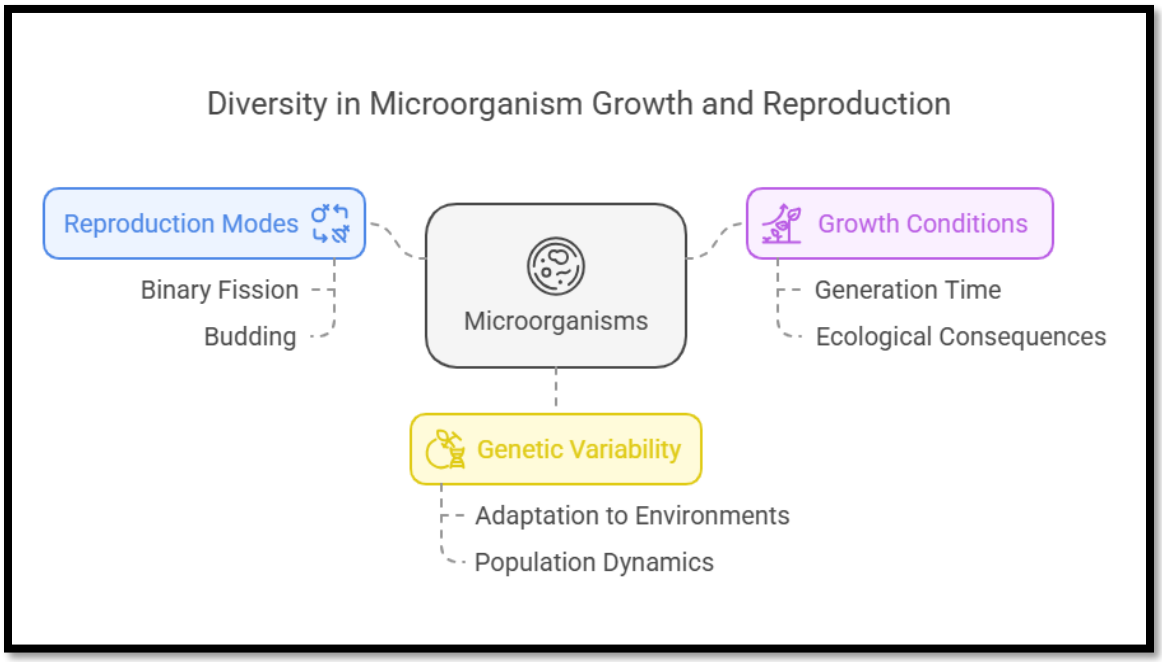
This class of metabolic processes can be classified in two ways: the generation of energy and molecules, anabolism; the use of energy and molecules, catabolism. All metabolic pathways were organized to these two functions: storage and use of chemical compounds and energy. Microorganisms are diverse in their metabolic abilities and are environmentally relevant because of key roles in biogeochemical cycles, bioremediation, and bioconversion (Dang & A. Chen, 2017). About 90% of all bacteria are anaerobic and have developed or acquired ways to generate ATP without the help of oxygen. Bacteria can respire externally or ferment, a process involving intracellular redox exchanges and limited energy yield. In a sense, fermentation is a rudimentary form of respiration. Bacteria possess a rich set of enzyme families that enable them to catalyze virtually any metabolic reaction. A majority of metabolic

processes in bacteria can be performed by multiple enzymes sharing the same function. Any reaction can be optimized by the evolution of different enzymes. The microbial metabolic system allows these organisms to adapt and grow despite varying environmental conditions such as changes in temperature and the chemical composition of their surroundings.

3.3. Reproduction and Growth

Microorganisms are a tremendously diverse group with respect to their biology. This also holds for how they reproduce and grow, as well as which conditions are optimal for their growth. Various modes of reproduction and growth are presented here, before various conditions favorable for the growth of a diverse set of microorganisms are discussed. This includes the importance of generation time as an aspect of growth and its ecological consequences. The role of genetic variability for the adaptation to fluctuating environments, larger and therefore slower growing bacterial populations, and thus the role of other bacterial populations in stabilizing the growing population dynamics are considered. Several experimental results expose the possibility to infer the intrinsic limits of possible growth and shape of populations from only basic in situ characterizations. Microorganisms are a tremendously diverse group with respect to their biology, which includes many different bacteria and archaea, various unicellular eukaryotic microorganisms such as yeasts and protozoa, but also multicellular forms of the latter such as fungal mycelium or even slime molds. Although it is nearly impossible to make broad statements that hold for all of them, a highly successful generalization by a lot of microorganisms rely on asexual reproduction, referring to this generalization seems useful to provide a common point of reference and to introduce standard terminology. However, many additional and more diverse life cycles could be discussed when expanding the view beyond the best-studied mammalian model. Given the very different biology of microorganisms as compared to elephants and the lack of a widely recognized common reference similar to binary fission, and given that no generality regarding e.g. offspring biomass size or yeast budding are anticipated, it seems appropriate to rather introduce the diversity of modes in which microbes can reproduce anew. Most of the common bacterial species, for instance, reproduce asexually by binary fission, meaning that an individual grows to a certain size, then divides into two (as equal as possible) offspring cells, which subsequently grow to that same size before dividing again (Pichugin & Traulsen, 2022). Budding, where a daughter cell develops as an outgrowth of the parent that eventually gets released and grows to its size, is an alternative route to this same end. Various species dispense with the need to grow between fissions altogether, instead budding directly at the time of cell formation and splitting it immediately after release. Hence, the daughter cell does not grow with respect to its size after division, but only to the extent that the parent did beforehand. There are unicellular yeast-like

species, however, that bud not as an outgrowth, but by symmetrical growth in only one location until the two parts are about similar in size.

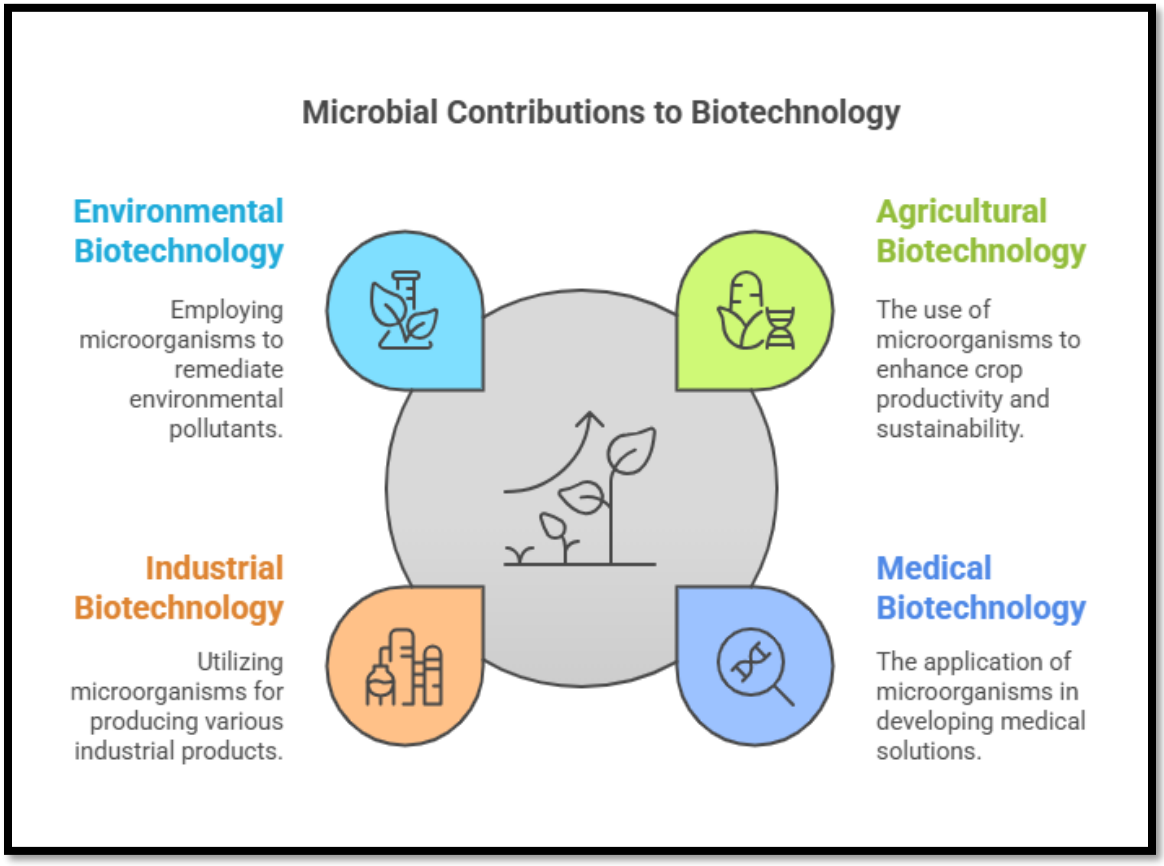


Even multicellular animal species branching off offspring as clonal individuals under many naturally occurring conditions lack a counterpart in the microbially dominated parts of the ecosystems. In contrast to this static clonality, some colonies are dynamic in space and time with parent and offspring constantly joining and disassembling, and yet none of them grow to a significant fraction of the parent's total volume.

5. Applications of Microorganisms in Biotechnology

Over the last few decades, there has been fascinating progress in the field of biotechnology. Presently, microorganisms are widely applied in myriad cellular and molecular approaches, exhibiting an impact on every sphere of life. The applications of microorganisms in biotechnology are vast. There are four categories associated with their application: agricultural, medical, industrial, and environmental biotechnologies. The use of microbial products, microbial measurements, and genetically engineered cells and microbes are classified as the latest application of microorganisms in the biotechnology sector. Efforts to maximize crop productivity and sustainability are driven by the application of biotechnologically developed biofertilizers, along with biopesticides. Modern agricultural biotechnology is aimed at genetic modification in crop plant species. This possibility has been supported by plant transgenes, which are manipulated by microorganisms. Hence, microorganisms are extensively employed in agricultural biotechnological processes. Biopesticides

enriched with Bt are currently among the most utilized pesticides. Downstreaming bacterial processes, such as bioformulated production of industrial products, have been established through biotechnology. This branch of science has been applied industrially since 1765, beginning with ethanol production. Industrial biotechnological applications have recently been extended beyond food and beverage to produce biofuels, chemicals, fertilizers, and energy. Modern products use living cells and enzymes for bioconversions. Such applications have been extended to waste management and waste health implementation. Pollution levels on the ground, in water, and in the air are dramatically rising due to rapid industrialization and urbanization, so there are new and significant implications of microorganisms in environmental biotechnology. Bioremediation is an environmentally friendly technology that uses various components of bioremediation to biochemically catalyze the degradation processes of environmental contaminants. Similarly, understanding the operation of these technologies in tandem with the actions of other technologies can represent a comprehensive solution for ecological spoilers.



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Chapter Two

Exploring the Microbial World

1. Introduction to Microbes

Microbes, or microorganisms, are widely defined as small organisms not individually seen by the unaided eye that include bacteria, archaea, fungi, protists, and viruses. Here, the spotlight is given to those which encompass domains Bacteria, Archaea, and certain groups of fungi, protists, and viruses alike, in some cases collectively termed protists. These are collectively considered as microbes, distinct from macroorganisms (eg. plants, animals, larger protozoans, most fungi and algae) (Maraz & Khan2021)(Singh, 2023)(Uluçay, 2023)(Gupta et al.2021)(Dhagat & Jujjavarapu2022)(Sharma & Yumnam, 2024)(Chanda & Joshi, 2022)(Asiegbu & Kovalchuk, 2021)(Muhammad & Saadu2023) (Muhammad & Saadu2023)(Asiegbu & Kovalchuk, 2021)(Dhagat & Jujjavarapu2022)(Chanda & Joshi, 2022)(Sharma & Yumnam, 2024)(Gupta et al.2021)(Ibiene)(Maraz & Khan2021)(Singh, 2023)(H. Youssef et al., 2015). In the ecosystem, microbes, including viromes, are more referable than macro-organisms to form, genetics, physiology, structure and metabolic behaviour. Although often unseen, they are critical in ecosystem behaviours and are experimental for further studies to understand an ecosystem completely. The question of microbial biogeography and its effect on ecosystem service is only further entertaining austerity of a regime viewpoint.

Microbes are key components of every ecosystem, performing a wide variety of nutrient recycling and energy transfer tasks. Microbes are also the most abundant types of organisms, single-celled prokaryotic bacteria and archaea, believed to be the earliest form of life that emerged nearly 4 billion years earlier, a billion years before plant and animal era (Mony et al., 2020). But the type and function of these microbes remain mostly unknown, so it is understandable that microbiology revelations in recent years have urged a proverbial storm. Although classical microbiological experiments on culture-based biology and microscopy already number more than one and a half centuries, it would not be until the 21st century that technical progress in genome sequencing and the growth of computational power making valuable connections occurred. In addition diversity and redundancy of the microbial ecosphere have posed astute obstructions to identifying a reality of how settings are handled by microorganisms.

1.1. Definition and Classification

Living organisms are traditionally divided into two broad groups, the micronic (or 'microorganisms') and the melanic ('macroscopical organisms'). Microorganisms (or 'microbes') are defined as living, microscopic entities not resolvable with the unaided human eye. A wide variety of microorganisms exists, comprising a great diversity in

cellular structure, physiology and thus also in metabolic capabilities. Microorganisms may hence be classified on a number of criteria. Traditional classification schemes divide microbes into either the prokaryotes (or Monera), those groups without a membrane-delineated nucleus, or the remaining groups, the eukaryotes that possess a nucleus. Over the years, through the application of improved microscopic techniques, other criteria of microbial identification have been suggested. These other criteria have recently been perfected through comparisons of base sequences of microbial 5S rRNA owing to techniques in molecular genetics. Such comparisons have lead to a reassessment of current classification schemes and the emergence of new microbial categories and insight into the scrutiny of evolutionary lineages. The discovery and insights into the peculiar archaeobacteria (species with a peculiar metabolism) shows the complexity and diversity of the microbial world.

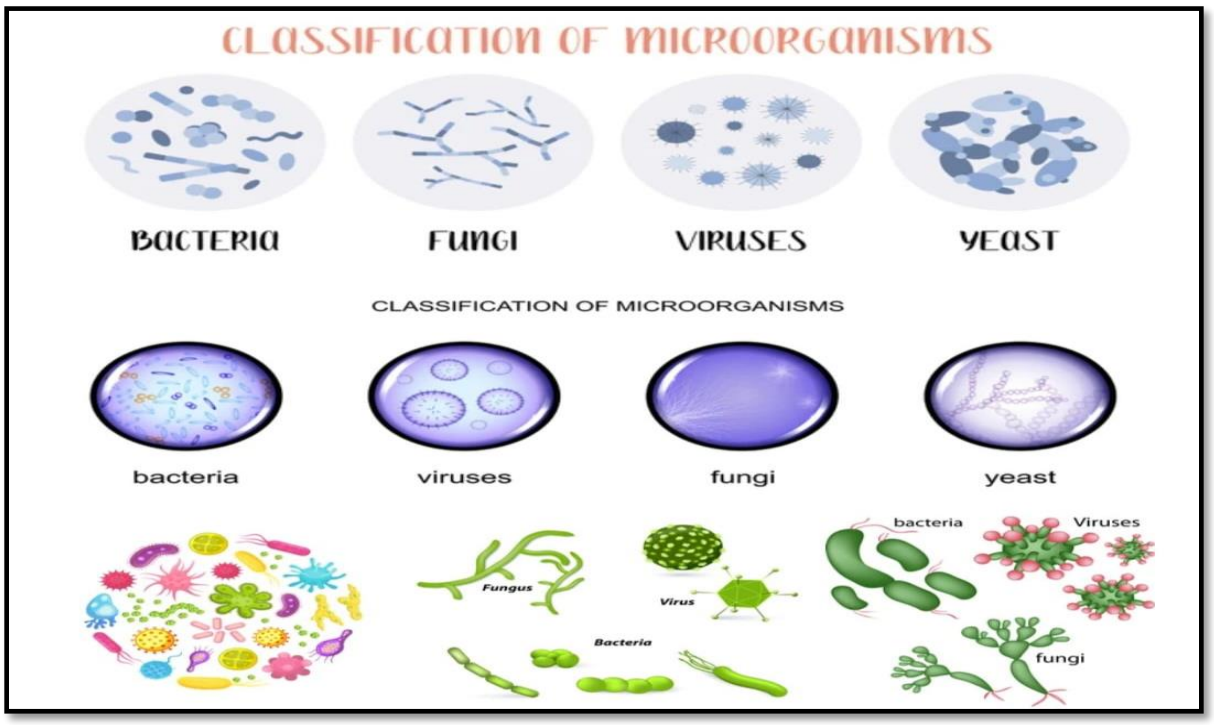

Figure1: Classification of microorganisms

To illustrate the complexity of the microbial world consider that the total number of species on earth has been estimated to range from 3 to 100 millions (Okafor, 2011). This is the equivalent to a 1000 different species of bacteria for each species of plant and animal combined; a truly impressive thought. Laboratory experiments suggest that a single gram of soil may contain thousands of different species of bacteria alone. To explore the complexity of life on our planet, let us consider a few case examples of significant microbial groups, representative of different habitats and phyla, and exhibiting a range of metabolic capabilities and growth requirements.

1.2. Importance of Microbes

Microbes form an enormous and diverse group of organisms that are omnipresent in the biosphere. They affect their surroundings through a range of mechanisms that allow growth and survival. Microbes also have a substantial impact on the environment, with effects occurring in various ways. The results of sensitivity profiling assays against environmental perturbations suggest whether the community is perturbed and, if so, what changes may affect the community (J. Blaser et al., 2016). The effects of microbes on their environment can be beneficial or detrimental. In an applied framework, increased understanding may lead to the management or promotion of specific changes in microbiota to enhance system resilience and functioning (Gupta et al., 2016). The most significant effect of microbes on Earth is their involvement in the recycling of the primary elements that make up all living systems, especially carbon, oxygen, and nitrogen.

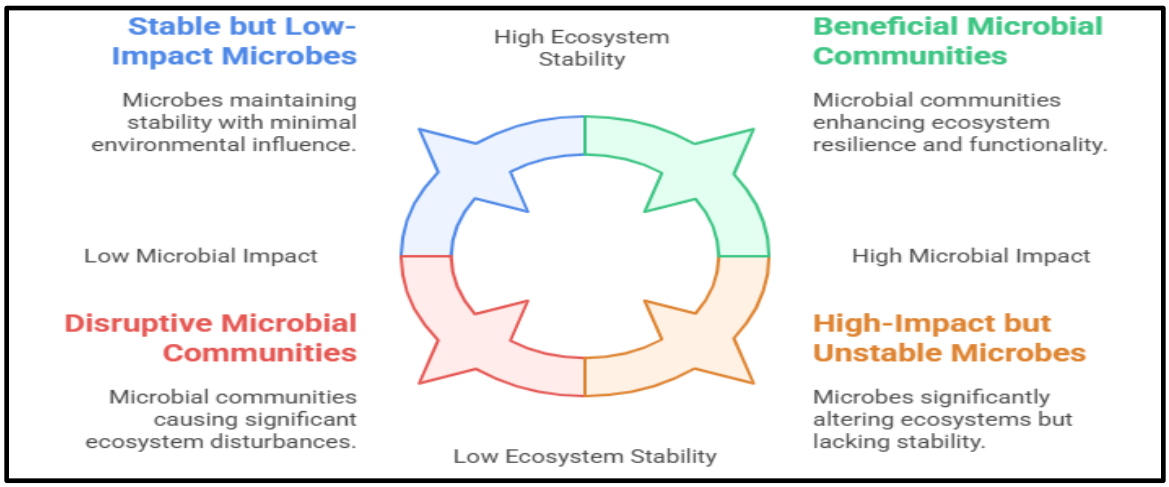

Figure2: Microbial Impact and Ecosystem Stability

Comparing natural settings in both species-rich and species-poor environments, researchers analyze the effect of local plant diversity on soil microbial community composition and resulting properties. Since microbes are the largest biomass on Earth, they also have the greatest propensity for affecting the environment.

An Earth-like knowledge of the microbial world that anticipates the operation of its ecosystems could realistically result in the end of hunger, the cease of all degenerative diseases, an unlimited supply of clean water, plentiful wildlife, the leisure to enjoy it without living in immediate fear of our lives from climate change. However, the nature of this may vary considerably with the experimental biome involved. The microorganisms that form these ubiquitous communities form 50% of the world's ecosystems and play a crucial role in supporting the life forms on Earth. Additionally, a closed chamber system could artificially create a consistent community that does not necessarily resemble real environment or could be a proxy for high N-bias of the resident microbial consortia. In the systems stability concept used here, the stability concept is expanded to define both the ability of microbiomes to resist large changes in function when perturbed and their ability to rapidly recover to original function.

2. Microbial Diversity

Much of the biosphere's microbial populations remain uninvestigated as these entities are rarely present under non-interference conditions. However, over the past decade, technological innovations have begun to illuminate the previously 'dark' microbial world (H. Youssef et al., 2015). Broad shifts in microbial populations have recently been discovered to occur over space through time. Understanding such shifts and the phylogenetic diversity within them is crucial for interpreting the dynamics of and predicting impacts on community composition.

There are multiple taxa in the microbial world with a scale of diversity not found elsewhere. Expanding our knowledge of microbial populations that range across ecosystems and impact nutrient cycling/energy flow is vital. Such knowledge would enhance understanding of the temporal and spatial scales at which microbial populations shift and the potential influence of these shifts on ecosystem functions. Techniques of next generation sequence and direct community DNA from lake sediment were used over space through temporal gradients for deciphering the complex dynamics of populated microbial communities. Changes were found in microbial community composition including key taxa not previously linked to observed system shifts. This material provides new insight on environmental factors and potential mechanisms driving the broad community shifts occurring. In combination with other recent results, this novel evidence implicates key microbial lineages in ecosystem scale transformation of ecosystem states. Broad instructions governing the diversity and dynamics of microbe-eukaryote symbiosis are provided. These findings offer a path towards understanding broad shifts in ecosystem structure across the macroscopic world.

2.1. Bacteria

The microbial world is composed of organisms that are too small to be seen by the naked eye. These include the archaea, bacteria, fungi, algae, and protozoa. This chapter focuses on bacteria, the most numerous and diverse group of organisms on Earth. Like all other microorganisms, bacteria have thrived on Earth for billions of years, contributing to the biogeochemical cycling of all elements. In humans, some bacteria cause disease, while others are beneficial and even essential to health, aiding digestion.

Bacteria are cosmopolitan and are found everywhere in nature. They exist in very large numbers and in places where most other living things cannot grow. As a group, bacteria exhibit the greatest metabolic diversity of the microbial world, reflecting their wide array of habitats (Okafor, 2011). Bacteria are the most numerous group found in both soil and water. In the soil, bacterial diversity of different ecosystems has been categorized, including cropland, grassland, and desert. Bacterial communities in the ocean change as the environment is altered naturally through seasonal changes, and artificially by the discharge of warm water from power plants. Freshwater bacteria are responsible for the removal of waste from treated sewage effluent. Because they exist in both wastewater and commercial waste treatment processing plants, bacteria have the capacity to remove much of the organic carbon from waste in treatment plants (H. Youssef et al., 2015). It is now known that many

of the changes in ecosystem health that were previously considered the result of shifts in viral or macrobial communities are instead due to shifts in bacterial populations. Land use activities exert a greater effect on the number of bacteria present in different ecosystems relative to other types of organisms. Some of the land use activities that alter the bacterial community structure in soil are irrigation, pesticide exposure, and high tillage intensity. As a result of increased enhancement of carbon cycling, land use changes promote the blooms of prokaryotic and eukaryotic organisms. Industries can significantly affect bacterial communities through the release of heavy metals, solvents, and other toxic chemicals, resulting in an increase of metal and antibiotic resistant bacteria in wastewater treatment plants. Major epidemics of humanitarian concern, such as cholera, were largely relieved via environmental intervention. Chemically synthetic agents created in the last century have proliferated on Earth's surface and have lived a profound impact on bacterial ecosystems. In contrast to the comparison with the marker genes from known eukaryotic organisms, a far greater number of PCR amplicons were obtained when using the 27F primer in attempts to amplify bacterial 16S rRNA genes. Fatty acid analysis has been less useful for discriminating between similar species of eukaryotic microorganisms than for bacteria. In fresh water, many communities of picoplanktonic bacteria with highly specialized roles have been described, such as purple sulfur bacteria found in stratified surface fresh water lakes.

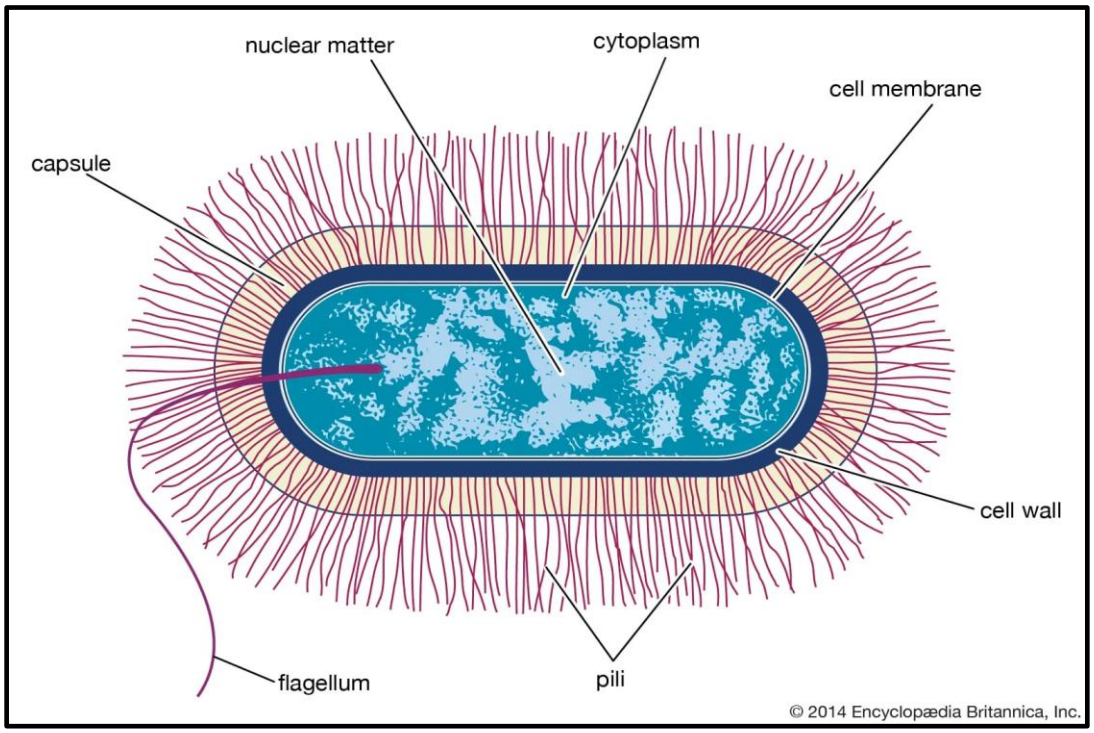

Figure3: Bacterial cell Schematic drawing of the structure of a generalized bacterium.

2.2. Archaea

The discovery of the Archaea has provided a wealth of information about the Tree of Life. Despite their unique structure and physiological characteristics, Archaea may have more in common with Eukarya. A hyperthermophilic member of the Archaea

was found that was related more closely to the eukaryotic cytoplasm via a common cenancestor than to the origins of the Eukarya, Bacteria, or mitochondrial lineages. Therefore, the discovery of the Archaea is indeed a remarkable find, enlightening about the organismal diversity and the physiology of the microbial world (H. Blum, 2008). Many interesting and important scientific questions have arisen. Previous notions about the Tree of Life have been challenged as well as strengthened. Gradually emerging are not only the idiosyncratic differences between the three major groups but also the ancient, conserved similarities and features linking all life together. Metagenomic studies of the microbial world will continue to rapidly expand and revise over traditional notions of the unobservable hidden majority. What will be resolved is the relationship and contribution of this uncultured majority to the ecology and function of the planetary biosphere. Not surprisingly, this hidden world forges phylogenetic and biogeochemical ties with domain of the Visibles, Archaea.

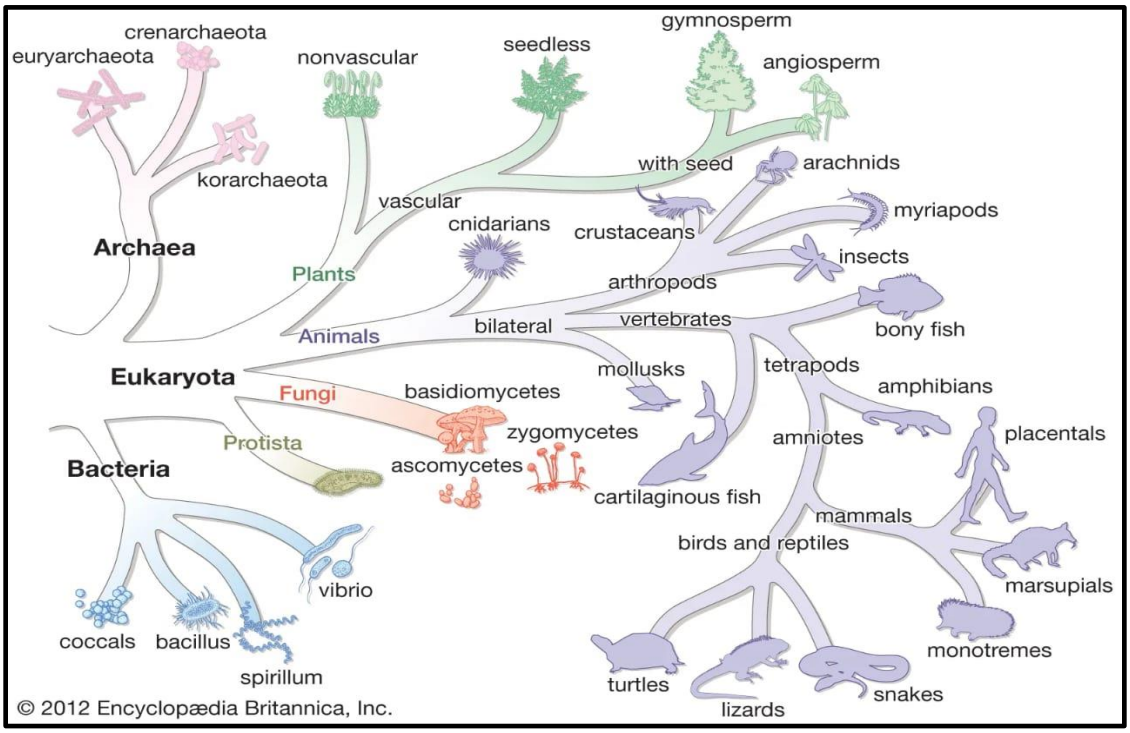

Figure4: Tree of life illustrating the three-domain classification of life-formsThe tree of life according to the three-domain system of biological classification.

Archaea play a pivotal role in biogeochemical cycles. Understanding the role of Archaea in soil systems is incomplete but emerging. Soil acidity can impact the presence of specific archaeal species and the relative amount of archaeal ribosomal RNA. In pH 5.5 soils, the amount of archaeal rRNA is low compared to forest soil. Soil acidity also influences the level and type of ammonia oxidizers, which are key players in the nitrogen cycle of terrestrial ecosystems. To date, little is known about the involvement of Archaea in ammonia oxidation in soils, and there are no clues about the gene expression through laboratory experiments, the aspects of the nitrogen cycle in archaeological aspects of commonly biogeochemical cycles. These approaches begin to expand the understanding of microbial diversity and biogeochemistry, otherwise related to recent studies and the science of ecosystem processes.

2.3. Fungi

Fungi are a critical, but often overlooked, component of ecosystems worldwide. Ubiquitous and forming a group of highly diverse kingdoms, Fungi are model organisms that can be used to understand broad areas of biology and research questions. They are proposed as mediators linking organisms and ecosystems, with interacting fungi facilitating, influencing or mediating the responses of a host organism. In contrast, they enable or constrain environmental conditions. The machinery and pathways that drive these ecological functions are influenced by factors such as horizontal gene transfer, gene regulation, and the structure of populations and communities.

The kingdom of Fungi is comprised of around 3 million species, almost one hundred times the number of plant species, each with unique ecological interactions and environments. Fungi form diverse communities on a range of hosts and substrates and have considerable environmental impact. Comprising as much as 25% of the biomass in soils and their network of hyphae can span vast areas. In other environments, such as the leaves of plants, fungi can cause devastating diseases. Recent estimates suggest that over 3 billion tons of biomass are turned over each year by terrestrial fungi, a rate higher than that produced by animals in all environments combined. Most of this decomposition is carried out by guilt fungi, the only organisms capable of breaking down recalcitrant polymers of lignin. In aquatic environments, parasitic chytrid fungi are responsible for global losses and extinctions of amphibians and fish. Another type of aquatic fungi colonize aquatic macrophytes, where they can degrade submerged biomass, and their subsequent colonization by bacteria can change nutrient cycling and food web dynamics. The kingdom Fungi, however, has been somewhat slower to join the party.

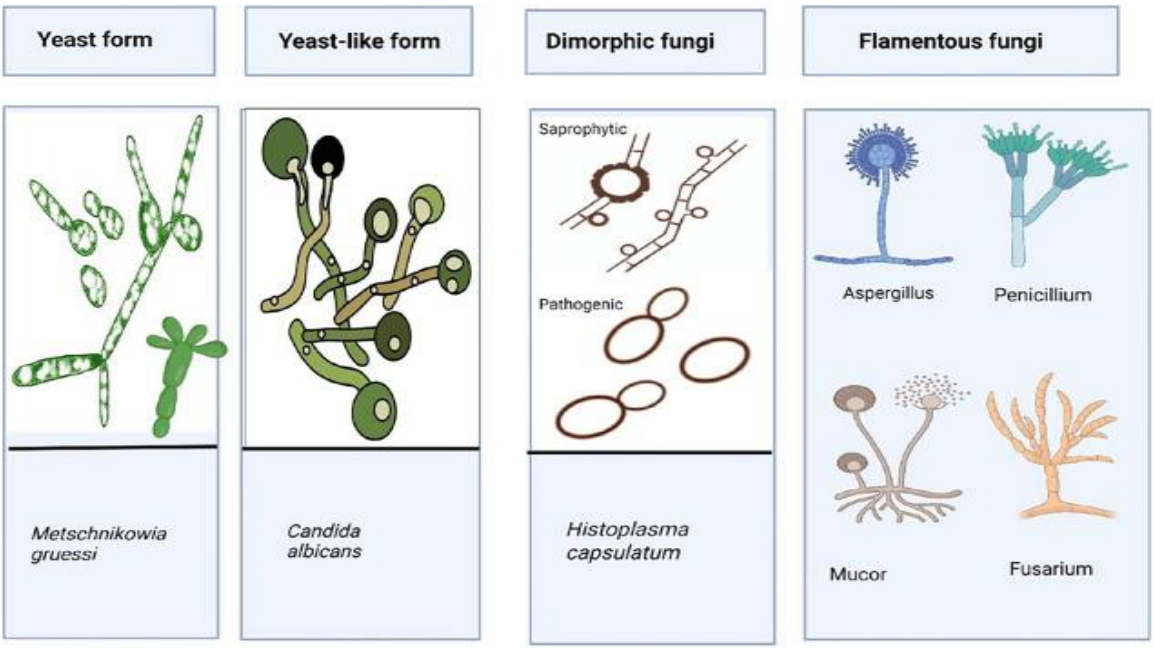

Figure 5: Different types of fungi and their cell shapes.

2.4. Viruses

Viral biology centers on entities that exist on the fine edge of living and non-living. Despite the great structural and genomic diversity displayed by viruses and other small entities, they are united by their extraordinary smallness. Much unlike cellular organisms, the existence of cellular life forms is bounded by lower limits of genome and protein size. Overwhelmingly, cellular organisms, including any life form easily recognizable as a creature, sit at a substantial distance above these limits, and are composed of tiny molecules termed proteins that can be synthesized in 64 or fewer representatives. Even in cases where viruses significantly outstrip the level of genomic complexity of tiniest cellular organisms, the much narrower confines of protein space they are forced to operate in presents them with constraints that do not limit cells. In this sense, it could be said viruses are not simply composed of protein, but by necessity are wholly-held by protein. At their most bedrock level of description, viruses are simply nucleoprotein: nucleic acid ensheathed by a shell of protein (D. Manuel & C. Snyder, 2024). This defining combination has deep structural and biophysical consequences which are able to couple in intricate and profound ways with cellular structures that define the reproductive and biochemical activity of cellular organisms. It allows viruses to leverage cellular activity in a fashion that simultaneously permits replication, yet ensures cellular resources can be re-purposed for their own ends.

From an ecological perspective, the existence and characteristics of viruses are closely twined with their role in the maintenance of microbial populations and how microbes function as major drivers of essential ecosystems on Earth, including those involved in global biogeochemical cycling. The co-evolution of microbes and their viruses has and continues to profoundly shape the development of all ecosystems, even human beings. While the simple decimation of populations is the most easily imagined aspect of a virus's ecological role, a more nuanced picture also is drawn from current perspective. Though viruses (not only bacteriophages, but eukaryotic viruses) effectively consume and lyse a large amount of microbial biomass, those subsequently burst cells then also serve as material support for the growth and development of the microbial community. Viruses stimulate host microbial community metabolic activity through production of metabolites and co-factors, and this is in turn thought to lead to enhanced transformation of organic matter (Roux, 2019). Oddly, infected cells may experience marked changes in gene expression leading to enhanced excretion of dissolved organic carbon, thereby increasing microbial "metabolic bagginess" and stimulating the growth or newly infectable hosts. Rich ooze, a thick plume of ectoplasm and free ribosomes, leads to a stickier and more nurturing environment in which the bursting of a majority of the biomass may actually lead to a stimulation in overall community growth by significantly beneath burst size classes. But there's an invert bells-on-it. Just as infection may lead an energetic expenditure in gene expression and diversion of host resources to viral production, viruses may manipulate their hosts so as to divert growing cellular biomass to otherwise unused cellular parts, therefore making it unavailable for viral material. The larger the total host population, the larger the fraction of the overall biomass becoming sequestered in quiescent cells, and closer to a zenith. With wastrel

cells sitting about, squalling for resources, but not toting them to viral factories, these cells represent maintenance function parasites, living fossil records of what they once were in the community.

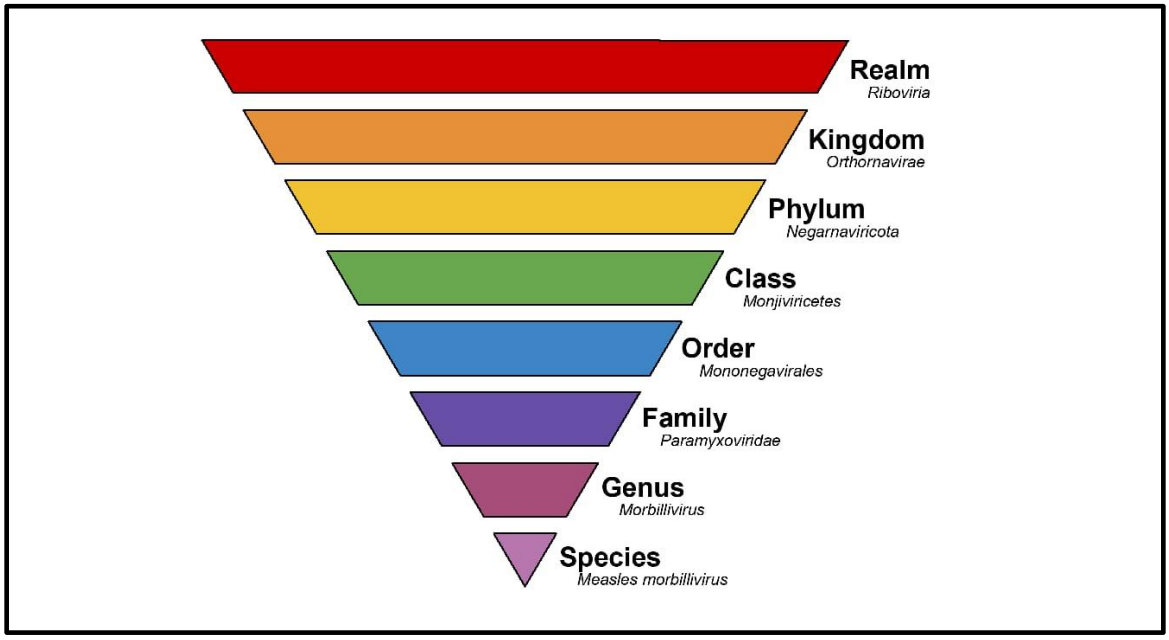

Figure6: Classification of viruses.

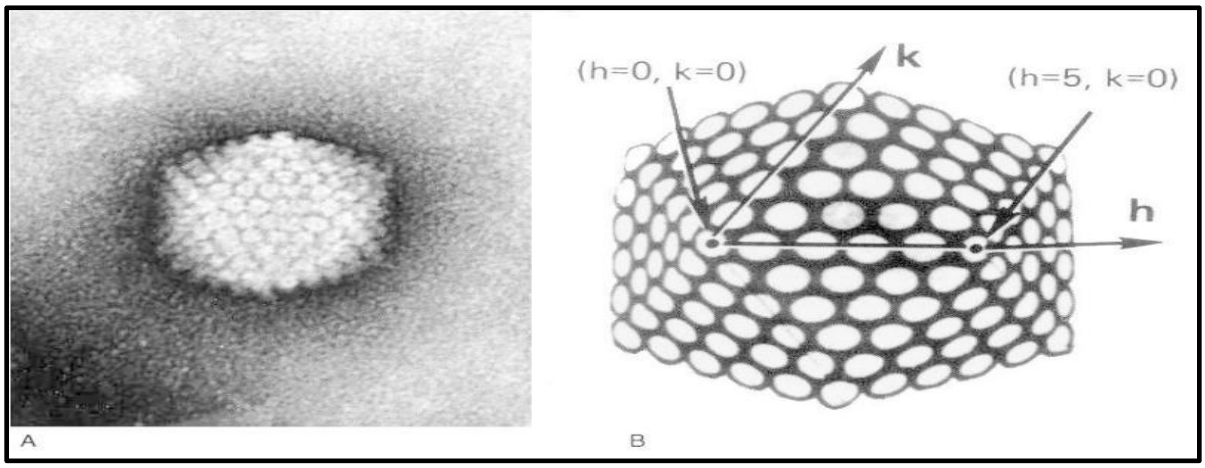

Figure7: Adenovirus after negative stain electron microscopy. (A) The capsid reveals the typical isometric shell made up from 20 equilateral triangular faces. The 252 capsomeres, 12 pentons and the 240 hollow hexon capsomeres are arranged in a T = 25 symmetry.

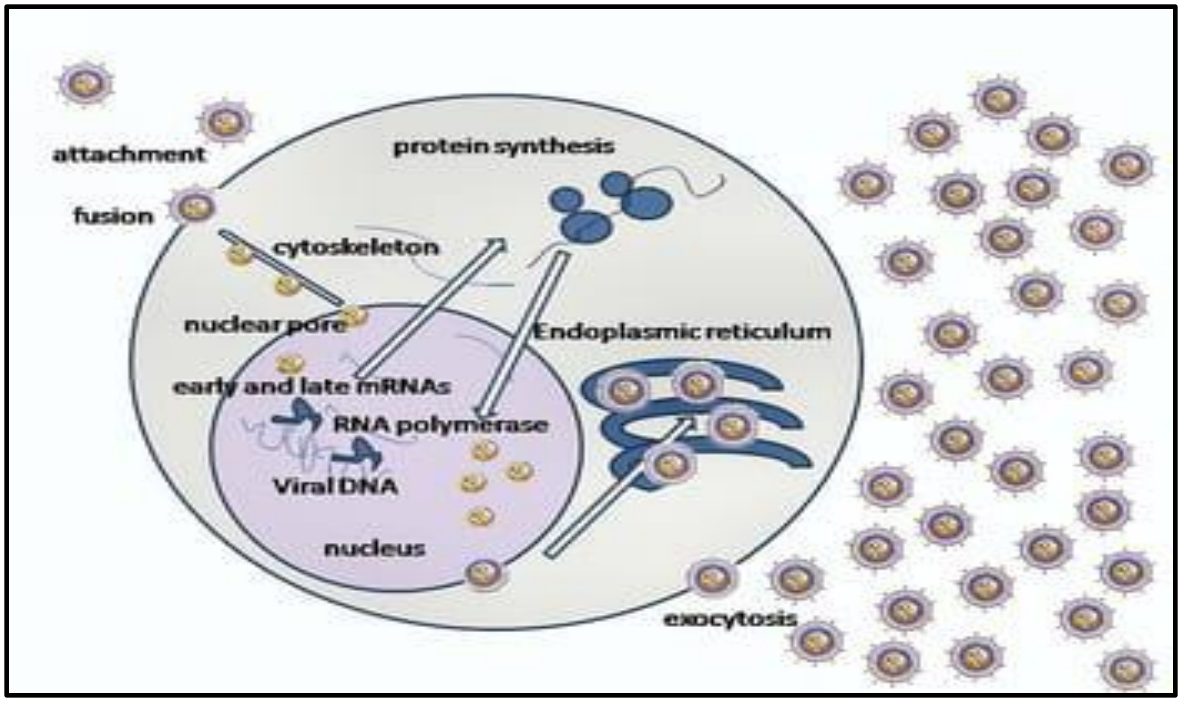

Figure8: Virus cycle: entry of herpes simplex virus (HSV) into the cell (on the left), multiplying within the cell and release (right) from the cell

3. Microbial Ecology

Microorganisms represent by far the largest fraction of biodiversity. Archaea, bacteria, fungi, protists, and viruses constitute the main groups of microorganisms. The amazing abundance of microorganisms on earth is now acknowledged due to molecular approaches, these minute organisms play a central role in every biogeochemical cycle of elements. In soil, microbes strongly affect fertility since they participate in all the stages of the N cycle, they release phosphorus by secreting chelating organic acids, they enhance the release of assimilable nutrients by solubilizing minerals, and they degrade organic polymers and thus determine the storage of organic C. This last point is of particular interest in the context of global change because it raises the question of whether the current C storage in soils saturated at the global scale, will be modified by human activities. Importantly, soil is a carbon source because of its function in terrestrial ecosystems but it is also subject to microbial degradation, this is central to the atmospheric CO2 pool. Hence, to predict possible feedbacks to the rate of CO2 release to the atmosphere, it is crucial to understand how the distribution of microbes depends on the different components of community assembly (Mony et al., 2020). Ecologically relevant questions naturally rise in this context: First, the resolution of this description is required for a better link with niche theory and coexistence mechanisms, as well as with a better understanding of how community assembly is related to the functions and functioning of these microbial ecosystems. Second, the interactions (competition, mutualism, etc.) between the different fractions of comicrobial strategies to answer to the questions posed by this high-throughput description. Two main approaches have been hitherto pursued to explore. Now attention will be paid to the abiotic and biotic factors that structure free-living bacterial communities, in particular their

variation along environmental gradients. The analysis will be based on the bacterium, which is clearly identified, and soil samples are taken from different sites. This studied system concerns ten sites differing in their biological and physical properties and representative of a given ecosystem. The sampled sites concern middle mountain alpine grassland located in the Vercors massif, France. Six of them are maintained by extensive sheep grazing, and four with high altitude pastures are characterized by the presence of the yak Mergetale breed. The data are most fundamentally compositional and this high predictive power can be shown on the spatialisation of individual samples, that is in the biplot. Here, datasets form a 5x2 matrix and this structure can be projected on principal component 1 and 2 which appears as the most retainable ones. PC1 is positively correlated with pH and organic C content and negatively with available N whereas PC2 is negatively correlated with total N content. Overall, environmental variables explain 47% of taxonomic bacterial variations. Negative spatial samples of sites showed significant Riyadh distance only for distance matrices. Thus, changes in geographic and environmental variables are significant in explaining variations in the community composition of free-living bacterial across sites.

3.1. Roles in Ecosystems

Microbes can be found almost anywhere on Earth, providing crucial roles in diverse environments. They determine the functioning and sustainability of most ecosystems (Zhu et al., 2023). The roles that they fulfill are numerous, varied, and often perform multitasks. Because of the large variety of ecological conditions and the variability in community assembly, soil microbes carry out a large range of functions which ultimately results in the ecosystems they inhabit.

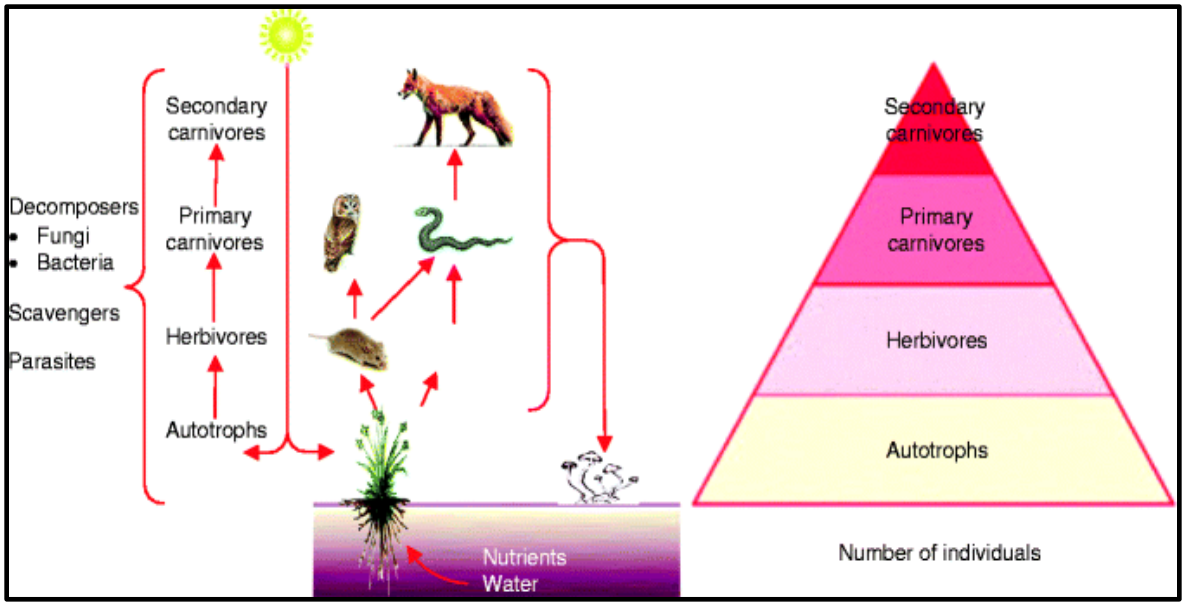

Figure9: The role of Microorganisms in ecosystem.

A large proportion of the food webs in almost all ecosystems are principally based on microbes. Their preeminence is rooted in the fact that they interact with both organic and inorganic materials, playing a leading role in their transformation. Organic compounds, which are the primary source of energy for most organisms, may be

transformed into inorganic and simpler molecules that can be taken by others. For instance, in a water ecosystem, microbes tend to decompose complex compounds found in fallen leaves of trees. When that happens, simple by-products derived from the breakdown can be taken up by plants or other living organisms. Thus, a mediator role in the ecosystem functioning is achieved. This mediator role is also necessary to close the cycle over time, as sine qua non material for the wellbeing of eukaryotic food webs. In this way, there is an increase in the rate of soluble nutrients so that plants can easily absorb them. Also, upper aquatic consumers make use of this material from which the cycle starts anew.

3.2. Biogeochemical Cycles

The importance of microorganisms in global ecology can never be understated. From oxygen production to waste degradation, such organisms act as key operators in most of the biogeochemical cycles that are constantly at work around the planet. It is arguable that the most important of these chemical cycles is the carbon cycle, but nitrogen, sulfur, and various other elemental cycles also require investigation in order to understand the full spectrum of microbial life on earth (Gupta et al., 2016). It is thanks to microorganisms that environmentally isolated reactions such as fixation are not only possible but widespread. The transformation of nitrogen gas into nitrates, the most biologically available form of this element, is accomplished solely by bacteria, as is the return of such gas to the atmosphere after denitrification.

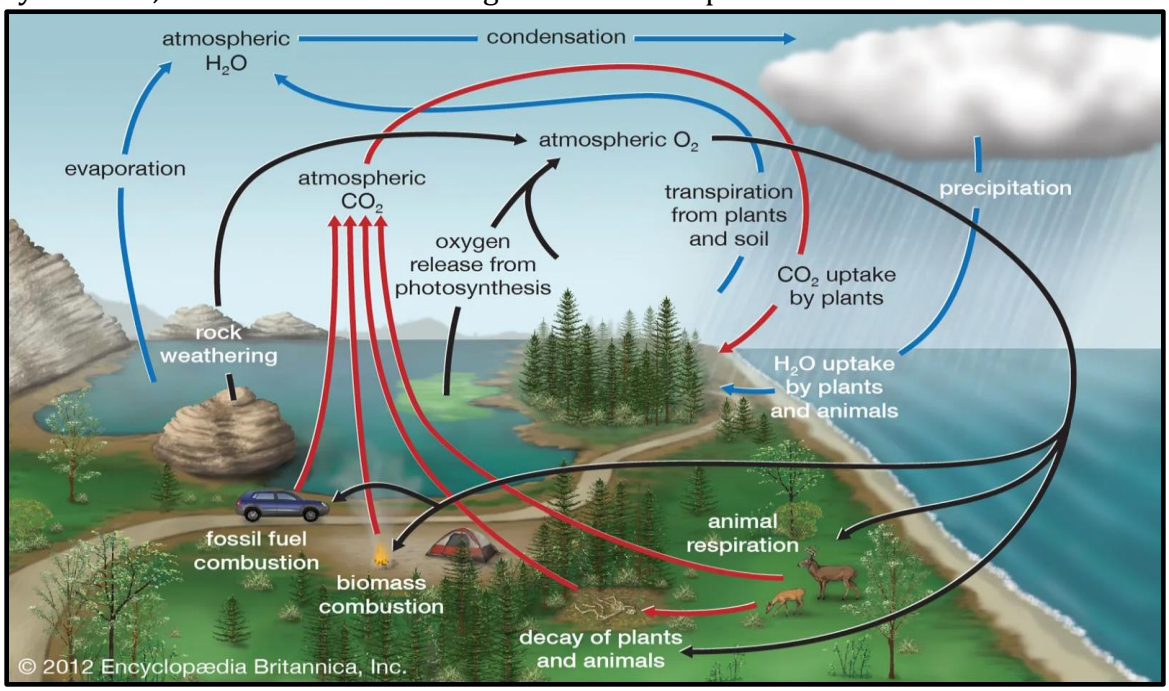

Figure 10: Carbon cycleThe generalized carbon cycle..

This tying of the biotic and abiotic components of an ecosystem is a prevalent theme in the study of microbial ecology. The variabilities in the nitrate and sulfate contents of water as indicators of wetland health that can be monitored remotely, and span a variety of temperature and pH interaction ranges. The acid and base of a solution are

used up on the formation of a salt, neutralizing the liquid and dramatically decreasing the environment's value as an indicator of ecosystem health. The L's are a series of lifts and crustal plate formations which move slowly, about the rate of fingernail growth, over geologic time. Earthquakes occur at transform boundaries, a point worth mentioning as another connection at the biological level could be drawn.

4. Microbes and Human Health

Invisible to our naked eye, microscopic organisms have long been veiled in the shadows of science. Since pasteurization, flooding, and the advent of penicillin, humans have established themselves as the ultimate competitors in the domain of the microbial world. So accomplished are we, that the very word "bacteria" is often mistakenly used to describe anything dangerous or "contaminated" in common parlance, when in reality the majority of those very small animals are actually symbiotic with us. No other entity plays a more important environmental role as microbes, yet no other kingdom are met with such fear and often unwarranted pessimism.

The microbial world is a two-faced Janus, and with some of its aspects it's as harmonious for humans as the elements are for themselves. An ecosystem's self-regulatory mechanisms can often appear ruthless and impersonal, and that is how humans see diseases. Roman fever, bubonic plague, cholera, tularemia, and many other infamous pandemics were caused and spread throughout history thanks to these "little animals," as Antoni van Leeuwenhoek first observed them. If the agents of each infection are to be listed in an attempt to understand the full scale of damage that can be caused by pathogenic microbes, a textbook would be needed. Whether bacterial, viral, mycotic, rickettsial, or with any other microbial nature - pathogenic agents are by far the biggest scourge to threaten Earth's dominant species, the Homo sapiens sapiens. Additionally, certain very resilient pathogens can quickly develop resistance to drugs in use, often making a disease impossible to treat. Yet, the goal of modern medicine is to fight not so much the symptomatic manifestations of diseases, but their causes - which, in many cases are the "simple" microbes (Ahmad Malla et al., 2019). In the early days of pre-antibiotic treatment, beating a bacterial infection could take months, or in many cases, lead to irreversible damage or death. While the days of using arsenic to treat syphilis and mercury to treat relapsing fever are long gone, killing bacteria en masse is still the essence of antibiotic action. And it works, as long as some resistance mechanism doesn't arise in the torn of a mutant gene or a plasmid exchanged someway. Yet, the golden era of antibiotics might soon be over, with the uncontrolled use of these drugs rapidly helping pathogens develop resistance, thus once again making many of them untreatable. This alone should focus research, to finally come to understand and engineer host-organisms (including humans) to provide a fine-tuned defense. Unfortunately, many pharmaceutical companies have turned away from antibiotics, as the production of new and effective drugs is very complex and expensive. Efforts should focus on other baits, such as phages for bacteria, and plastic films with fungal spores to replace antibiotics and pesticides.

4.1. Pathogens and Disease

Pathogens are a category of microorganism defined by what they do to humans (and other animals); they infect and cause diseases. There are four broad classes of pathogens that cause disease in this fashion: bacteria, viruses, fungi and protozoa. Pathogenic bacteria normally colonize the host using a specific tissue tropism or niche and possess defined virulence factors to establish infection (Fierer et al., 2016). Once established, they implement events that often take place in distinct stages, including colonization, local infection, invasion/translocation, dissemination, and finally infection of target organs. The outcome, i.e., whether infection results in overt disease, depends on many factors: the host, the pathogen and the environment. Pathogenic bacteria produce a wide range of infectious diseases in humans. They typically run a four-to-six-week course, during which bacterial density in the bloodstream is uniform and often helps clinical diagnosis. Some acute bacterial diseases, however, not only break this general rule, but also can be crowned with pronounced septic shock signs. There are many examples of severe bacterial infections that can become systemic just days after initial conditions and kill the patient hours later. This spectrum of diseases could be produced by distinct bacteria and the underlying molecular mechanisms used range from a general nature to the pathogen species-specific strategies. Treatment of bacterial infections normally involves antibiotics, but also there are other therapeutic tactics, such as the use of bacteriophages. As a consequence of the huge burden of bacterial diseases, vaccination campaigns have been established in multiple cases, yielding spectacular results. Despite all these efforts, some bacterial diseases lack prognosis, and new cases continue to emerge through history, as illustrated by the epidemic emergence of bubonic plague in the fourteenth and also other centuries.

Microbial-based infections recurring from time to time originate plagues or pandemics that are responsible for devastating consequences. Out of contagious diseases, tuberculosis, cholera, leprosy, and the like, became grim threats to public health. This situation lasted until the post-WWII years, when antibiotics and vaccines started to become widely spread and lifesaving resources for human societies worldwide. Currently, this struggle, however, seems to take a hard turn, as the acquired resistance to antibiotics by many pathogens and the emergence of new diseases outside the grasp of current treatment methods are putting us again in a vulnerable position. Worldwide, infectious diseases remain a major cause of death, particularly in the developing countries, where malnutrition and poverty compound the problem by impairing the immune system and as a result increasing susceptibility to pathogens. Vaccination has proven to be one of the most economical, effective and safe ways to fight pathogens causing infectious diseases. Communication among multidisciplinary bodies is vital to build a comprehensive approach to some of the medical world's most challenging problems. This understanding must include how bacterial pathogens regulate pathogenesis, as a means essential to develop new, rationally designed drugs. Importantly, human population's immunity to many bacterial pathogens is poorly understood, with consequences on vaccination strategies. Evolutive analysis of human pathogens is necessary to understand better the emergence of new diseases and devise ways to anticipate epidemics, as

exemplified by the threat avian influenza poses currently. Finally, an integrated approach is necessary to understand the complex communication system between pathogen and host and try to control it.

4.2. Probiotics and Health Benefits

The establishment of the Human Microbiome Project as the extension of the Human Genome Project has recently sparked a fervent exploration throughout the scientific community: a deeper, novel organizational model of the human body is taking shape, bestowing an in-depth portrait of the symbiotic relationships between humans and the microorganisms that colonize it. This burgeoning fertile cooperation has piloted an upsurge of probiotic studies, and it has become increasingly evident that such studies are conferring new emerging facets of microorganisms for their advantageous utility to the host (Kiousi et al., 2019). Correspondingly, this comprehensive review accentuates the potency of probiotics in moderation of specific physiological, immunological functions or ailments along with highlights on derived trials operating in vitro, on murine models and clinical trials.

Fermented foods have existed for centuries within the bedrock of the eastern populations like China, Japan, and India, engaged by food fermentation systems creating staple dietary traditions such as sauerkraut, kimchi, tempeh, miso, natto, fermented lassi, and yogurt in contrast to the western norm that lacks comparable, related traditions. The appearance of fermented foods within western dietary traditions upon emerging scientific findings about the health benefits of probiotic-rich foods has inspired novel product developments within the functionalities of probiotics, now comprising an extensive range of products including cheese, ice cream, salad dressing, smoothies, cereal, instant food, and energy and snack bars. Furthermore, the marketing strategy for commercial promotion now extends beyond the regular food market into other arenas such as health, diet supplements, drugs, and veterinary utility (Fijan, 2014).

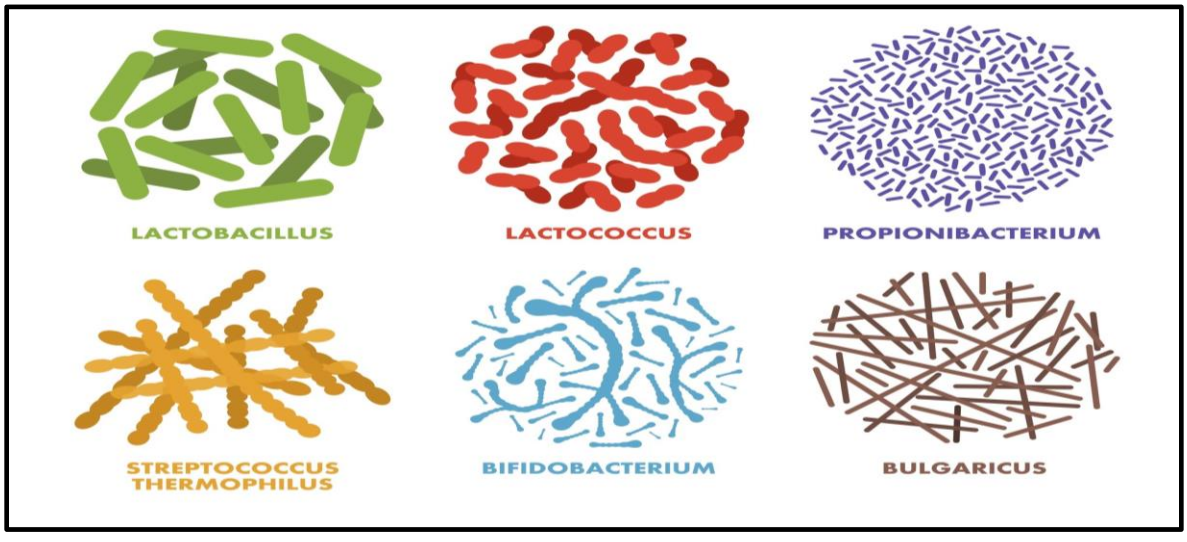

Figure11: Types of probiotic bacteria.

With the increasing appreciation of the role played by probiotics in promoting health the confirmation of market growth prospects has hailed of monetary investment by well-known food MNCs such as Nestle, Danone, Coca-Cola, Pepsi, and the Kellogg Company.

5. Current Research and Future Directions

Exploring the microbial world is a thrilling journey. With advances in technology and shifting approaches, the microbial world has yielded to the inquisitive in unprecedented ways. From the era of Leeuwenhoek to the times of genomic and metagenomic explorations, a new window continues to open every day about the ordeals and triumphs of microscopic beings that sustain, destroy, or ingeniously modify to help other life forms. Organized existence on Earth is bound to the microbial world—microbes inhabited Earth long before other complex macro-organisms. Still, most of the microbial worlds out there are yet to be known. Microbes have evolved as a whole lot among themselves—bacteria, archaea, eukaryotes, and recently characterized candidate phyla.

A considerable number of human diseases, either infectious, chronic, psychotic, or developmental, have been connected to microbes. The relationship between medical cure and microbes started many centuries ago and molded myriad medical outlooks and practices that continue to evolve. In recent decades, mounting antibiotic resistance has marked the resurrection of infectious diseases from the perils of the past, unconquered new human pathogens were uncovered unexpectedly, while the visionary optimism of using microbes as crack poisoning hit back both pharmaceuticals and hopefuls (Natarajan & S. Bhatt, 2020). This toll has re-energized acute mortality and morbidity. Moreover, the fearful, but largely ignored, effects of climate change on the microbial world—particularly on infectious disease evolution like malaria spread—are yet to be seen. On the other hand, the prospective uses of microbes in increasing climates challenges like carbon recycling and drug synthesis still nag its way for the unravel. To tackle these great and meticulous microbial issues, interdisciplinary and far-extent collaborations will indeed be ubiquitous. A good deal of collaborative works still awaits to smoothen the road of humanity in this microbial decade and the upcoming centuries. The benefit of humanity also highly demands that while seeking to modify microbial worlds, the uncharted worlds must be pursued harmoniously like all the other great worlds. The vast majority of microbial worlds out there are yet to be there to decide.

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Chapter Three

Handling and growing microorganisms

1. Introduction to Microorganisms

Micro-organisms are microscopic, single-cellular organisms. They are very small and can only be observed with a microscope. Micro-organisms are found almost everywhere, living in soil, water and the atmosphere. This realm is extremely diverse and encompasses bacteria, fungi, viruses, and protozoa. Micro-organisms contribute to the balance of ecosystems as they can decompose organic material into inorganic nutrients, generating fertile ground for plants. Theses organisms can also prevent disease by outcompeting pathogenic micro-organisms with harmless ones. Viruses contribute to the evolution of other forms of life, enabling genetic mixing among different cells. The concentration of molecular building blocks was low when life on Earth started, and there was a need of small enzymatic molecules to jumpstart metabolism with constrictions. To address these, it has been proposed that underground production of flextronic molecules can lead to the emergence of tentacle protocells. Micro-organisms can be classified utilizing a phylo-genetic approach, grouping them in four classes. The field of microbiology investigates micro-organisms' applied relevance. Due to their multitude of applications and the essential forces they carry out in the environment, it is crucial to have a microbiological background. Most of biochemical processes of living organisms are conducted with the collaboration of micro-organisms. Bacteria and fungi also largely contribute in the evolution of Earth's microbial ecology, and their diversified metabolism can supply sustainable solutions to tackle future challenges such as the climate and resource crises (Estoppey et al., 2024).

Micro-organisms have many applications in research and industry. Their easy growth and short replication time make them suitable models for experiments in subjects such as genetics, cell biology, or biologically-active substances production. Regarding the latter, for example, the production of antibiotics is mostly done by molds, which are abundant and easy to grow. Moreover, micro-organisms are indispensable for other types of productions, such as the processing of food. Notable in this respect is their use in fermenting processes like the one which yields bread. They can also cause spoilage, though, and are highly responsible for the discarded food. Living entities must constantly adjust their surface, interior, and interchange with their

environment to match all the dynamically-changing influences of time. In reaction to very diverse scenarios, uni-cellular life acts as an efficient universal consisting machine for tremendously longer and much more complex entities, such as animals and societies. If not dangerous for them, these adjustment morphologic deformations of a living thing offer a unique way to express and transmit information. This article suggests a paragon of events, where re-considering the integration of the morphological adjustment makes it possible for bio-cruising agents to achieve purposes or offer the chance of reading a communication.

1.1. Definition and Classification

This subsection is focussed on handling and growing microorganisms. Prior to any such practice, it is necessary to define what microorganisms are and to know the criteria by which they are classified. There are many different definitions of the term 'microorganism', defining them as 'living microscopic bacteria, fungi, protozoa, and microscopic algae, precluding generally larger forms. A more common approach is to base their definition on their size, with microorganisms generally taken to include all living things that are 0.1–0.3 mm or less in diameter. There are four main groups of microorganisms, which are distinguished largely on historical/phenotypic grounds, even though they do not form a coherent natural grouping: bacteria, archaea, fungi, and viruses.

Traditionally microorganisms have been classified by a number of criteria such as shape, size, growth characteristics and metabolic properties. These criteria were based on the defective knowledge of the time when microorganisms were first being studied. Classification by biochemical criteria remained dominant until the 1970s, when genetic studies became prevalent. The basis of the significance of biochemical classification is discussed. The importance of classification lies in a) the need to identify a particular microorganism in any given situation, b) encouraging study of a particular type of microorganism by identifying it in a sample. There are different taxonomic systems in operation at the same time regarding the same or similar group of microorganisms. A taxonomy at a higher level usually has a bigger impact on research and practice than that on a lower hierarchical level. Terms such as corona, cladistics, monophyletic group, synapomorphy, paraphyletic group, and polyphyletic group are not just intellectual abstractions but have tangible consequences for applicability when it comes to the phylogenetic position of a particular microorganism. There is also a need to emphasise that accurate understanding of the classification of microorganisms allows for better communication between scientists and is a prerequisite for a targeted application, for example understanding harmful effects of a particular strain of E. coli on human health necessitates knowing that strain's taxonomic classification and general physiological properties. This in turn has

significance for industry (danger of human infections from poor food processing, symbiosis of cows with E. coli O157:H7 leading to effective curing of cattle, and in consequence reduced contamination of soil and water), healthcare (need to control opportunistic infections by knowing which bacteria are able to act as pathogens), and environmental concerns (addressing the dangers of the introduction of genetically modified organisms to the environments in which they are not naturally present, and the requirement to timely detect potentially hazardous exposure to genetically engineered microorganisms).

1.2. Importance in Research and Industry

Microorganisms are unicellular organisms, and they can also live in multicellular communities. Microorganisms have an importance in hierarchies of food chains. They are of great importance for research, an area that deals with diseases that cause pathogenicity and quality assurance studies in production, and food and water controls with rapid, easy and cheap methods. Their use in the production of beneficial products and the elimination of harmful environmental pollutants has become a serious necessity. Microorganisms are widely used in industrial production because they can produce a wide variety of enzymes in the quantities needed within a short time. Furthermore, as a result of the studies performed on their cells, they also produce a huge amount of useful pharmaceuticals and nutrients that cannot be produced synthetically. Also, today one of the important issues is to determine foreign particles in the environment and industrial products and to do them quickly and reliably. Another use of them is as bioindicators as a response to pollution and disaster (Singh et al., 2017). These can be used in environmental monitoring to facilitate the realization of cleaning and monitoring studies at low costs. At the same time, beneficial in food, useful bacteria kill the pathogenic bacteria and ensure the retention of food. Beneficial and catalytic potential is huge.

Explains it as follows: On fermentation of milk by lactic acid bacteria, it shows that pathogenic bacteria cannot grow and multiply in acidic medium. Thus, reversible conversion is made to a degree that acid can grow and the food remains healthy. Alkali production of moulds is harmful to bacteria and that this effect has been used to extend the shelf life media used in the preparation of culture media. Another important area to be noted is the genetic studies on microorganisms. Most of our knowledge about DNA and genetics is known from bacterial studies. The analysis of model organisms also provides significant benefits. In addition, the studies of microorganisms such as Schizosaccharomyces, Aspergillus, Candida and Saccharomyces cerevisiae, which grow easily, proliferate quickly and have a lot of genetic information, provide a significant contribution to scientific researches in genetic and biotechnological studies.

2. Sterile Techniques in Microbiology

Aseptic techniques and productive microbial cultures are the obligations of anybody involved in microbiology. Generally, the laboratory work with bacteria and viruses is completed under the regular atmosphere. Therefore, if the samples are not correctly managed, they may get contaminated with the microorganisms in the surroundings (R. Sanders, 2012). At the same time, any microorganisms getting analyzed may also ruin the surroundings in case of infections. The aseptic and sterile handling practices are fundamental to microbiology and all work must be carried out in the aseptic or disinfected environment. This implies that air, tools, water, solutions, glassware, and the work surface must be sterilized. Individuals engaging in the laboratory handling cultures and disposables are necessary to use the necessary PPE and must be conscious of the associated hazards.

To stop the bacteria and yeast virus from nascent crops, individuals will have to maintain the brew and foodstuff in a sterile environment. Scientists must close the bouffant flask, impose a teat at the top of the flask - these are used to receive the aseptic coffee and foodstuff. To dupe the microbial upscale, if individuals choose to pour the broth onto the agar dishes. The respective matrices need to have been boiled in the autoclave for sterilization. When the barbed stricorn coffee requested, the Erlenmeyer flask lid shall be controlled to maintain an aseptic environment. Do not be polite to the syringe used for coffee routes - they should have been sterilized using alcohol and flames (P Reis et al., 2012). Trim the external leaf of the flask and razor for boiling will also have been previously sterilized through the flames. When laterally tilting the flask from an aseptic environment, use the third small device to grab it. Only posture the request cups - conic flasks are extremely hot, hence they are not a securities device and validly achieve their purposes. Boil the external flats in fire to sterilize rather than autoclave.

2.1. Principles of Aseptic Technique

The first exercise raises awareness of the importance of aseptic technique when handling and growing microorganisms. Designing and manipulating experiments that involve the culturing of microorganisms is a routine academic task; the best practices and key considerations in minimizing chances of contamination are outlined here. The basic concepts underpinning aseptic methods are introduced, and an understanding given of why it is necessary to prevent contaminants from disrupting the original experimental aim. At its heart, aseptic technique for the culturing of non-pathogenic microorganisms is the formation of a sterile field, i.e. one entirely free of contaminating microorganisms and their spores. In practical terms, this sterile area encompasses the working area, equipment, and solutions. Because the skin is host to many millions of bacteria and fungal spores, it is important to minimize the number

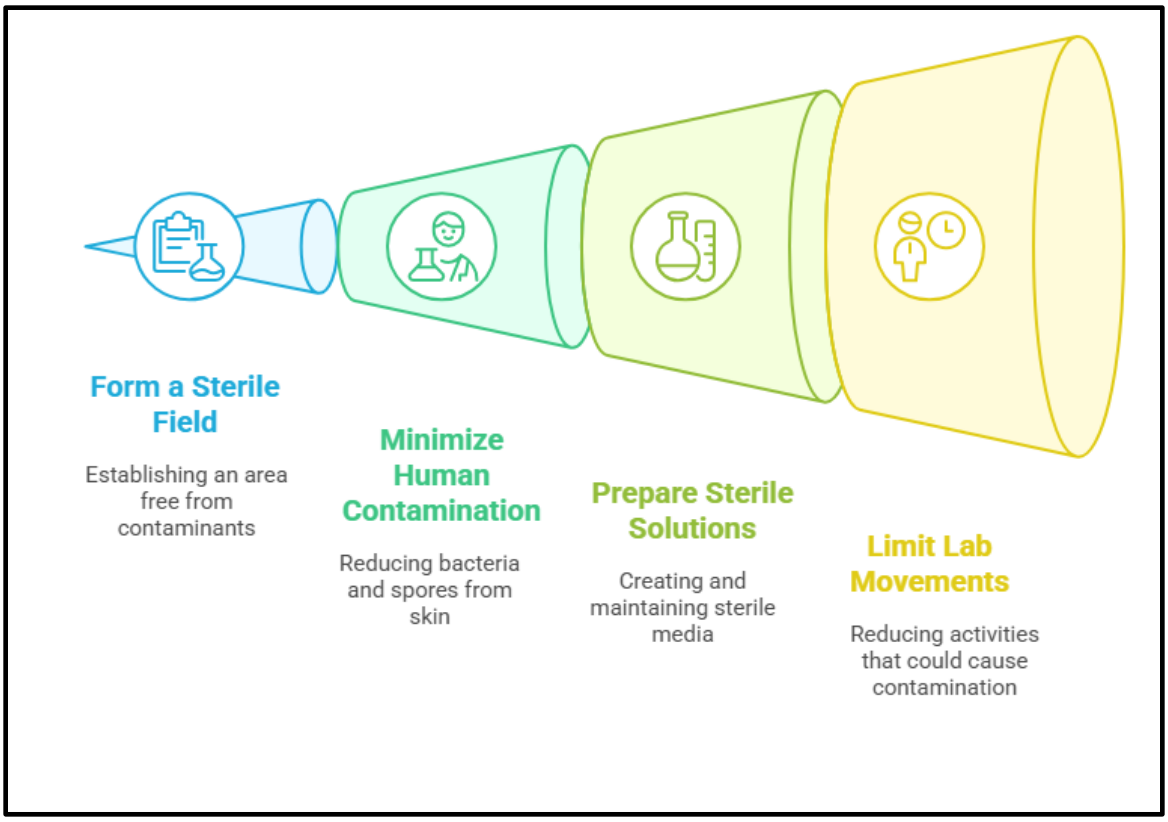

Figure 1: Ensuring Aseptic Techniques in Microbiology

of human microorganisms present at the bench. One (albeit extreme) way to do this is to work entirely naked, although the resulting exclusion orders would likely draw suspicion from enforcement officers. A much more acceptable practice is to wear dedicated lab coats and, where expected to come into contact with biohazardous materials, gloves. No guarantee is made as to the quality/cleanliness of lab coats, so if you do choose to pursue this option, it is recommended that you bring your own and take it home to wash every so often. It is important to avoid activities that promote the dissemination of non-particulates, and to limit movements in the lab. Clearly, typing up reports or completing some rock polish while the agar sets is not an option, and it is important, therefore, to be prepared and ready to go before assembling a work area. Nonetheless, dedication to tasks should never eclipse good safety practice – if this involves leaving a pinning session to attend to something urgent elsewhere, it is best to start afresh. This section discusses various ways to prepare sterile solutions and media. The best method is decided by the specific requirements of an experiment and the facilities available; for this, it is first necessary to understand how to aseptically handle liquids. Behind this lies a basic understanding of what 'sterility' in liquid terms is and what can be done to ensure it is maintained. Further considerations are in minimizing risk of exposure to harmful microorganisms and techniques for their disposal.

2.2. Sterilization Methods

Sterilization methods are very important in making sure that your media and apparatus are free of unwanted microorganisms. There are several techniques to be used for sterilization. There are physical and chemical techniques that work to kill or inhibit the growth of microorganisms. The most commonly used is an autoclave, which heats media and equipment to the point of killing, then destroys by heat. Although it is essential to make sure that what you are sterilizing actually gets sterile. Plastics and waterproof papers can't be autoclaved, which gives the means to use chemicals as a sterilant as equipment. Incinerators can be used for sterilization of lab coats, waste, and contaminated organic materials.

Autoclave is the most widely killed by medical attention, isotope glassware, Steveny, and polypropylene by means of heat and pressure (Yoo, 2018). Hot air sterilization is effective in Dry heat kills by oxidation and is suitable for aqueous and oily liquids, glass, metal powders etc. Filtration can be used for sterilization for non-chemical and non-heat resistant liquids for animals medium media. Chemically, ethylene oxide is used for sterilization of heat-sensitive equipment like tape, fernite, and IV rubber products.

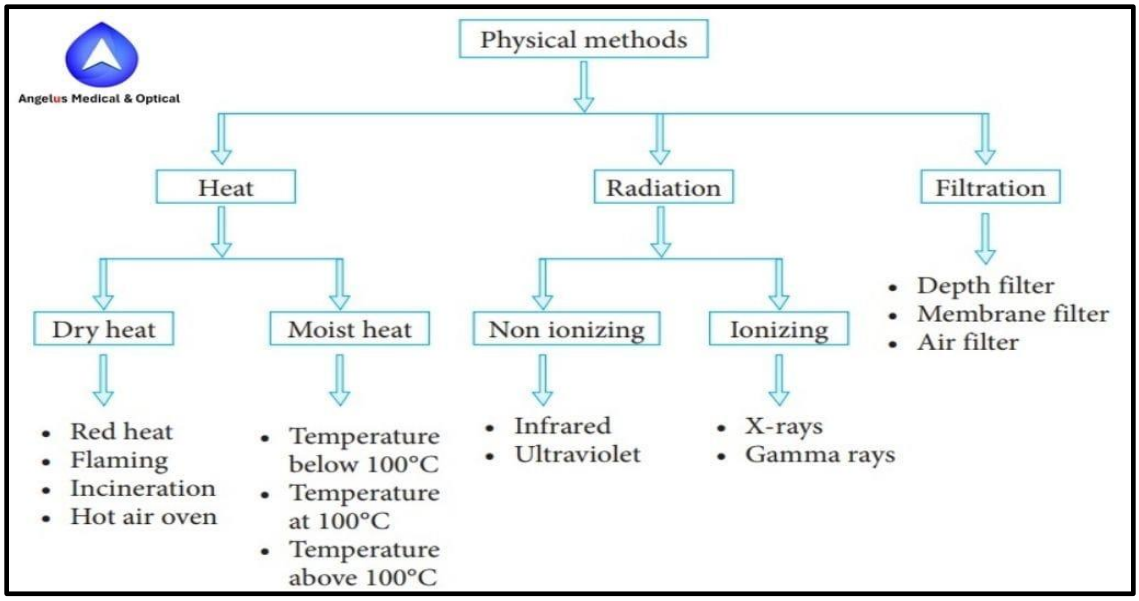

Figure 2: physical methods of sterilization.

Hydrogen peroxide can also be used for sterilizing heat-sensitive apparatus. More importantly, be sure to use sterilization methods based on materials and equipment used for experiments. Each sterilization method has a degree of efficiency based on the amount of time, temperature, and pressure used. The presence of creation of steam is also important in the heating and autoclaving process (Chifflet et al., 2019).

Better knowledge about sterilization will be useful for maintaining an uncontainment environment.

3. Culture Media Preparation

A culture medium is a useful growth medium for microbes. As a result, successful cultivation is important. This medium is composed of agar, peptones, beef extract, yeast extract, water, and salts. The composition depends on the organism to be grown. This report covers the fundamentals necessary for the formulation, precautions, and autoclave of the culture medium (Bonnet et al., 2019).

Culture media are divided into liquid media, solid media, and semisolid media. Liquid media include Tryptic Soy Broth, Heruva Broth, and Yeast Extract Broth. Liquid culture can serve as a basis for confirmation tests after the growth of the organism because it is suitable for confirming that a sufficient quantity of the target organism exists. However, liquid media are unable to gain competitive growth against other bacteria. Solid media include Tryptic Soy Agar, Luria-Bertani hijelly, and Tryptic Soy Agar hijelly. After 24 hours, it can be isolated by streaking cultivated food bacteria. Hence, the bacteria to be grown are selected from liquid media, propagating is performed on a solid medium, and the confirmation tests to confirm the results are performed on liquid media after the organism grows successfully. The selection of a solid or liquid medium can selectively grow the microorganisms of interest and ensures the normal performance of the confirmation tests. Therefore, the selection of a proper culture medium for cultivation is important. The formulation, precautions, and procedures of these culture media should be described.

Figure 3: Conical flasks containing dissolved culture media

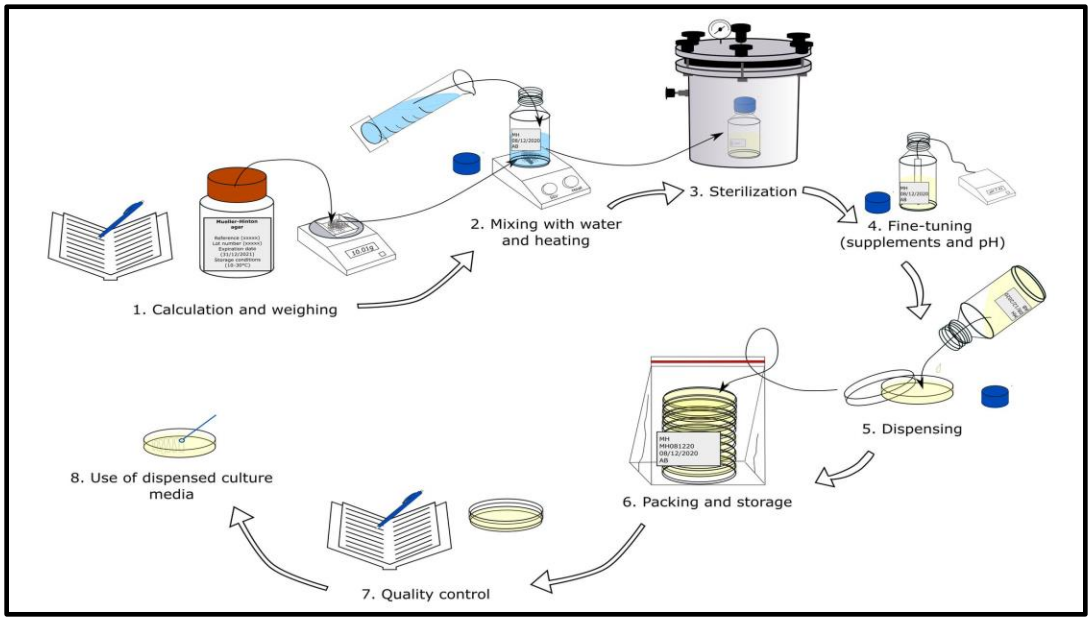

Figure 2: Steps for preparing culture media for growing microorganisms.

3.1. Types of Culture Media

Of all the components necessary for successful cultivation of microorganisms, appropriate culture medium is undoubtedly one of the most important. There are many different types of culture medium, each designed to fulfill specific growth requirements for the enormous range of bacteria and fungi present in nature. It is important for the microbiologist to understand the composition and use of different types of media, particularly when selecting/developing methodology, as the type of culture medium used will affect the growth and physiology of the organism(s) being cultured. One major way in which culture media can be categorized is by function. Broad groups of function include: selective media, differential media and enrichment media (Bonnet et al., 2019). Selective media contain components which, on the basis of different growth requirements, specifically inhibit the growth of certain organisms without affecting others. Combining selective media with different temperatures and gas conditions can enable the growth of a wide range of taxa, even within a complex polymicrobial sample. There is a large range of available robust and novel selective media choices, with thoughtful consideration of both a priori knowledge of the sample and an awareness of the range of selective media options assessed in the literature.

Differential media contain compounds that allow identification of different organisms based on differential growth characteristics, a metabolic end product, change in pH, or a change in appearance. They can be used to differentiate colonies of the same species or between different species during cultivation. With over 700 commercially available culture media in production, only a limited number are routinely

implemented. The growth of bacteria and fungi on some of these media can be affected when incorporated together, and growth abnormalities have been reported, including the alteration of colonial morphology. Finally, enrichment media, as the name suggests, are aimed at enriching the growth of a particular organism that may be present in lower numbers. Enrichment media are used when attempting to culture bacteria out of complex environmental samples where the target organism(s) may be present in low numbers. Enrichment cultures are often followed by use of selective and differential media to isolate and enumerate pure cultures of target organisms. The importance of appropriate culture media is undoubtedly recognized by most microbiologists, yet it is still underappreciated in the wider literature. Nonetheless, during experiments aimed at growing microorganisms, media composition is controlled, manipulated, assessed, and reported. Due to the vast number of available culture media, simply providing the name of the media used is often insufficient, as growth of an organism(s) on two different types of media will yield different results (Kawanishi et al., 2011).

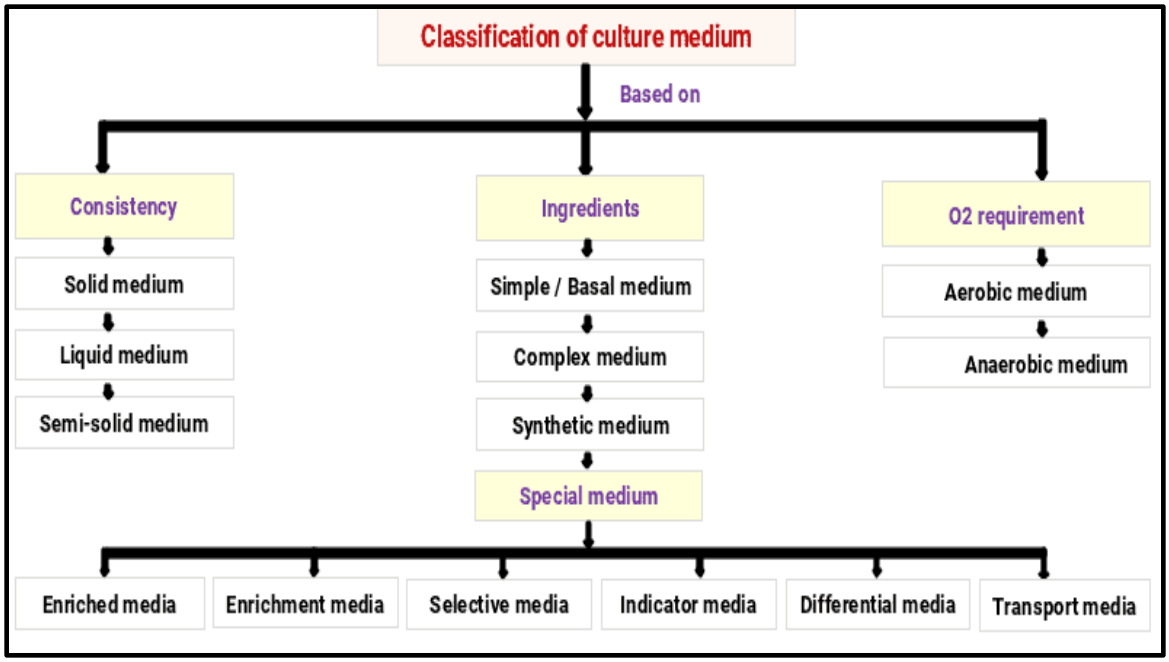

Figure 3: Classification of culture media

Table 1: Summary of commonly used culture media for various types of organisms.

Culture Media	Main Ingredients	B	F	A	N	MB
Blood agar	Peptone, tryptose, 5% sheep blood	Y	Y	–	Y	–
Chocolate agar	Similar to blood agar, but with lysed blood	Y[a]	Y	–	Y	–
Sabouraud agar	Dextrose, peptone	–	Y	–	Y	–
Potato dextrose agar (PDA)	Potato infusion, dextrose	–	Y	–	–	–
Non-nutrient agar with *E. coli* overlay	Peptone, yeast extract, beef extract, *E. coli*	–	–	Y	–	–
Lowenstein Jensen (LJ) medium	Potato flour, asparagine, malachite green, glycerol, potassium, magnesium	–	–	–	–	Y
Thioglycolate broth	Sodium thioglycolate, L-cystine, glucose, yeast extract, casein	Y[b]	–	–	–	–

3.2. Components and Preparation Techniques

This subsection explains the various components which make up culture media and techniques involved in their preparation, an essential aspect of successful microbial culture. A culture medium is a liquid or gel designed to support the growth of microorganisms or cells, and consists of a number of components that provide the nutrients necessary for growth. A typical medium will include a carbohydrate energy source, a protein nitrogen source, growth factors, vitamins, and minerals. The concentration and form of each component is also crucial to the medium performing as expected. Measurements will need to be extremely precise, typically calibrated to the milligram or micromolar level. The exact formulation of each characteristic type of medium will also vary between suppliers because their composition is protected as proprietary information, making it essential to use only the designated media and to only weigh out the amount specified. Inappropriate preparation can explain an experimental error or failure to replicate a protocol exactly. Components may become contaminated after being prepared, storage for long periods can affect media, or the pH can drift out of range (Bonnet et al., 2019).

Before use, the solid ingredients should be added to distilled or deionised water and dissolved by heating, recrystallised chemicals will need gentle heating, while some heat-sensitive components should be microwave- or nitrogen flow-dispersed. Media can either be vacuum filtered or mixture kept warm to minimise solidification and ensure effective sterilisation. The item needs to be added to media after it has been dissolved and autoclaved so as not to denature or degrade it, though it is often sterile-filtered instead. The fresh media should be kept warm at medium heat or in a water

bath (45-50°C) while a stir bar is used to ensure its homogeneity. The pH of the media can have a critical affect on the growth of certain microorganisms because it governs the solubility and ionisation of many chemicals. A buffer system should be added to the water of the media before any other salts to aid in maintaining the desired pH throughout sterilisation and once the medium is inoculated. A number of antibiotics or other selective agents can also be added to prevent unwanted contamination by non-target bacteria, media may also instead need to be supplemented with additional vitamins, growth factors, or a complex mixture of organic sources to promote the growth of certain strains further.

4. Isolation and Identification Techniques

Two main objectives when working with microbial cultures are to be able to separate and isolate any specific microbe from a mixed culture and to obtain a pure or axenic culture of it. This is necessary in order to study and identify its properties. The first techniques used in this are simple, but are designed to reduce hugely the number of candidates so that further work is more successful. Most techniques used are a variation of the streak plate method, films and imprisonments being alternated. It may be necessary to stain the agar with a neutral dye so that the films can be dispersed clearer.

It is important to select only colonies which are typical, and to ensure there are no more than one species. If different types are seen in a plate it is not sensible to test any. When one type, if available, may have reduced in number, showed to have the desired property. If a mixed culture is not considered visually, then there are a number of techniques to verify. Colonies bred from a pure culture should be shown identical in type. On a circular dish, colonies from inoculations of the disk seen in an increasing ring, stationary types are probably of a single parent. Everything reverse occurs on a rectangular plate. When the lesser colonies are selected also tend to be purer after a meal. It is wise to make a first re-strike to an aseptic culture.

With the advent of the plate technique of Petri, it was possible to produce cultures in which each cell comes from one population. Here, an example of the chestnut roaster's method, led contact with a chestnut roaster, has an affair with a chestnut roasting man, meets other roasters on the south bank and obtains from this individual a culture of motiles.

4.1. Streak Plate Method

Since the time Antonie van Leeuwenhoek observed microscopic single-cell organisms in pond water, the methods used to handle and grow microorganisms have developed and vastly improved. One of the first hurdles to overcome when trying to study these

organisms is how to handle them in a way in which pure cultures can be obtained. This is a fundamental concept of microbiology, as the ability to work with pure cultures is a necessity for specific microbial study. From there, an entire field of research has grown around isolating and identifying these microorganisms. For these methods remain effective, yet they often rely on a handful of tools and techniques. The goal of writing this article is to give an overview of some of these common techniques, beginning with the streak plate method (R. Sanders, 2012). The intention is to provide a basic explanation of these methods where they can be performed, and what they are used for. With a better understanding of the methods and purposes, it should be clear why it is important to perform these techniques correctly because it will affect the research results in the future. Moreover, having a basic understanding will help in identifying common challenges and errors that may arise, thereby making it possible to improve and build on these methods.

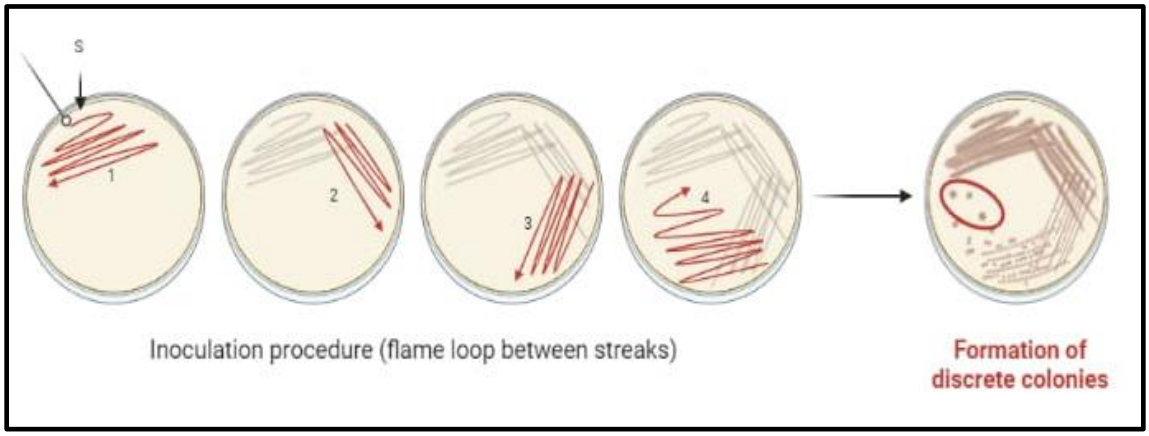

Figure 4: Streak plate method**.**

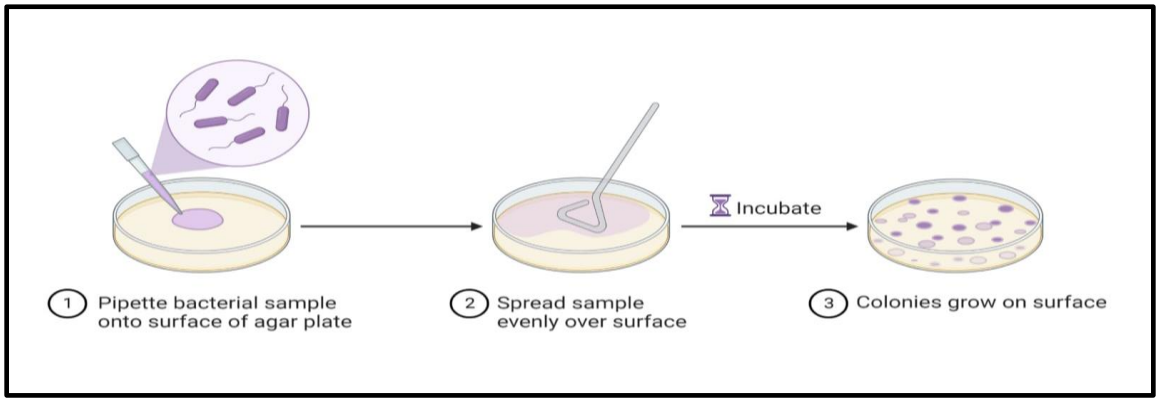

Figure 5: Spread plate method.

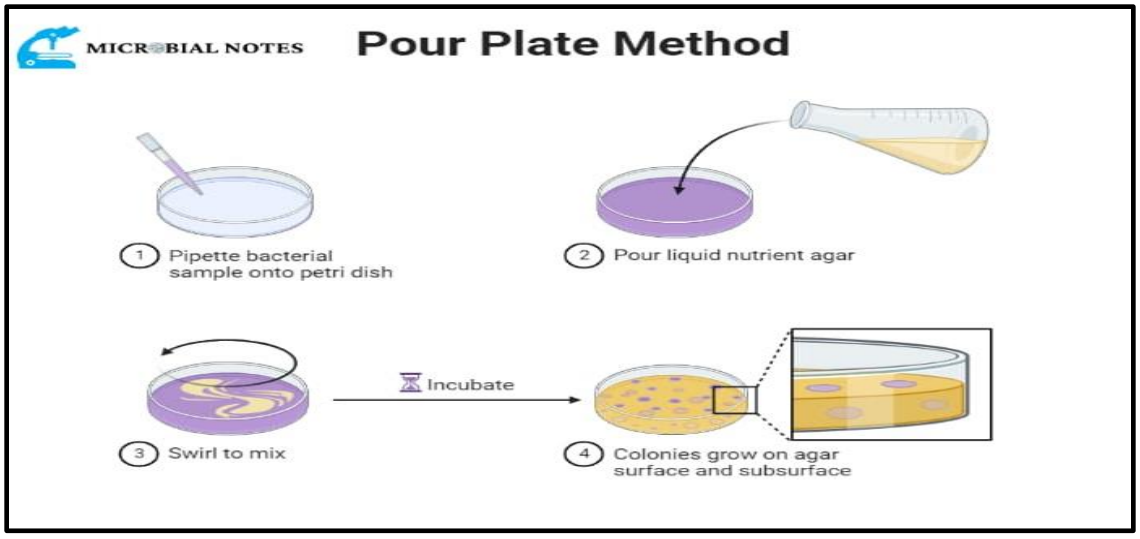

Figure 6: Pour plate method.

4.2. Biochemical Tests

Biochemical tests are commonly used for the identification and classification of microorganisms based on their biochemical properties. Fresh clinical, industrial, or environmental isolates need to be characterized and identified because they are usually medically important or could be of use in industry or the environment. The interpretation of biochemical tests is important because it generates information that can be used when making choices or recommendations. Different species of microorganisms have differences in ability to metabolize nutrients and to produce certain waste products. The results of biochemical tests provide clues to various differences in the physiology of the various species of microorganisms used in the tests (A. Pence and Liesman, 2020). These differences can therefore be used, from the results of the tests, to make decisions about the identity and characteristics of the unknown organisms used in the tests. Such basic decisions could be of use, for example, in choosing a possible treatment for a clinical infection suspected to be caused by an unknown bacterium.

Figure 7: An example of Biochemical tests (Voges Proskauer).

Specialized medical assays, using specific sets of tests, can be performed to clarify and confirm the results of more general tests. These (Galar et al., 2013) assays provide the clinician with more and accurate guidance in the choice of treatment. On the other hand, clinical assays (including these specialized medical tests) can uncover the characteristics of the infecting organism which allow more targeted research in the discovery of new antibiotics. Such further analysis could allow treatment of the infection with drugs specifically targeted to the infected organism. Biochemical tests could also be of importance to the rapid establishment of new identification systems for new emerging pathogens. Such rapidly emerging pathogens often have no information about them in a standard identification system. Hence, specialized tests, either clinical or environmental, could be thought out that would lead to a rapid identification. Early identification can be of obvious importance to either medical aid of sanitation of a general environment. Any of these decisions likely to have substantial personal and financial effects if conducted in industrial setting, where unknown or unwanted species of organisms are suspected to be present would have to be made based on the results of biochemical tests. Due to the wide range of microbial species, and the extensive range of possible nutrients, the availability of tests is not necessarily adequate for confidently making the decisions needed. Thus for each biochemical test performed on an unknown bacterium includes a consideration of the limitations of the test and a possible recommendation for further testing.

5. Troubleshooting in Microbial Culturing

To succeed in experimenting on microbial growth requires the ability to troubleshoot problems. Contaminating microorganisms tend to rapidly outgrow the growing form of interest, and a wide variety of potential contaminants are omnipresent. It is important to be aware of the common types of contamination so that steps can be taken to avoid or manage them. Microorganisms and microscopic particles continuously circulate in the air. A sample open at crucial times, such as when taking a culture from a flask or streaking it out on a plate, makes it likely that some unwanted organisms will land on your culture. Touching tubes or other sterile items together, failing to sterilize serological pipettes, or other items can make it even more likely that the unwanted microbes succeed. Sometimes contamination is due to a poorly cleaned surface or a culture placed in the incubator containing a lid wiped with a non-sterilized material. The only way to be sure is to streak contaminated cultures out onto plates to isolate the different unwanted organisms present, and then to eradicate them by autoclaving all contaminated cultures and other disposables (Nikfarjam and Farzaneh, 2012).

Contaminating sources of microbes could be: Airborne. Microbes and microscopic particles cannot be seen but the likely port of entry can be observed. Cracks and uneven edges, not air-tight fitting caps, or cotton covered test tubes are problematic. Amino acids from skin as well as microbes. Improper handling. Coming into contact with an item not washed thoroughly enough with ethanol or a different solvent leaves abundant microbes on the hands. This is particularly so after opening a fridge or a stock solution cabinet. Using serological pipettes without sterilizing them in an ethanol flame. This is more dangerous because the pipette contaminates permanently. Other important items to properly sterilize are glass rods used to assist the streaking out of cultures, tip holders, and their respective disposable tips. Inadequate sterilization. A powerful source of contaminants is failing to properly sterilize the instruments. A flame must be used to produce sufficient heat for the destruction of all potential contaminants 100 %. An example of a mistake is not inverting the tube of a stock culture after flaming the rim. If contamination is a recurring issue the best approach is to strengthen precautions and change techniques in order to prevent its occurrence. This will result in a more precise and cleaner work. An approach to confirm the presence of bacterial contamination on an observable level is by letting cultures grow for longer, up to weeks even, on a plate with the appropriate medium. To achieve similar experimental responses it is necessary to have designed a similar environment; that is similar pH, salinity, temperature, etc. Adapt the environment. For instance, different strains of the microorganism growing in slightly different conditions; to reproduce this change one or more variables and see if this helps the unwanted strain to disappear from the resulting culture.

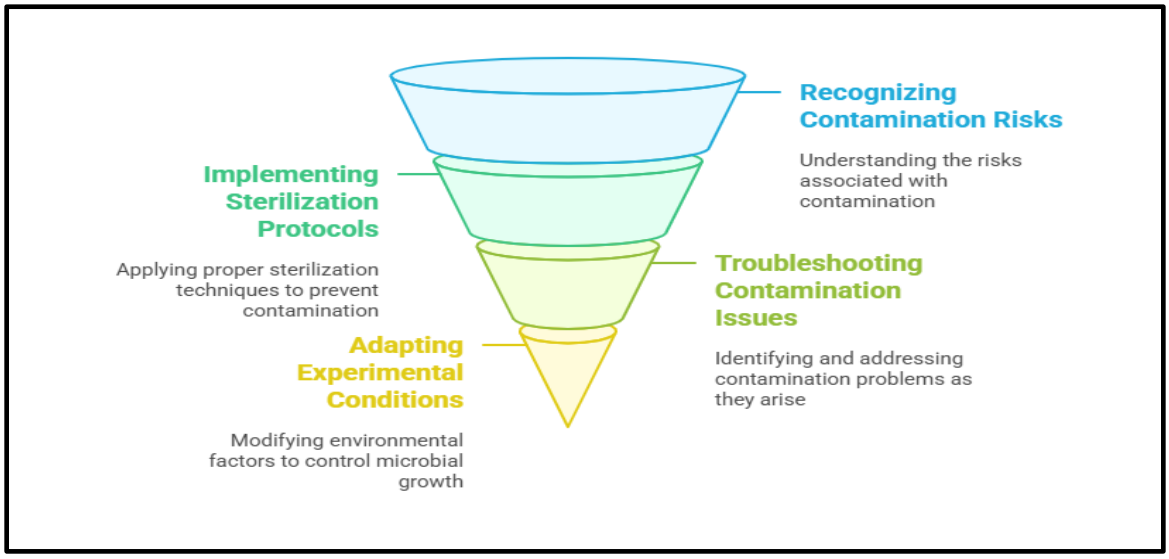

Figure 8: Microbial Contamination Management Process.

5.1. Common Contamination Issues

Common contamination issues have presented significant challenges to the increasing unregulated microbial culturing done by bioartists. A variety of contaminants challenge the pure field of growth and lead to false, even dangerous, results. It is expected that by thoroughly understanding what these common contaminants are, a first step in cultivating better lab skills might be taken. Outside of the common fungal and bacterial contaminants found in bioart laboratories, there are also community ones, such as in the process of isolating bacteria-and viral. Nearly all cells grown in monoculture can be ruined by unwanted contaminants. Fungal spores can spread in the air, so even one contaminated petri dish can destroy a whole lab's work. In general, seeing contamination is not hard, but recognizing it for what it is can be. Common contaminants will be enumerated, and some stress will be given to the peculiarities of signs and symptoms. The discussion will also touch on the sources of contamination and give a cursory discovery of some tips on avoiding them. Early detection of contamination with vigilant intervention can quickly salvage work that might otherwise be lost.

Life in the lab is fought everyday with the unseen. Work is engaged with blade-angles, liquid pressures, smells, small words, exact durations, perfect measurements, rare electrics, squat apertures, negligible emulsifications and rates of onset. Microbial culture comes in near the top of the easiest things to ruin. Single cells are capable of ruining-a willful recombinant dug out of a lab book; a brilliant chemical, painstakingly refined from costs and reviews; a species discovered as a byproduct of some zeros or a careful, tenable cycle of ecology as wholesome as a business plan. Worst of all is that the ruin will not be fact until late, until many hours have already been spent, long planning and re-planning, war weeks or months gone when the simple growth looks to know better (Nikfarjam and Farzaneh, 2012). So it will be useful to stress how difficult, how outstanding is the way to the klondike slant of pure life. The aged and rusted maxim holds truest, truest to the anxious aspiring practitioner: Watch your work. But no one knows the way to keep vigil, midnighted in black glass, on the small meats of pneumatics and fire. There are titrinos with burning, minikin hats with glass eruptions, and great umbilicals of wets and ices: the art beside the act, that chamber of recondite executors. Acts and inferences, material textured quite with concept, must be kept in array, righted, dusted, handled with skill, greased or alumed, silver'd or cinnabar'd, wove with great ache. Such Pacificas are in an art more dorm than nature, and this is why life in the lab is treacherous; smaller is more costly and fallible, quick hands make poor aleat. And sense this poor alignment, the long spool of these anxious reflections concerning contamination.

5.2. Optimizing Growth Conditions

Growth Conditions Handling and growing microorganisms is a fundamental activity in microbiology and enables the completion of many classical experiments. However, growing microorganisms is actually not a trivial task, due to the large number of factors influencing microbial growth that have to be systematically optimized. This includes the choice of appropriate growth media, adjusting pH-values, adjusting the temperature, maintaining anaerobic conditions etc. In the following, general techniques to assess and adjust growth conditions with the focus on: Checking (and adjusting) the temperature, Checking (and adjusting) the pH-value, Avoiding exposure to light, and Checking (and adjusting) the presence of oxygen will be given. (Hemmerich et al., 2017)

Optimizing growth conditions for the microbes of interest is a fundamental task for successful culturing, yielding the desired microorganism products and preventing contamination by unwanted species. The optimization of growth conditions involves the selection of growth media and appropriate environmental factors including temperature, pH, oxygen levels, and nutrient availability. Growth conditions must be tailored for organisms of interest since they exhibit diverse metabolic processes influenced by the conditions. Understanding the behavior of organism types is important in order to maximize their productivity and health. On the practical part, it is described how to check and adjust growth conditions and how to monitor growth progress. A theoretical understanding of these processes allows one to manipulate environmental parameters such that the microorganisms of interest thrive, while the growth of unwanted species is inhibited. This knowledge is highly relevant for microbiologists working in research environments but also for industrial microbiologists growing high-value microorganisms in large-scale fermenters for the production of bioactive compounds. The utilization of these techniques helps to grow the desired microorganisms in axenic conditions.

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Chapter Four

Bacterial Structure and Function

1. Introduction to Bacteria

Bacteria have profound impacts in ecology and the environment, as well as on their roles in health (KRELL and BEVERIDGE, 2014). Although mostly unnoticed, and generally associated with disease and contamination, bacteria are in fact essential for the working of most biomes and complex organisms. The first prokaryotic microorganisms appeared almost 4 billion years ago, eventually rolling the stage of a habitable Earth, and seeding the planet with the resources and conditions necessary to the emergence of multicellular organisms. Bacteria were (and remain) great sculptors of the planet's landscape, having created and reshaped its atmospheres, lakes, rivers, soils, and oceans, over immense sections of geologic time. Most biogeochemical cycles are bacterial-centered, such as the carbon, nitrogen, sulfur, or phosphorous cycling, and some complex organisms are known to be completely dependent on their bacterial symbionts to survive, as is the case of most herbivores and ruminants.

The structure of bacteria is complex, despite its apparent simplicity, and it is responsible for their success as living systems (E. Harper and J. Hernandez, 2020). One of the most successful life forms, very abundant in Nature, bacteria have diversified enough to occupy almost all ecological niches. Their body plan, the prokaryotic cell, is nevertheless somewhat rudimentary for today's standards. A 1000 polymeraxes intracellular bacterial microtubules, bacteria tend to be, at maximum development, a few tens of polymeraxes on size, and some 10 or 100 times shorter. Like bigger living cells, they are enclosed in a closed membrane compartment, the cell wall, that brings a heavy influence in the cell general aspect and properties. It is known the existence of several types of bacterial cells, due to the diversity of the cell wall. The main discriminating characteristic is the capacity of synthesizing a sort of biopolymeric mesh, the called peptidoglycan or molecule-bin, which is responsible for the cell wall structural resistance and buoyancy. From the structural viewpoint, bacteria may then be divided in two groups, gram positive and gram negative.

1.1. Basic Characteristics

The bacteria are members of the Monera, the single-celled prokaryotic organisms (KRELL and BEVERIDGE, 2014). They are the most numerous organisms on earth; of the approximately 2 x 10^30 prokaryotic cells present, it is estimated that upwards of 99% of those are bacteria (or bacteria-like organisms). The physical characteristics of a bacterium that distinguish it from other prokaryotic cells include its lack of a nuclear membrane, its generally rod or spiral-like shape, the presence of ribosomes in the cytoplasm, its small size, cellular morphology, and its reproduction by binary fission. One English naturalist has referred to bacteria as "the small things that run the world." The bacteria were identified and first named in 1676. Many scientists believe these organisms to be among the first life-forms on earth.

The basic cell structure of a bacterium includes a cytoplasmic membrane and a cytoplasm; most have a cell wall, an external layer and flagella. Many cells also have intracellular inclusion bodies. Some also possess a capsule which is basically a thick cell wall. All of these structures, with the exception of the intracellular inclusion bodies, are referenced in the drawing of the Rhodospirillum rubrum bacterial cell. The cell wall is compound, which means it is composed of many layers; many commercially important antibiotics target this structure. This structure also includes a potentially health-related issue; if antimicrobic agents disrupt the cell wall, the cell will lyse and die. Bacteria do not have a nucleus, peroxisomes, mitochondria or pyrenoids; the genetic material is often incorporated into a plasmid. A plasmid is a covalently closed circular DNA molecule(s); the bacterial chromosome is a similar but larger double-stranded circle. The ribosomes are smaller than those found in plant or animal cells. In fact, 70S ribosomes can be found in bacteria, mitochondria and chloroplasts.

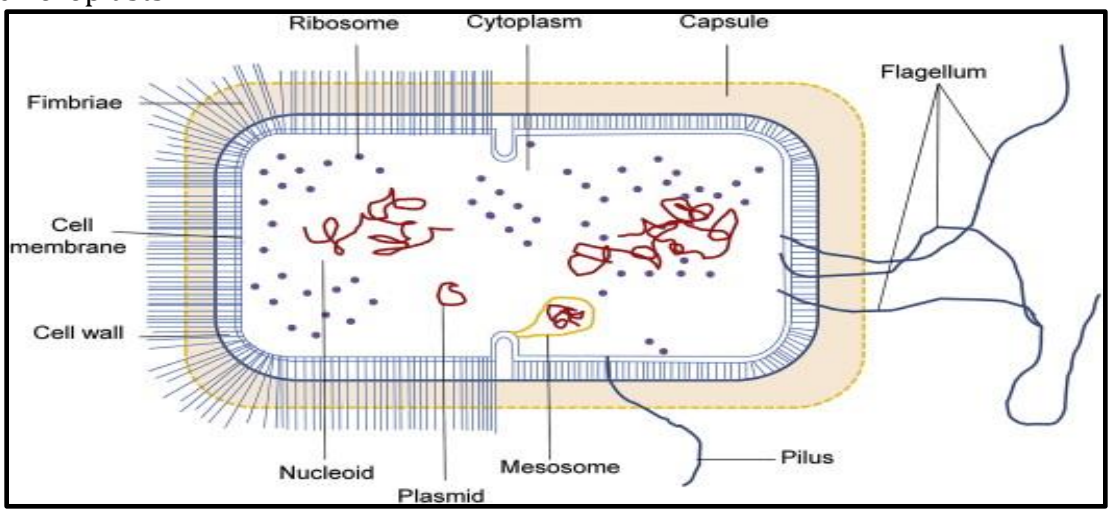

Figure1: Schematic representation of bacterial cell structure.

Compared to the 80S ribosomes in plants, animals and the ER, there are significant molecular differences between the two ribosome types. The genetic material found in bacteria is much less organized than that of eukaryotic cells. Bacterial genes (DNA) are not found on paired linear chromosomes. Instead, the genome consists of a single continuous double-stranded closed loop, typically from 1000 to 4000 times smaller than the multiple linear chromosomes of eukaryotes. Genes are found on this double-stranded DNA molecule.

1.2. Classification of Bacteria

Bacteria are a diverse domain of prokaryotic microorganisms. Although they are not as morphologically diverse as eukaryotes, bacteria vary widely in terms of special structures, appearance, and requirements for cultivation. They are ubiquitous and colonize a wide range of habitats, ranging from frozen lakes in the Dry Valleys of Antarctica to hydrothermal vents in the ocean's thermal zone (Okafor, 2011). There are also a number of species which thrive inside other organisms like insects and mammals. This makes classifying bacteria rather challenging. In medicine, microbiology laboratories use a number of criteria to classify bacteria; the most important of which are shape, Gram-staining characteristics, and metabolism. From these three criteria, bacteria can be classified into a number of different groups, and the implication of this in the context of medical microbiology and environmental studies will be discussed here. By understanding how different bacteria behave and interact with their environment, it is hoped that a better appreciation of the broader evolutionary implications of microbial life will be achieved.

Bergey's Manual of Systematic Bacteriology is the definitive resource for the classification of bacteria and, over five editions, has organized the most well-known of them into six groups. The features most commonly used to classify prokaryotes are their shapes (cocci, bacilli, and spirilli), Gram-staining properties, and metabolic processes. According to the Bergey's classification, the Domain Bacteria are currently classified into 18 groups. Small, spherical bacteria are styled cocci or coccus, whereas bacilli are rod-shaped and spirilli are spiral-shaped. Other shapes do exist, such as the comma-shaped Vibrio species. The width of bacteria varies greatly, with the smallest ones measuring around 0.2 μm, and the largest being somewhat over 1 μm. Their length ranges even more, from about 0.1 μm up to several centimeters in length. With some, the length-to-width ratio is important, while other shorter species approach a more cubic shape. Certain species exist that are only one diameter in length and therefore are often mistaken for cocci. Bacteria can also form clusters of varying sizes. Django-shaped chains are microbes in which the cellular division along one plane never completely separates.

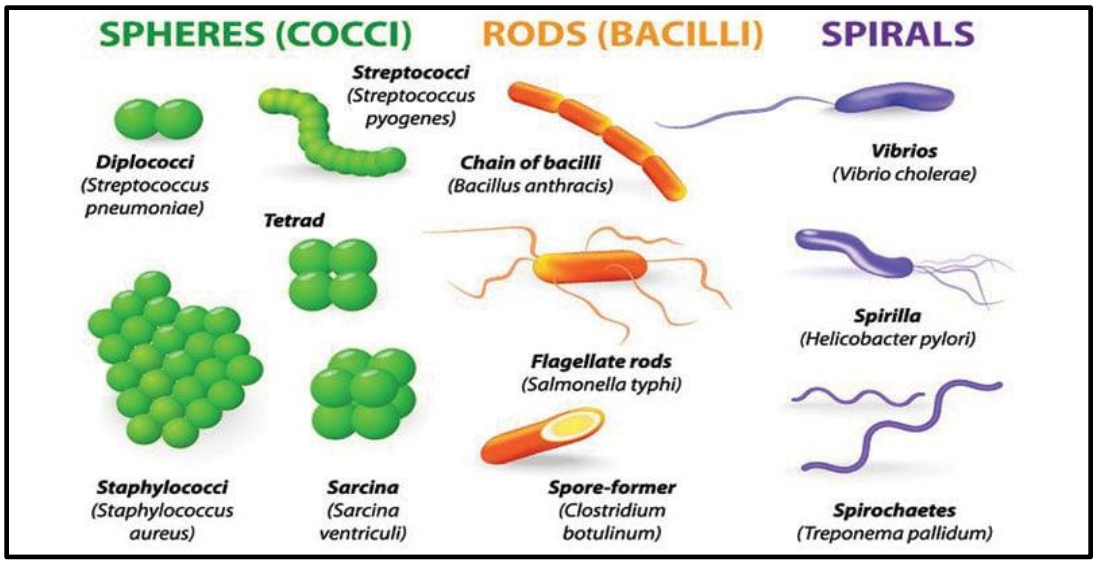

Figure 2: Classification of Bacteria on the Basis of Shape.

2. Cellular Structure of Bacteria

In this section, the prokaryotic cell of bacteria and its structural organization are explained. The starting place is with the cell envelope. This is because the cell envelope is pivotal in maintaining the structural integrity of the cell, controlling the exchanges that take place with the external medium, and mediating the interaction of the cell with other cells or with external agents.

The cell envelope of bacteria is a very complex structure. The basic structure of a bacterium is described as a composite object, in other words made up of several distinct elements. Most elementary of these is its “sacculus” which, is the “shell”. This word is derived from the Latin word saccus which means a “leather bag or sack”. The sacculus is not a membrane as it is colloquially taken to represent, but a very fine mesh-like bag in which the cytoplasm is cradled. It is, however, true that the usual depiction of the bacterial cell makes it look like a stack of envelopes or membranes more or less parallel to each other, or one inside the other, comparable in this regard to some of the other prokaryotes. The innermost “envelope” or membrane is then designated as “plasma membrane” (PM) (KRELL and BEVERIDGE, 2014). The bacterial sacculus is an immensely strong and resilient structure. In the naming conventions of bacteriologists, the sacculus is also referred to as the cell wall. It is this structure that gives the bacterial cell its shape and, to a large extent, its other features such as its motility or the nature of its associations with the biotic and abiotic entities that share its environment.

The complexity of the cell envelope does not reside in the shell alone. Not immediately obvious is that the outer task of the cell wall is covered with yet a more or less continuous layer that is essentially a molecular mesh and is made up of a class of biopolymers. These organic molecules are not easily visualized using standard methods in molecular biology. The pertinacious scientist can, however, try to deprive the biopolymeric mesh-like layer off the cell in other ways, and then adjust the formulation of the new question to the range of methodologies that can be brought to bear on such a pre-treatment.

2.1. Cell Envelope

The cell envelope of bacteria is an essential multi-layered shield-like structure which surrounds and protects cells from their environment. This structure also helps the bacterium to interact with the environment. The most outer layer of this system is the outer membrane (OM). The outer membrane of most bacteria is asymmetric, consisting of an inner leaflet of phospholipid and an outer leaflet of lipopolysaccharide (C Gilmore et al., 2021). SubM complex is composed of three proteins TamB, YbdH, and YhdP. TamB and YbdH physically connect the inner (IM) and outer membrane (OM) for the localization of outer membrane protein sorting factor TamL in E. coli. Mutation of tamL, ylhA, or yjlC is sick in a strain where the expression of tamB is repressed. This growth defect is suppressed by deletion of any of the three genes. These results show that TamB, YhdP, and YbdH are redundant but essential for the growth of E. coli (Ruiz et al., 2021). Below the outer membrane is the cell wall. The cell wall consists of peptidoglycan, a macromolecular mesh-like structure. The cell wall acts like a turgor pressure-resisting exoskeleton, protecting the bacteria against osmotic pressure, osmotic shock caused by shifts in the osmolarity of the surrounding medium, disruptive components of the environment, and preventing it from bursting or collapsing as a result of negative cell turgor pressure. In addition to protection from external mechanical and osmotic threats, the cell wall of the bacterium, through its myriad of transport proteins, channels, and pores, also separates the cell from its environment by selectively allowing substances in and out. This selective permeability is also influential in the cells signaling with the environment. Communication is achieved via the exchange of neurotransmitters, hormones, and small-signaling molecules. These can enter or exit the cell via the porins, channels, and transport proteins on the outer membrane. Activation of these proteins triggers intracellular signaling pathways or induces gene expression in the cell. Subsequently, the last component of the cell envelope surrounding the bacteria is the plasma membrane (also known as the inner membrane). The plasma membrane is composed of a phospholipid bilayer, protecting the cytoplasm from the external environment.

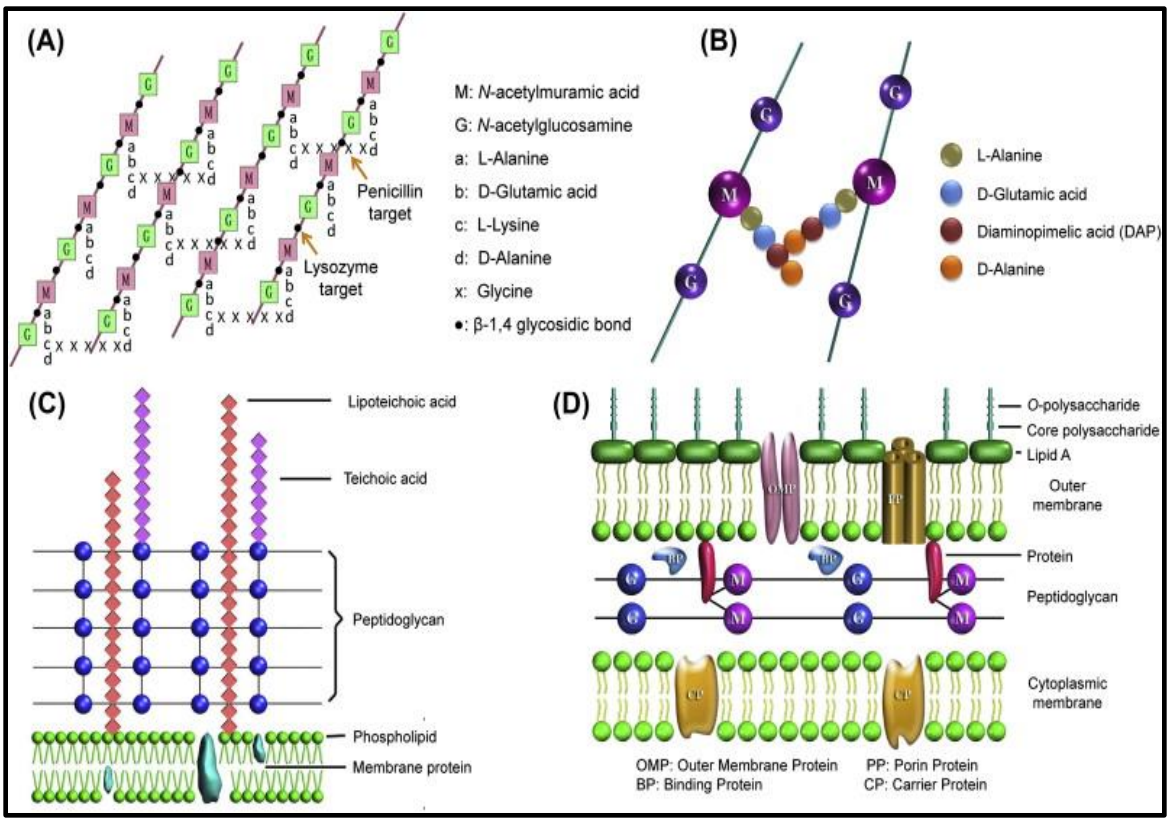

Figure3 : (A) Schematic representation of Staphylococcus aureus cell wall peptidoglycan. (B) Schematic representation of Escherichia coli cell wall peptidoglycan.(C) Schematic of gram-positive bacteria cell wall. (D) Schematic representation of the cell wall of gram-negative bacteria.

2.2. Cytoplasmic Structures

Bacteria are primitive, single-celled organisms with relatively simple cell structures. However, while their cell structures may not be as complex as those of other organisms, the function and metabolism of bacteria are far from simple. A few syntheses of simple cytoplasmic structures enable bacterial cells to increase in mass sufficiently for growth and resist environmental changes to ensure survival. The cell structures involved become essential for bacterium-cell vitality. The understanding of the function and malfunction of these structures represents the foundation of bacterial cell physiological activities (Pilhofer and J. Jensen, 2013).

Bacteria can also survive in an environment of constant fluctuations because of the presence of passive RNA, which is primarily related to the ribosomal activity. Ribosomes, however, represent only one aspect of cytoplasmic space found in bacteria. Several other of the sub-cellular pieces are also situated in non-random locations. This suggests that, besides the circularity of macromolecular structures (like those of the ribosome), other mechanisms are also involved in bringing a biologically functional unit into a non-random place. Bacteria are surrounded by a

lipid membrane, engulfing the cellular take held by the cytoplasm. As bacteria do not possess complex transport mechanisms found in eukaryotic cells, several areas at the outside of the outer membrane display spike-like structures that extend into the extracellular spaces. Similar structures on the nucleus provide anchoring places for cytoskeletal proteins. Although f-actin is still excluded from the nucleoplasm in the eukaryotic cell, it clusters at nuclear pore complexes and there forms a kind of diffusion barrier (Dersch et al., 2022). Due to the intracellular spike-like structures and the absence of nucleoporins bacteria are thought not to contain a regular meshwork-membrane pore complex arrangement. Instead, the bacteria exhibit a meshwork membrane curvature. The cytoplasmic protein of B (PopZ) binding directly to the f-acting of the bacteria also forms filament-shaped structures. In the pure in vitro setting PopZ filaments are supplemented in a uniform micron. In vivo, however, B. subtilis PopZ forms two types of filaments, one of which has a diameter comparable to f-acting assemblies. Remarkably, the wider filaments in vivo were scarcely detected in previous super-resolution studies on PopZ.

3. Bacterial Metabolism

Microbial cells live in an environment surrounded by attempts to halt their growth and division. They live in an acidic, salty environment with a high temperature and high ultraviolet (UV) exposure. In order to survive, cellular functions have developed in bacteria. A wide variety of metabolic pathways are contained within the chemical processes that make up metabolism. Catabolism of organic and amino acids involves the acquisition of raw materials along with other monomers leading to the production of protein polymerization. It resembles a two-carbon unit derived from organic acids, acetate in particular. Other monomers, such as those that make sugar, pyruvate can be derived from organic acids. The metabolic role of amino acids is varied and complex, both in nutrient utilization for the production of proteins and for the biosynthesis of non-protein amino acid derived molecules. Minerals have important roles in metabolic processes, including those facilitating the uptake of ligands or functioning as metal cofactors of enzymes (Judge and Dodd, 2020).

The process of metabolism involves utilizing nutrients and direct consciousness towards preservation. Bacterial energy needs are met by the processes that convert nutrients into power. The microbes were first colonized on the Earth due to their ability to develop the anaerobic environment prevalent at that time. And thus they are well suited to living in an anaerobic environment. The end product of nitrate reduction or sulfate decrease may be the same kind, but the molecules involved in the pathway vary from bacteria to bacteria. The greenhouse gas methane is made using the largest known enzyme complex that encodes the gene. Methane is one way for CH4 making and CO2 is oxidized to CH4. It includes indirect light absorption through

long CO2 pathways. Phototrophic prokaryotes can fix their CO2 and use it as a sole source of carbon as most autotrophs (anaerobic processes are called prokaryotes). Bacteria photosynthesize and have the ability to use light as an energy source.

3.1. Energy Production

Energy production is the process that powers all biosynthetically expensive cellular processes and, as such, it is a critical phenomenon to understand when considering a bacterium's metabolic capabilities. Bacteria have a wide range of strategies to generate ATP, the standard currency of the cells, and can exploit a variety of different metabolic pathways. Most bacteria produce energy through an electron transport chain (ETC). The electrons are shuttled from a donor to acceptor via redox reactions, ultimately generating a proton gradient across the cytoplasmic membrane that is used to synthesize ATP. Generally, most bacteria can generate ATP via either a respiratory or a fermentative pathway. The substrate-level phosphorylation is the only method of energy production in anaerobic conditions and involves a high ATP yield per glucose molecule (Lila Koumandou and Kossida, 2014). It should be noted that, while energy production in bacteria is often displayed as schematics of separate pathways, cells rarely operate a single pathway in isolation.

Probably a better way to view the metabolic capabilities of bacteria involves considering the electron acceptors they can utilize. Following the most macroscopic distinction, there are bacteria that utilize either O2 for respiration and those that rely on other electron acceptors. Most bacteria with a genome and a lifestyle similar to pathogenic or free-living bacteria belong to the former group. They can carry out both aerobic or anaerobic respiration, although some bacteria preferentially use O2 even under anaerobic conditions. As a rule of thumb, for similar substrates, aerobic or nitrate respiration is more efficient than the use of alternative electron acceptors. On the other hand, respiratory pathways that rely on Fe (III) or other electron acceptors with low redox potential tend to have a lower yield of energy production per substrate molecule. In contrast to common belief, lactate fermentation is quite inefficient for most bacteria and only yields 2 ATP per glucose molecule. Nonetheless, bacteria can be extremely flexible and they can shift pathways in response to environmental changes. The ability to adapt the metabolic strategy in response to environmental shifts has substantial applications in biotechnology and environmental sciences. Ultimately, the ability to constrain energy production strategies is a useful but simplifying categorization; and, for instance, it is not universally possible to classify bacteria as fermentative or anaerobic only on the basis of the acceptors they can utilize. In contrast with respiration, fermentative bacteria do not rely on an ETC to generate a proton gradient but instead use substrate-level

phosphorylation to generate ATP and redox balance to re-oxidize the electron carriers.

3.2. Nutrient Uptake and Utilization

Nutrient uptake and utilization are essential processes required for bacterial growth and metabolism. All bacteria must scavenge and take up nutrients from their environment to survive, which is achieved through the action of a variety of membrane transport proteins that catalyze the movement of small molecules across the phospholipid bilayer membrane (S. Davies et al., 2021). Bacteria take up essential nutrients such as carbohydrates, amino acids, peptides and metals from the environment and so exert a profound effect on their surrounding biotic and abiotic environment (J. Tanaka et al., 2017). There are many different families of bacterial transporter proteins, with differing folds and substrate specificities, numbering up to thousands in any given and sequenced organism. To help with overall informativeness and conciseness, the families of transporter proteins will not be discussed thematically but ones with known functions will be considered. Facilitated transport using transport proteins play an important role, particularly in the uptake of quaternary amines and branched chain peptides. The uptake of free carbohydrates in bacteria is also clearly shown to involve the action of specific transport proteins, the phosphotransferase system in enteric organisms and the binding proteins of the ABC transporters for other Gram-negatives.

Bacteria are able to scavenge and use a diversity of different organic and inorganic substrates for growth that are not readily available for eukaryotic organisms. Examples include methyl- and halides important for the utilization and detoxification of such substrates. Others are specialized to use more complex substances such as hemicellulose, animal waist products, saxitoxin or acetate oligomers released during the degradation of polymeric lignocellulosic products. Controversy exists as to whether the transport systems are (in their macro-level organization) built around the specific needs of each kind of substrate or are instead arranged as multicomponent systems that would allow freeing any given substrate from the encumbrance of its co-utilizers. Bacterial ability to scavenge a broad spectrum of potential substrates will be contrasted against a limited capability in utilizing many of those such as cellulose and some polysaccharides.

4. Bacterial Reproduction

Division is a crucial aspect of a bacterium's life cycle. Its population size reflects, among other things, a balance between division and loss (Pichugin et al., 2017). Bacteria reproduce rapidly through binary fission, allowing them to divide and increase their population size quickly. This simple mode of asexual reproduction also

creates two genetically identical daughter cells. Procedures have been shown to have at least one binary fission event, though some rod-shaped bacteria can frequently divide accurately in multiple planes. For species that divide in place one dimension is the location of division, with B. subtilis, for example, dividing across their shortest axis. Micrococcus tetragenus divides in two perpendicular planes to form packets of four – tetras, meaning 'four' – coccoid cells. Bacilli such as Mycobacterium austroafricanum split in half, yielding two offspring cells. E. coli divides in two planes perpendicular to one another at the center of the cell differently sized daughter cells. This process is elaborated by the formation of the septum, a double membrane that grows inward from the cell wall until the two daughter cells are completely separated.

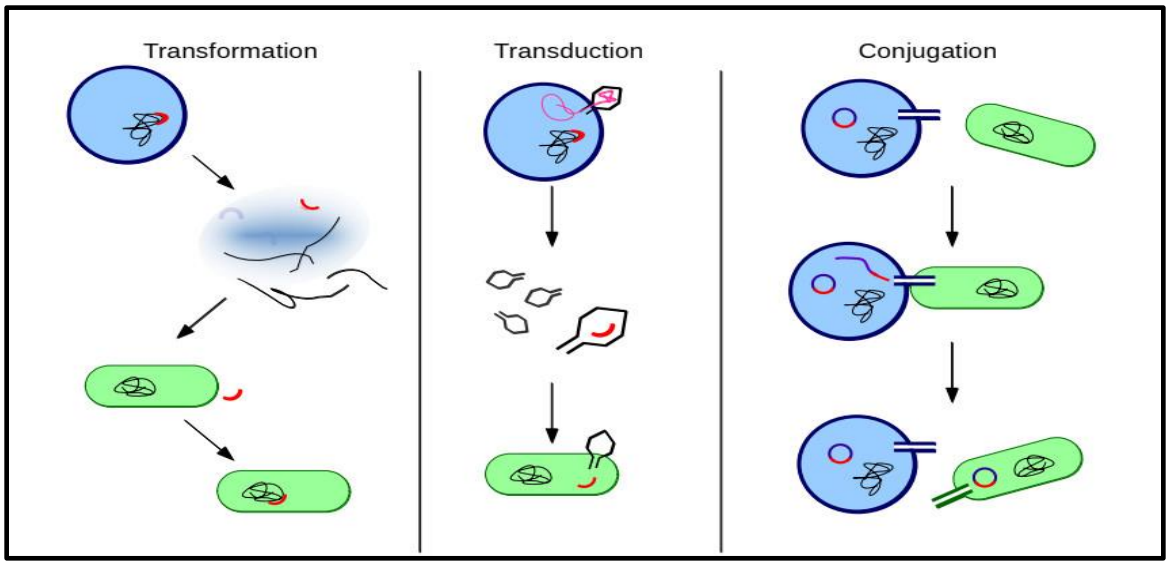

Figure 4: Reproduction in Bacteria (Horizontal Gene Transfer).

Binary fission is a highly efficient method of reproduction and populations can double in a matter of just 20 minutes. A single E. coli bacterium can divide to about 75,000 organisms in a period of only 24 hours, provided that sufficient nutrients are available. Knowing that bacterial reproduction is chiefly asexual, with simple cellular division producing identical offspring, underscoring both the significance and interest in bacteria, whilst also emphasizing the range of replication found in the world's most abundant life form. Other, more complex means of reproduction, however, form an essential part of the subject, particularly the amalgamation of genetic material possible through conjugation. This notion of other reproductive strategies raises question around the subsequent adaptability mechanism fuelled by such genetic diversity, essential to a decimation of evolutionary dynamics. An understanding of both the rates and methods of reproduction amongst bacteria consequently forms a robust groundwork in many penetrations such as the modeling

of growth, and the investigation of bacterial adaptations in changing environments. Thus, an evaluation not only of reproductive strategies, but the repercussions of conditions under which such strategies are deployed, is warranted.

4.1. Binary Fission

The extraordinary rates of population growth experienced by bacterial populations are enigmatic for non-specialists in the field. The combination of a thorough understanding of the underlying biology and simple mathematics explains the large number of offspring in a new perspective on the robustness and efficiency of the fundamental biological processes underpinning bacterial growth. Consequently, the relevance of the divisive septum in bacterial cell biology makes more sense along with the developments of association of bacterial cell division with cell polarity and growth. A lockstep progression along the process of division, while bacterial growth proceeds even faster, makes the divisome more prone to directly or indirectly cue to anything that favors cellular growth, at the possible expenses of a lower efficiency of bacterial cell division. Finally, the evolution of a group of molecules prioritizing faster cell division may contribute to explain the loss of function of full inhibition in eukaryote cells.

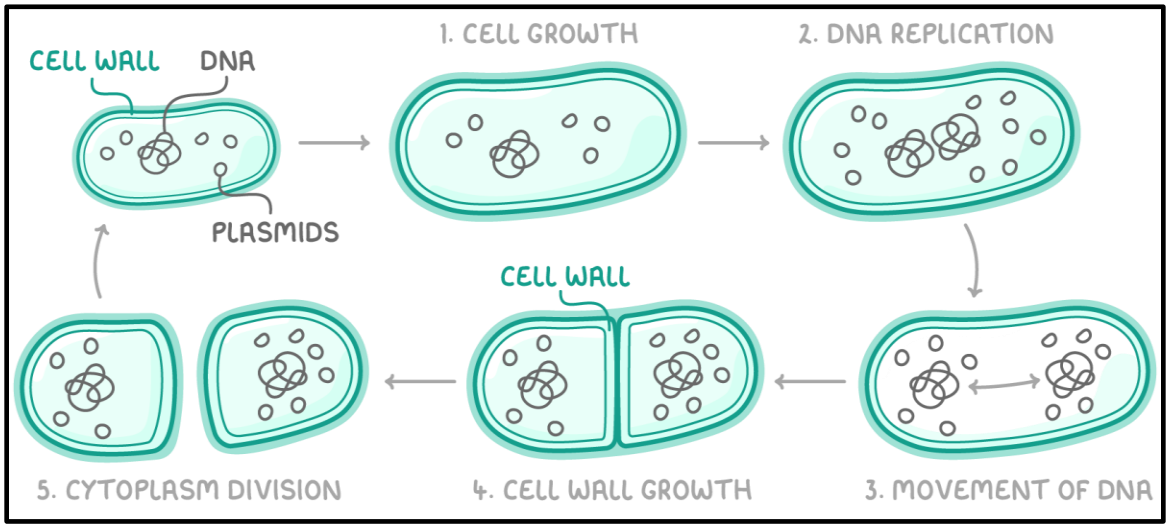

Figure 5: The steps of binary fission.

Bacterial cell proliferation is very efficient, both because bacteria grow fast and multiply with a low failure rate. Several processes contribute to this efficiency. First, the robustness of the bacterial cell cycle and its tight synchronization with cell growth and cytokinesis ensure that most cell cycles are successful and generate nonviable products only infrequently. Second, bacteria have evolved an exquisite coupling of their genome with the cell cycle, such that DNA replication occurs once and only once every cell cycle and is systematically completed before the onset of cell division.

Third, septation is initiated when the cell length has reached a threshold number of generation and only after the majority of cell division sites have been properly placed, ensuring the reproducibility of cell size at division (Meunier et al., 2020).

4.2. Conjugation

Conjugation is a unique way of genetic exchange among bacteria. Although most bacteria reproduce asexually, they often exchange genetic material to expand their genetic diversity, usually by transforming or transducing. One bacterium transfers genetic material to another through direct contact in conjugation. Pili, namely conjugation pili or sex pili (hereafter simple pili), are needed to establish this contact. One bacterium with a conjugal plasmid, called a donor (F+, usually referred to as donor), develops pili and binds to a bacterium in the recipient (F-), opening a channel for transferring single- or double-stranded plasmid into the recipient. These plasmids are then re-circularized and function as a template, or are unrolled and integrated into the chromosome of the recipient (Virolle et al., 2020). Compared with transformation and transduction, conjugation is more efficient than the other two methods because 5-30% of the recipients acquire plasmids. Conjugation also seems to be involved in the spread of antibiotic resistance, as plasmids carry genetic factors that provide resistance to various antibiotics. Since the gain of these selective advantages compensates for the metabolic costs of plasmid biosynthesis, conjugation plays an important role in the maintenance of these conjugative elements in bacterial populations.

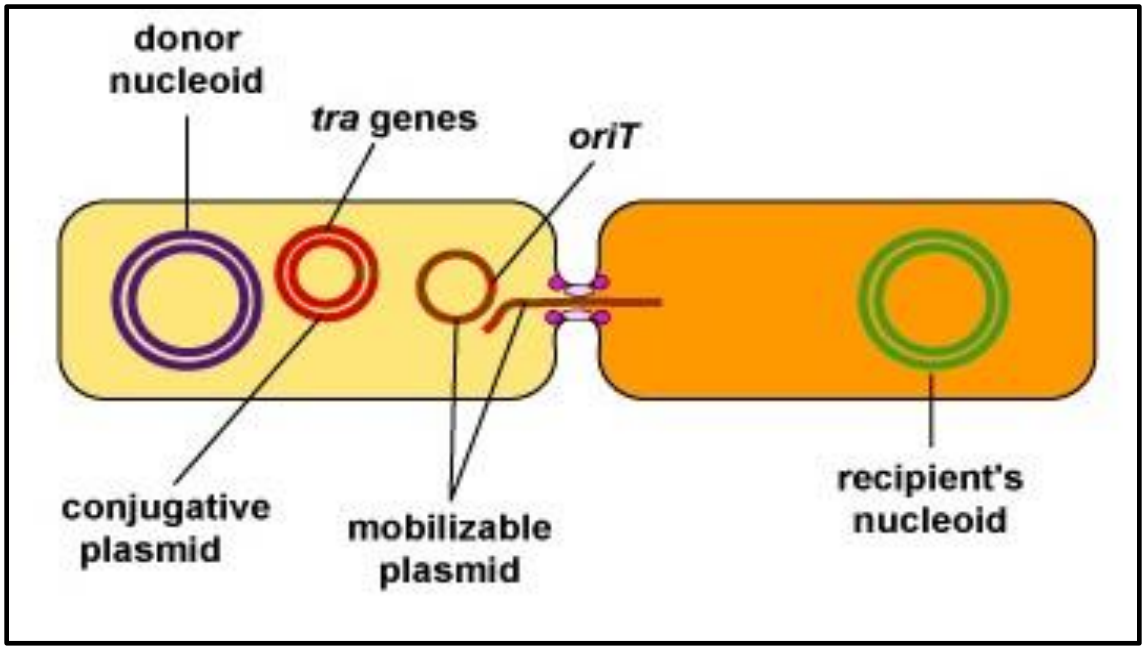

Figure4 : Conjugation in Bacteria.

Various bacteria carry redundant immobile conjugal plasmids, even though conjugation requires high-energy investment and could result in a metabolic cost when host populations are cultivated in environments where metabolic resources are limited. Also, it risks the loss of plasmids in both the donor and recipient, when broad host range plasmids often spread resistance to unrelated pathogenic species. To provide an explanation for this paradoxical observation, a mathematical model has been constructed that takes into account several deterministic and stochastic parameters of plasmid biology. Suitable combinations of plasmid parameters and host densities increase the contribution of the conjugation cost to the meta-energy landscape. This makes conjugation dichotomous in most circumstances, while behaving either as a sink mechanism or promoting the maintenance of plasmids. This phase-like behavior accounts for the apparently futile replication of R-plasmids in commensal and clinical environments.

5. Bacterial Adaptations and Survival Strategies

In a dynamic environment, adaptation is driven by selection. The survival of a lineage depends on the particular changes it can invoke in its phenotype to benefit from forthcoming conditions through a competitive advantage or, conversely, be sufficiently buffered against potential alterations in the environment. The balance between stress resistance and growth is tilted at different settings for bacteria depending on their specific niches, and adaptive mutations (both structural and regulatory) allow bacteria to shift this balance depending on the growth- or stress-related selective pressures in their environment. In nutritionally fluctuating environments, adaptive protein expression is known to play an important role in adaptation to future conditions.

Survival of natural isolates and mutants of diverse species is measured in a range of particular stresses. Whilst the stresses are generally harmful, bacteria survive by activation of stress response pathways, allowing for copious production of proteins that otherwise protect cells. There are many stress response pathways that are conserved across Enterobacteriaceae, which reveals similarities in the stresses that different bacteria encounter in their environments (Chen, 2019). Study of these Enterobacteriaceae is relevant as these bacteria occupy diverse but overlapping niches, and understanding differences in stress response pathways, and how they affect the broader network of biological responses to environmental challenges, offers insight into the composition of physiological signals and stresses in their distinct niches.

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Chapter Five

Mycology: The Study of Fungi

1. Introduction to Mycology

Mycology is the study of fungi, and is often overlooked in the realm of microbiology. The secret world of fungi is revealing itself, however, with intriguing possibilities. Recent studies are showing fungi to be ancient life forms, already populated the planet, hundreds of millions of years before plants. It is predicted that of the four million species estimated to be in existence, we have only identified five percent of them. The function of mushrooms has been explored in traditionally flood-plain research areas too. Such research is just beginning however, the mainstream investigation of fungi has been relatively small, and the emerging perspective is that of most to come allies have identified a world largely unknown. It is important to inform, educate, and understand the roles that the "Forgotten Kingdom" play in the larger picture of the planet. Efforts are to understand how fungi operate, change, and dominate a world of decay, and yes, even some of life's fundamentals (Bahram and Netherway, 2021).

Fungi are very different from plant and animal bio-forms, both anatomically and physiologically. Numerically, fungi lie between the depression of known and unknown body forms, so it is keyed that to understand fungi and the unique ecological niches they inhabit is to place them as a stepping-stone in evolutionary perspective. There has been a large gap in ecological studies comparing bacterial or animal ecosystems, the forms being either too primitive or too large to examine. Many of the oldest life history armaments are either fungal or viral. In a world of symbiotic beginnings, along with nutrient leaching animals, fungi reign. There is good reason intercellular networks are far from new. Though they are largely invisible, the comprehend ecosystems either through advanced morphological changes in intimate settings or indirect repercussions in macrocosmic ecosystems (A. Naranjo-Ortiz and Gabaldón, 2019).

1.1. Definition and Scope of Mycology

Mycology is the scientific study of fungi (including lichens) and fungal-derived molecules and materials, but there is no consensus on exactly how fungi should be defined, and so on how mycology should be framed. Fungi are found at anywhere

from the poles to the Equator, from freshwater to marine habitats, from rainforest canopies to the abyssal trenches, and including an extraordinary range of associations with other organisms. Kingdom Fungi includes over 1.5 million species, some with enormous impact on the environments that they inhabit, and some with significant impact beyond their own environment. This influence includes environmental effects (both positive and negative) on abiotic and biotic parameters such as local and global climate, soil and water structure, and inter- and intra-specific interactions with other (including non-fungal) organisms. They influence the success of many plants, and in turn are affected by successional changes in plant soils and competitive exclusion in microhabitats. Fungi also have many cultural and economic effects on humans including their role in the discovery of antibiotics, the development of foods and beverages, and the control of plant and invertebrate pests (Lange, 2014). Conversely, they can contaminate human foods and feedstocks as a result of mycotoxin production, and fungi are also the cause of emerging diseases of plants, wildlife and humans. However, this wide range of interests and importance is not easily contained with the confines of one discipline. In one sense mycology may be seen as coextensive with biology, but it intersects with many other disciplines at a level deeper than most specialties. Mycology therefore faces a number of specific challenges including isolation and culture of many species, the relative scarcity of diagnostic phenotypic characteristics, and a paucity of markers for sub-specific genetic analysis. On the other hand a number of technical advances have facilitated the study of fungi, including many molecular techniques, and many of these technical advances are likely to shape an improved understanding of mycology in this century.

2. Fungal Morphology and Structure

The identification and classification of fungi crucially depends on a knowledge of their physical characteristics or morphology. This discipline reveals the astonishing variety of form in the fungal kingdom, indicative of functions related to spore dispersal, habitat exploitation, and complex symbiotic relationships with plants and animals. Some forms of fungi are microscopic and unicellular; filamentous species range widely in colony shape and aerial development; mushrooms and other macrofungi can be strikingly large and complex (D. Harris et al., 2005). The lower fungi show an even wider range of unique forms, often adapted to aquatic habitats. In contrast to animal structure, which is essentially determined by the development of specific tissues, fungal anatomy is a function of the growth of specialized hyphal structures. Understanding these adaptive structures and the interactions of fungi with their environment and other organisms is essential to a deeper appreciation of their ecological niche and the role they play in diverse habitats.

The morphological form of true fungi, which excludes slime molds and oomycetes, is determined by the growth and shape of the hyphal thallus. Hyphae have a high surface area to volume ratio and are well adapted for absorbing nutrients and water.

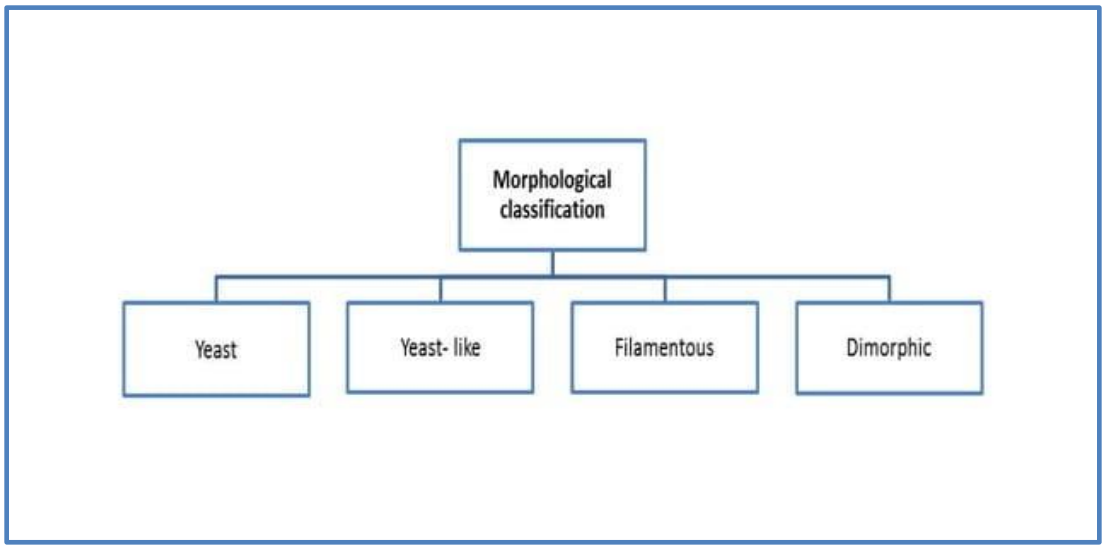

Figure 1: Fungal classification according to their body shape.

The branching growth of mycelia acts to secrete enzymes and acids into their substrate, aiding decomposition and nutrient absorption. The hyphal thallus grows indeterminately at its tips, and forms a vast system of mycelium of theoretically infinite extent. The complexity of fungal morphology is realized in the templates of space and surface on which it grows. A fungal thallus may be unicellular, in the case of yeasts and other lower fungi; many are multicellular filamentous structures composed of microscopic tubular cells or hyphae.

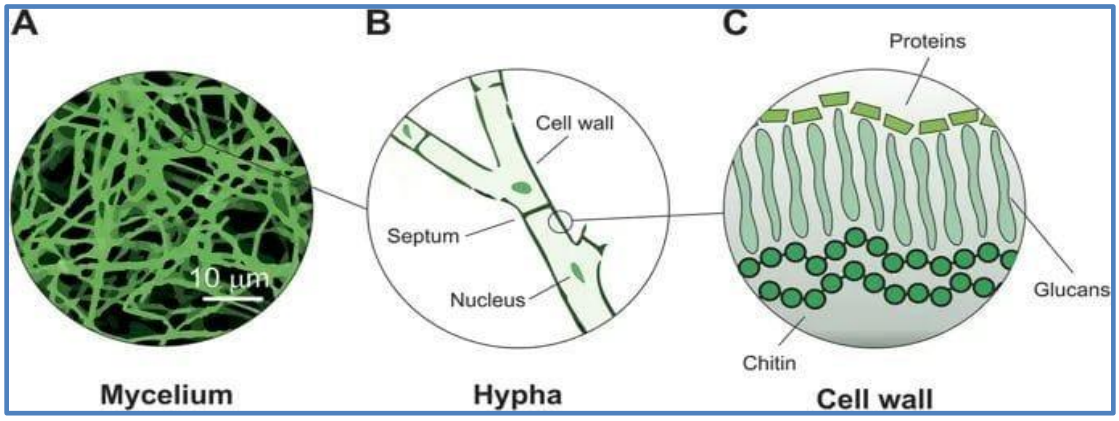

Figure 2: Representation of mycelium structure.

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Cells or hyphae may appear only in longitudinal section, in which case the hyphal thallus may appear as strands or cords, with growth limited to the tips of the strands. A more complex, felt-like structure is created by the growth of hyphae in more than one plane, matting together to form a colony. Common mushroom composts exploited by Agaricus spp. are an example of this form of growth.

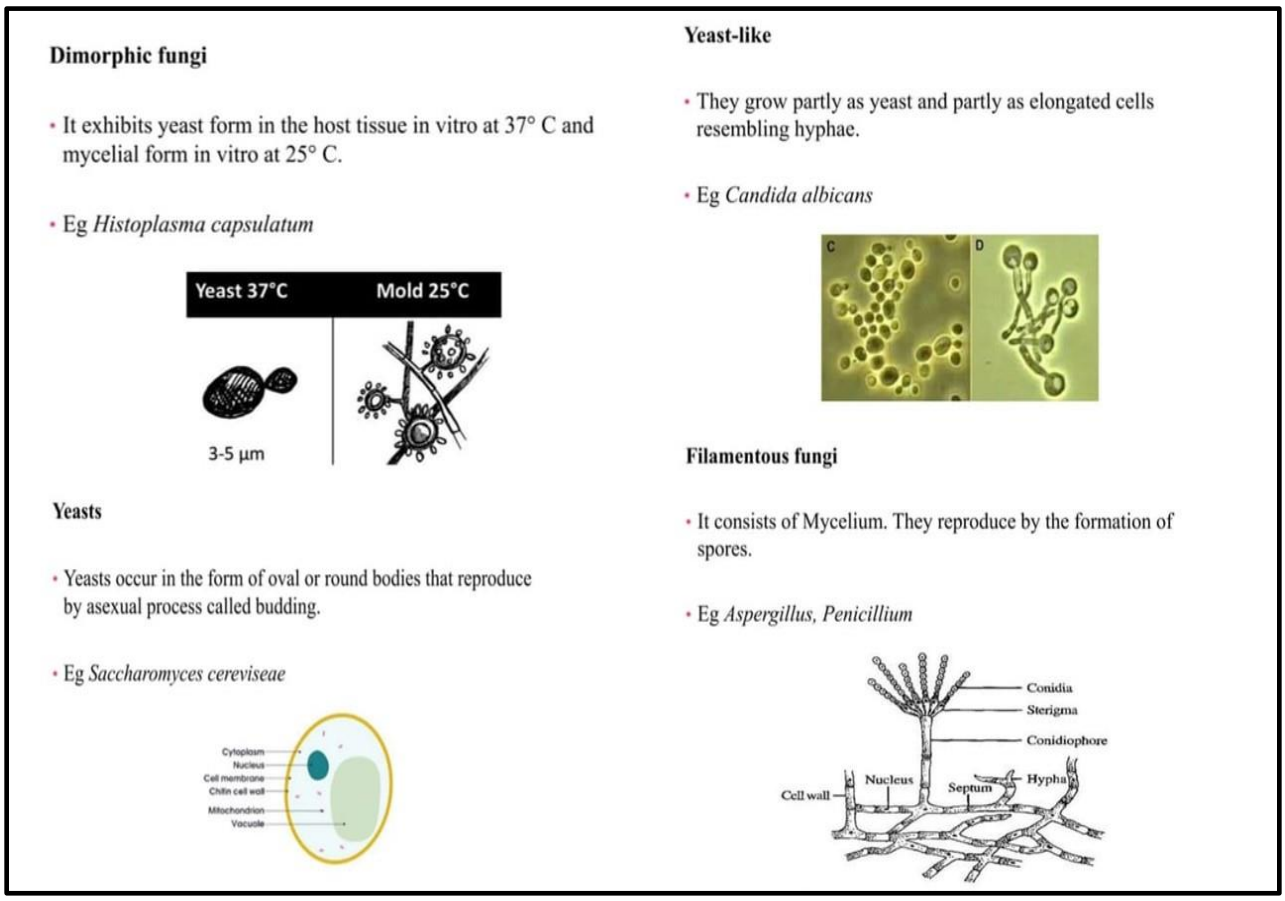

Figure 3: Fungal classification according to their body.

2.1. Cellular Structure of Fungi

The multi-cellular organisms that comprise the distinct kingdom of fungi are the focus of mycology. Macroscopic manifestations of these organisms have been known for centuries and are sometimes referred to as the "higher fungi" to distinguish them from other eukaryotes such as yeasts and molds. True Fungi are a diverse group of organisms that are ecologically important as decayers and mutualists, and they are significant components of a variety of food chains. A diverse group of metabolites and indole alkaloids as well as enzymes that humans use for industrial applications are produced by fungi. Some fungi are parasitic upon plants and animals and are,

therefore, involved in significant loss of food crops. The majority of fungi can be categorized into one of several groups or phyla.

The most ubiquitously recognized feature of fungi is their production of hyphae. The other unique cellular feature of fungi is their composition of chitin and glucan as their cell wall. Not only do fungi have features that define them at the cellular level, they are also uniquely distinguishable from other kingdoms by the presence of specific enzymes for nutrient uptake, specialized organelles, and distinctions in the cell cycle. Intercellular and intracellular communication inevitably play huge roles in the life of a fungus. The signaling pathways and regulatory genes of fungi are also subjects of study within mycology. Data concerning these diverse research areas is revealing much about basic biology as well as suggesting possible practical uses in biotechnology and disease treatment.

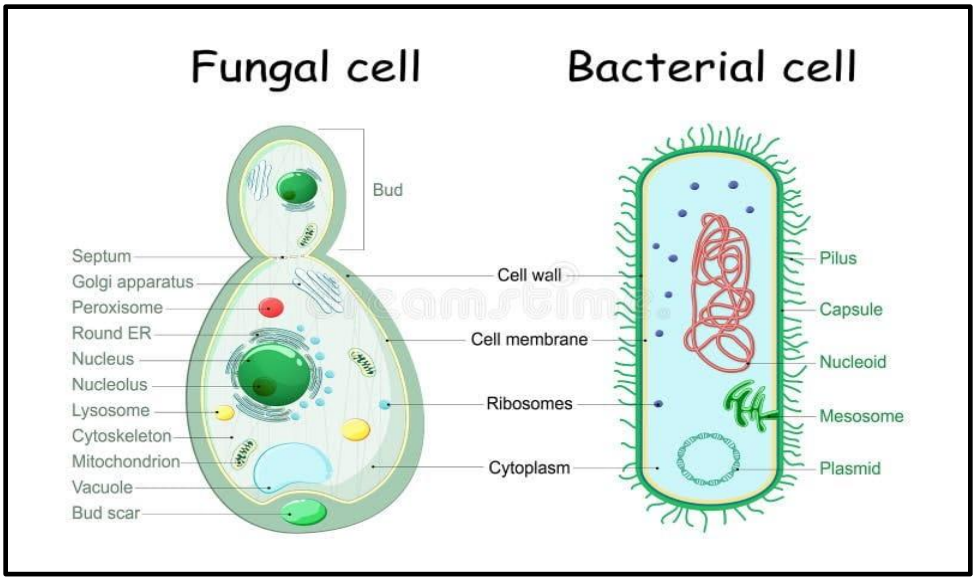

Figure 4: Bacterial and fungal yeast. Comparison of cell structure.

3. Classification of Fungi

Fungi include a wide variety of organisms ranging from yeasts, molds, and mildews, to mushrooms and puffballs, and many other lesser-known groups. Fungi are a kingdom of usually multicellular eukaryotic organisms that reproduce sexually and/or asexually via spores. Traditionally, fungi have been classified on the basis of spore color and structure, spore-releasing structures, and the morphology of the mycelium.

Fungi have been classified using traditional methods based on morphological characteristics. These studies group fungi based on similarities in spore color, spore-

releasing structures, and the overall morphology of the mycelium. However, a growing understanding of fungal phylogeny and the results of molecular studies have refined current classification systems. Fungi belong to the kingdom that bears their name. Like plants and animals, fungi are eukaryotic. This means that each of their cells has a distinct nucleus containing DNA. Additionally, like all eukaryotic cells, fungal cells are bounded by a plasma membrane. Fungi represent a distinct lineage of life. They are not plants. They are not even in the same domain of life as plants. Modern classification systems have a very specific hierarchical structure. The broadest classification is in Kingdoms, which gets more and more specific until you reach Species, the smallest classification (A. Naranjo-Ortiz and Gabaldón, 2019). Phylogenetic analysis has identified the evolutionary relationships among the many fungal groups by studying their phylogeny. This has led to a reevaluation of the criteria used to group different types of fungi. Characteristics such as the presence or absence of certain structures during the lifecycle, genetic makeup, and even substrate preference have been used to distinguish different fungal taxa. The growing understanding of fungal phylogeny and the results of molecular studies challenge mycologists to refine current classification systems. Some well-known groups, such as the fungi imperfecti, do not fit into the established classification system. Other, lesser-known but ecologically widespread fungi, such as the mycorrhizal fungi, have peculiar features that make classification difficult.

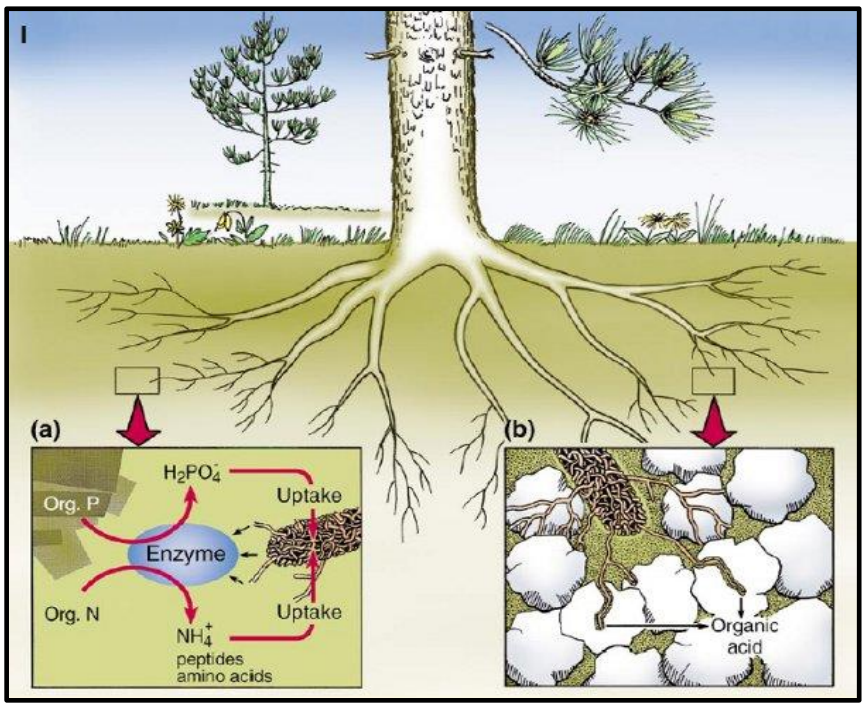

Figure 5: Mycorrhizal Fungi.

3.1. Major Fungal Groups

Fungi is a kingdom of complex and enigmatic organisms that includes some of the most important and diverse members of the biosphere. Conventional classification schemata recognize the existence of multiple phyla of fungi, but learning all about these phyla can be a challenging task. Therefore, the study of these fungal groups is systematized in Phylum view format. The major fungal phyla are grouped together with similar and related fungal phyla, and their ecological roles are discussed. Finally, general challenges and new perspectives in mycological research are detailed.

Ascomycota and Basidiomycota form the most diverse and commonly known and recognized phylogenetic lineages of fungi. The phenotypic diversity of eukaryotic organisms is believed to have originated in fungi, and this diversity has presumably spawned the vast array of adaptations with which ascomycetes and basidiomycetes are endowed. Early diverging members of the Ascomycota occur throughout Fungi, with predominantly marine representatives in the subphylum Pezizomycotina. Beyond the subphylum Pezizomycotina, there is an additional marine clade referred to as the Lichen-forming Clade. The nature of this clade remains poorly understood because there is a dearth of described species and a paucity of molecular data. Diverse basal branches are also found within the Basidiomycota, distributed in the classes Ustilaginomycotina and Exobasidiomycetes (A. Naranjo-Ortiz and Gabaldón, 2019). Basidiomycota is an early diverging lineage and includes two poorly studied marine lineages: the Cryptomycota and the Rozellomycota, two well-known but primarily terrestrial lineages with aquatic representatives, the Pucciniomycotina and the Agaricomycotina, respectively. The bulk of the fungal lineages on earth consists of unsampled, uncultured or unknown organisms which are referred to as 'The Fungal Dark Matter'. Despite being overlooked, their importance is paramount because their widespread distribution might significantly impact the biosphere. Symptomatic of our profound ignorance about the biology of the Hidden Kingdom, every year about 1000 fungal species are newly described that are either novel or previously known (Bahram and Netherway, 2021).

4. Ecology and Interactions of Fungi

Fungi are a diverse group of organisms exhibiting diverse lifestyles, reproductive and dispersal strategies and physicochemical traits, which could play important roles in their success as saprotrophs and other roles. There is growing support for the hypothesis that worldwide saprotrophic macro-fungi contribute to nutrient cycling in ecosystems. The assemblage of saprotrophic macro-fungi in an ecosystem may also reflect the critical transitions or stability of the ecosystem and could be useful as a long term monitor of ecosystem health. This is further enhanced by the strong

phylogeographic structure seen in macro-fungi. Many fungi are organic matter (OM) decomposers (saprotrophs), as is often learned in school.

Perhaps as many as 90% of higher fungi on Earth are purely saprotrophic, though a much smaller fraction of micro-fungi are responsible for most of the worldwide decomposition. In general, fungi play a critical role in the decomposition of complex organic material for total ecosystem health. However, fungi also play important roles in maintaining the health of the ecosystem, or in providing benefits that support an ecosystem. Commensalism, parasitism and mycorrhizal symbioses are key examples of this. Leaf litter decomposition not only affects the organic material in the environment, but more importantly is an essential part of the organic carbon recycling process (Bahram and Netherway, 2021). Fungi are vital players in nutrient cycling and, as saprotrophs, are among the first along with a great number of bacteria to colonize newly fallen leaves. Soil tilth, or the physical and biological conditions of the soil, is important for the incorporation of organic matter into the system. SAP fungi, which hydrolyze cell walls, can help to break down large litters, making tissues more accessible for other detritivores and microorganisms. The effects of macro-fungi will be more pronounced at later decay stages when lignin becomes an important consideration. Numerous species of higher fungi invade living leaves, penetrating directly through an epicuticle via hyphal emanating from the spore or conidium, and producing white or brown thin crust-like, circular kernels or dark lesions. Broadly, basidiomycetous fungi were found to have a significantly greater effect on their hosts than either ascomycetes, or pyrenomycetes or anamorphics. At a continental scale, macro-fungi display strong phylogeographic structure, in contrast to expectations that the chitinous spores should be widely dispersed by atmospheric currents. Fungi also have strong mutualistic relationships with many organisms. This is especially true of higher fungi, where commensalism, parasitism, and mutualism are seen. Bird's nest fungi release their spores protected in an outer membrane called a peridium. Upon hydration, the internal membrane swells until the spores are all simultaneously toppled out, shooting them for distances up to 1 centimeter. Fungi also form illustrious relationships with algae, both are necessary for the survival of Lichenics. These relationships are extremely varied. Parasitic fungi release secondary spores that only grow on already infected leaves, providing potential competition between pathogens. On the other hand, with parasitism, a few pathogenic fungal spores can germinate on a plant surface and cause tissue damage that favors the fungus. Many ascomycete fungi interact with plants, one very obvious way is as pathogens. On the other hand, in many endomycorrhizal associations, fungi penetrate roots and facilitate the uptake of nutrients, especially phosphorus. At least 8,000 species of higher fungi trap nematodes in species-specific spring traps. Another window of opportunity for a parasitic fungus is associated with the formation of lignin

defensive tissues by a plant in response to a wound. Strong mutualistic relationships exist also, for example, with Mycorrhizae. More than 80% of vascular plant species form mutualistic relationships with this symbiotic soil fungus. Furthermore, emerging evidence suggests that many processes are shared between dinoflagellates residing in corals and AM fungi colonizing plant roots, resulting in competing planetary health effects. This can be collapsed into a couple of pillars if the reader is not familiar with the data, AM fungi provide P to plants and receive photosynthates in return, increasing plant fitness in the meantime. Conversely, coral dinoflagellate-zoosporic fungi exchange inorganic P via fast-absorbing adenosine phosphate and are host to aerobic and fast-growing common bacterial genera causing inflammation. This issue is exacerbated in coral reefs as they are situated within oligotrophic areas and consequently show higher risks of exposure to elevated sea-surface temperatures due to their reduced cooling from turbid discharges. Evidently, heatwaves can trigger the disruption of coral-dinoflagellates synthesis resulting in temporary, and eventually irreversible, symbionts' loss, i.e., the so-called coral "bleaching." To understand the fungal contributions to direct or indirect mutualism between organisms is a fascinating theme that spans micro to macro-organisms. Fungal symbioses with a broad range of hosts, such as plants, algae, corals or animals, can further affect ecosystems and hasten the evolution and adaptation of interconnect afflicting organisms at multiple levels.

4.1. Symbiotic Relationships

There are many ways in which fungi interact with other organisms in their environment. In fact, complex symbiotic relationships are often formed that cross both taxonomic and ecosystem boundaries. While the most well-known symbiosis might be the mycorrhizal symbiosis forming between the vast majority of plant species and fungi, fungi can interact symbiotically with a slew of other organisms as well, from the cyanobacterial phycobionts in lichens to the yeasts blooming in fruit. Apart from their frequency, such partnerships showcase the versatility of fungi. The types of symbioses can be divided into three broad categories: mutualism, where all partners benefit; commensalism, where one partner benefits without influencing the other; and parasitism, where one partner benefits at the expense of the other . Fungi can exhibit all three forms of symbiosis, forming intricate ecological lattices with their partnering organisms.

Mycorrhizas, the most prevalent form of mutualism on earth, are by far the most intensively studied type of symbiosis involving a fungus. A mycorrhizal symbiosis forms between the hyphae of soil-inhabiting fungi and between the roots or, sometimes, the above-ground parts of plants. The complex hyphal network vastly extends the nutrient-absorbing compartment of the plant roots, and mycorrhizal

association generally enhances plant health by allowing nutrient exchange, ameliorating water relations, aiding in stress tolerance, reducing plant susceptibility to pathogens, and often even directly inhibiting pathogens. In return, the plant supplies the fungus with reduced carbon in the form of sugars, storage-products, or other metabolites. It should be noted, however, that the involvement and benefit of fungi and plants are more intricate than just this brief elaboration might suggest. Mycorrhizas generally grow in an intimate and symbiotic organic mixture known as humus, but the importance on a global scale is hard to overstate. It has been suggested that without mycorrhiza plant-dominated land systems could not have evolved on Earth. It has thus been asserted that mycorrhizas are the unifying theme in global terrestrial ecology and that they affect the functioning of entire biomes and whole ecosystems. In aquatic ecosystems, mycorrhiza-like associations form in some wetland macrophytes, but the rate of successful colonization of a potential host is generally lower, and the importance of these relationships in the functioning of ecosystems is less understood. In marine ecosystems, the formation of a true mycorrhiza is limited, but associations akin to some mycorrhizal functional traits form in seagrass.

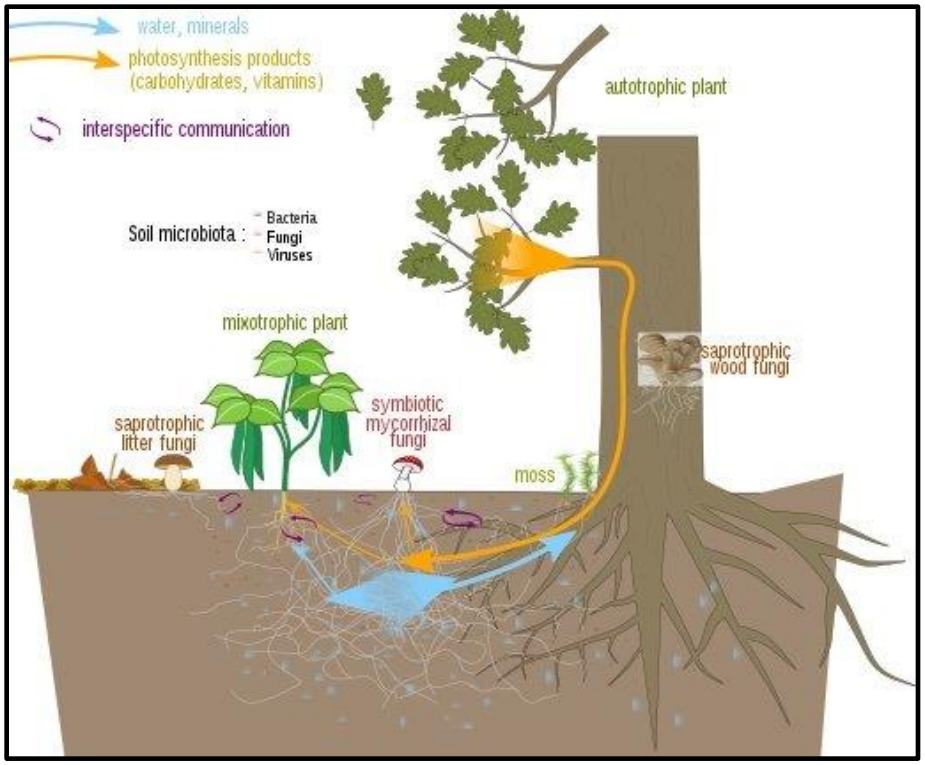

Figure 6: How Fungi & Plants Benefit One Another.

5. Applications of Mycology

Mycology is the study of fungi. It is a scientifically established field with a long history that is likely to have been initiated by the first agricultural endeavors of early human societies. In contrast, the field of microbiology has only recently become established.

An effort is made to address the former—the study of fungi. 10 of the approximately 94,000 published studies on fungi between 1966 and 2000 are summarized, selected because they capture the breadth of current research. They do not capture the scope of research, however, that jobs as work with yeasts, lichens, and mycorrhizae or the extreme diversity of fungi: an estimated 1 million species compared to 250,000 species of flowering plants.

Fungi are possibly the oldest complex life forms on Earth. As with bacteria and viruses, fungi interact with all currently known life forms. As decomposers, they allow some plants to complete their life cycle. Ancient humans quickly learned to use the sparks created by the spongy red fruiting bodies of the conk, Ganoderma applanatum, for human activities such as the therapeutic burning of incense. They are the most common cause of death in humans and their livestock, responsible for 70% of all cultivated plant diseases (Lange, 2014). On the other hand, edible mycophagy has a long tradition in human history.

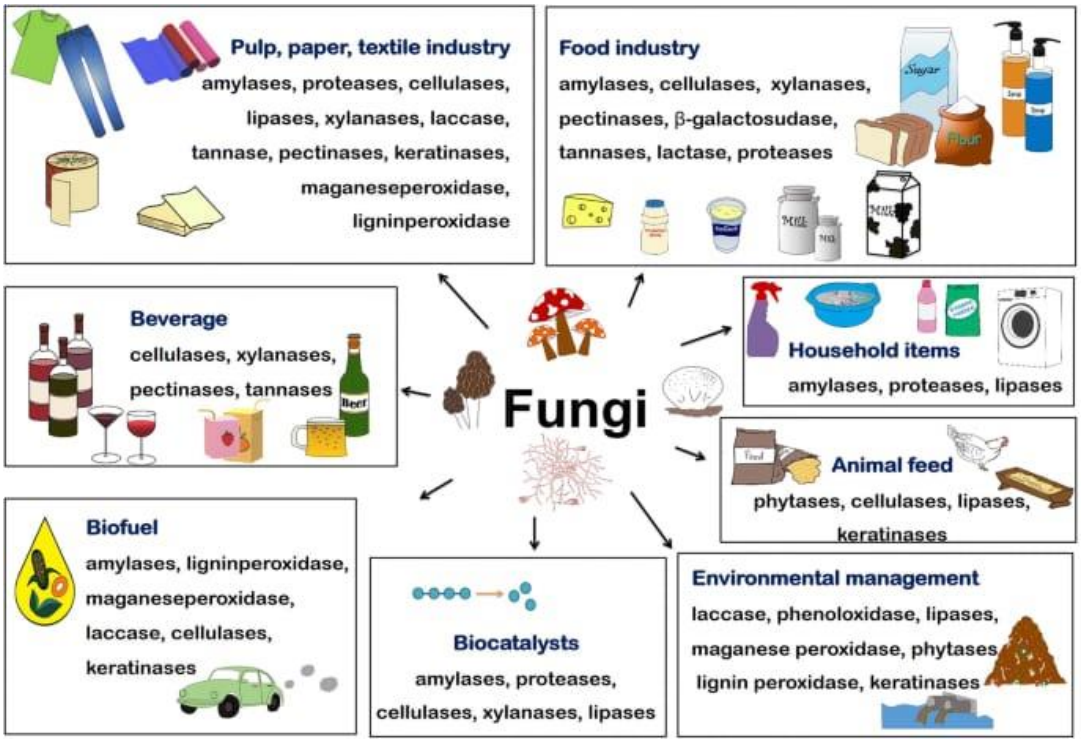

Figure 7: 50 ways we can exploit fungi industrially

Reached an age of mouldy bread, the isolated synthesis of natural chemically modifying substances produced by fungi, and detailed the structure of mouldy synthetase. Living in an era of rapid development of techniques, including the emergence of the synthesis machine with a computer, has led to the emergence of molecular biology mycology after the study of biologically interesting metabolite synthesis machinery. New fields, such as biodiversity research and the exploitation of fungal genes, have emerged, focusing on the exploration of biologically interesting fungi.

5.1. Medicinal Uses of Fungi

For most people, the word "fungi" typically brings up images of mushrooms and yeasts. However, fungal diversity is vast, estimated to contain at least 2.2 million different species, and the functions that fungi can carry out are even more diverse. The field of mycology is dedicated to the study of fungi and encompasses researchers from diverse fields such as biology, chemistry, and even meteorology. This article aims to briefly introduce some of the key areas of mycology, very much like the tip of the iceberg, and provide a more in-depth exploration of perhaps less well-known aspects of mycology (e.g. plant pathogenesis and symbiosis, and the use of fungi in scientific research).

Two key fields of mycology are largely symbiotic and beneficial to fungi, looking at the various ways in which fungal organisms interact with other species and exchange resources. Some of these interactions are antagonistic, such as pathogen-host relationships that underpin a devastating amount of fungal diseases across many crop species (Conrado et al., 2022). On the other hand, mutualistic relationships have significantly shaped biological evolution and allowed life as we know it to flourish. In mutualistic interactions, both species involved in the relationships benefit. Fungi have established these relations with myriad other organisms, with arguably one of the most important mutualistic interactions existing with plants.Before getting into the weeds of studying plant-fungi interactions, it is of utmost importance to first have a firm understanding of the basic science. The description of fungal morphology and life cycle above would fall under basic mycology. Additionally, having the tools to study fungi is absolutely needed before one can perform research and make discoveries. Thankfully, those tools are largely available, easy to use, and relatively inexpensive. The remainder of this article will dive into more depth on the development of curricular and experiments to study plant-microbe interactions, with a particular emphasis on fungal pathogens (Dai et al., 2021). But this also is a Special Issues devoted to the field of mycology, more generally, as this work is just a small piece of a much larger science.

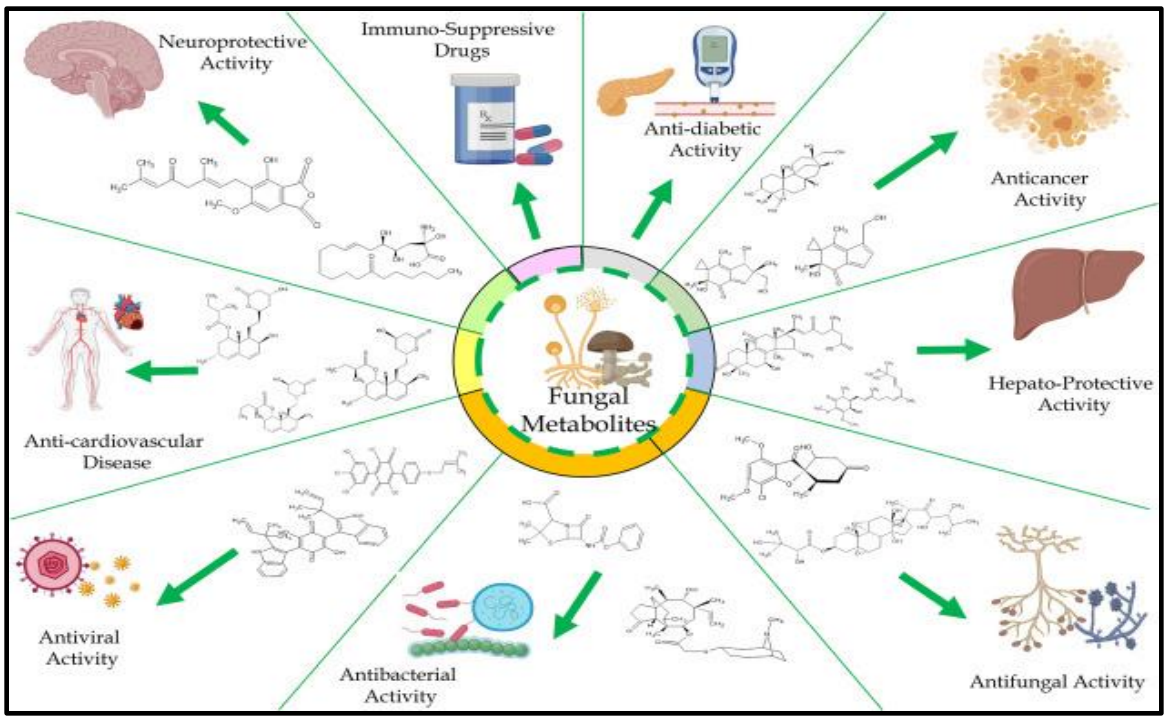

Figure 8: Fungal metabolites and their importance in pharmaceutical industry.

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Chapter Six

Protozoa

1. Introduction to Protozoa

There is a diverse group of organisms that falls within the classification of protozoa. This group is defined by a single cellular structure with eukaryotic cellular arrangements as well as separation from the algae and fungi that also exist in this stratum of the biological tree of life. Within the scope of these simple looking creatures is a vast array of complexity and they have very large impacts on various ecological systems and human health (T Larson, 2023). Historically protozoa are considered to be small animalcules and represent an animal-like protist. When the microscopes used to observe these cell sized organisms were further refined, it became apparent that these various simple looking organisms were actually very complex and therefore were considered proto-animals. However, as more molecular studies have been conducted, it has been recognized that this group of organisms is an artificial grouping of many diverse organisms. Scientific description, classification, and biological knowledge is still in its early stages of development. The research and volume of scientific information on these complex single-celled organisms is vast and varied, so this document will provide a comprehensive outline of a course in protozoology.

Protozoa are a monophyletic group with a single common ancestor. This is clear from both molecular and morphological data even though studies disagree concerning the most basal group of protozoa. However in a broad sense all eukaryotes can be divided into two groups with each group retaining only part of the prokaryote endosymbionts shared by the host for its mitochondria. The most basal group are the Archaezoa, which lack mitosome and have as mitochondria hydrogenosomal derivatives. But if the hosts lost their hydrogenosomal before the descendants of each group diversified, it would obscure the monophyly of eukaryotes. Anyhow their symbiotic ancestors are so deeply rooted that they may well defy attempts at classification. In contrast, all the groups in Corchuelo's proposal fall within the range of typical archaeozoan diversity. Nevertheless it is straightforward to pull one group Sarcomastigota out of the Archaezoa. It is coherent both in terms of what they have in common with each other and what they share with the rest of eukaryotes.

2. Classification of Protozoa

Protozoa, a paraphyletic group of eukaryotic organisms, includes a variety of living patterns. Among them are free-living forms in the water and soil, as well as in the bodies of plants, animals, and other protists. A systematic approach involves arranging these organisms in an order and a hierarchical system, ideally using a multiple approach. Thus, the classification usually depends on the organization, and only a phylogenetic system is a preferable one. But since a very consistent system of classification has not been established, the arrangement of protozoa is presented based on several aspects. Jointing corresponds to the motion of living beings, From this point of view, there are two groups of protozoa: 1) those that move freely; 2) those that do not need movement. Free-living forms, which represent the vast majority of protozoa, and among non-malicious forms predominate (T Larson, 2023). Locomotor features are very diverse, including cell changes, cilia and flagella. In parasitic forms, there are basically two groups: 1) those that distribute among habitats and do not need parasites; 2) those that can move with the help of a parasitic host, such as ciliates of the Ruminites group.

According to the mode of nutrition, the regulation of protozoa can also be divided into two parts. Many possess chloroplasts and synthesize photosynthesis. Algae such as phytoplankton are abundant in various freshwater habitats and play an important ecological role. Among the fungi, there are forms that mainly feed on low organic matter from complex internal parasites and macerates in the Biotrophic extracellular digestive form. The remaining harmful forms are classified into groups of food bacteria, with two possible sub-groups: 1) that feeds on the living bacteria and is mainly located in the water; 2) predation of living bacteria spirals, such as erythrocytes, completely engage them with care (Zhang et al., 2022). These groups also have subclasses. The classification framework of Percival can be used to determine harmful forms at class level. In general, there are six basic categories if you explore all the possible variations streamed or absorbed. Consistent paper suggests classes of harmful forms that are separate on the basis of motion and feeding habits, and can facilitate the identification and study of species.

2.1. Based on Locomotion

Protozoa are unicellular, microscopic, eukaryotic organisms, usually found in moist habitats. The choice of moist habitats is mainly due to problems of locomotion that this group of organisms experiences in the dry state, although parasitic forms have adapted to live in relatively dry environments. Protozoa exhibit an extraordinary diversity of structures and forms, and they have been classified in various ways, on the basis of different criteria. Protozoa can be classified according to various types of criteria. The main protozoans are classified according to two more or less

traditional criteria: according to their modes of locomotion and according to their habitat (freshwater, saltwater, and hematic). The way protozoa move is directly dependent on their structure, particularly on the presence of one or more of the following. First is the flagella, thread-like formation used by many cells to propel themselves through media. Flagelates are classified by the complex of flagella, group to which they belong. The classification of flagella is also performed by the presence of additional formation, such as coccal flagella which consists of thread, giving the impression of ciliates. Right now, coccal flagella and spiral flagella are regarded as the same and are in a group of the abdomen, while coccal flagellates are classified in the flagella group. Flagella can be located in the catching moment, extend only forward, or spread out in the abdomen similarly. Drawing the flagellae from different groups of groups show a great diversity of structure. This time the threads are attached with the membrane at the edge of the beet on the later edge and are connected with the zigzagoon. The flagella are moist-shaving membrane structures, in which the filaments extend tebally into the plasma beagle, covering the whole surface filleted with plasma membrane. These flagella can be in the form of two or more parallel, long, and short layers. Some flagella are covered by the clothes of D adhesive substances, covering the cell like a mau snak. Flagellates commonly show holopharg or phargocytic but some types of plant one photosinths and follow one osmotic paths of nutrition. The flagella in food equipment filter the food from the medium that is flowed and accumulated on the flagella. Examples of flagellates include leishmaniasis leishmania, giardia, trichomonas, euglena.

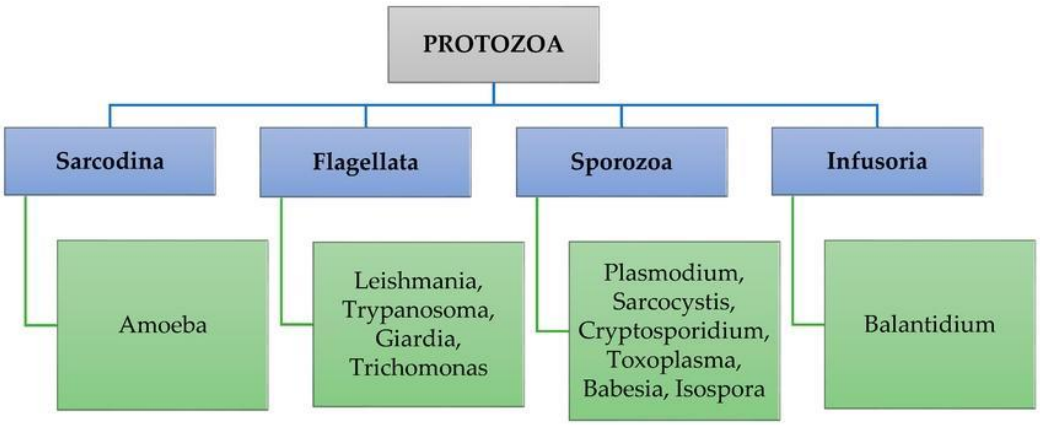
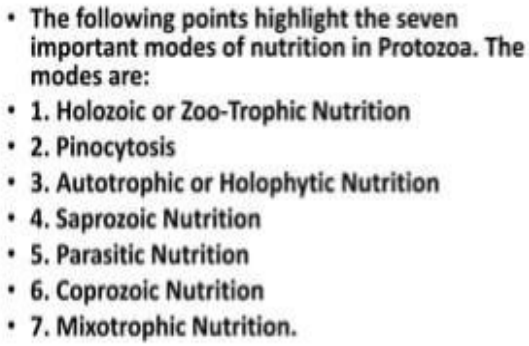


Figure 1: Classification of protozoa.

2.2. Based on Mode of Nutrition

Protozoa are classified according to their mode of nutrition. The classification of organisms is important since the principal community roles are the result of the organisms' life strategies. Therefore classifying organisms according to their means of acquiring energy and synthesizing protoplasm is fundamental to understanding their ecological roles. Based on how they obtain their food, protozoa are divided into two categories: autotrophic and heterotrophic organisms. Autotrophic protozoa contain plastids and perform photosynthesis for extant use, while heterotrophic ones acquire energy and biomass from dead or living organic matter. There are far more types of heterotrophic protozoa, and these organisms are those usually thought of as protozoa. The feeding strategy, a subset of the trophic strategy, of an organism, is how that organism opts to extract food particles or soluble food resources from bulk liquid media (F. Edwards et al., 2023). The most important mode of a feeding strategy for an organism in any given natural assemblage depends largely on the abundance of that edible item in the assemblage. There are three basic modes of feeding strategy exhibited in protozoa: phagotrophy, osmotrophy, and absorption. The term phagotrophy is applied to filter-feeding on particles captured from the environment. It is treated as covering all modes of feeding in which particles of diatom-size or larger are engulfed. Osmotrophy involves the intake of liquid containing dissolved organic molecules. A protozoan that feeds purely by the osmotic uptake of organic molecules is considered as a fluid-phase feeder. Even if the source of the organic molecules is quite particulate, the feeding mechanism is termed osmotrophy if the liquid phase is consumed with the solute. A protozoan that ingests particles and solubilizes the ingested material to derive dissolved organic molecules is classed as a mixotroph with respect to trophic strategy. An absorptive feeder is an organism that ingests particles containing organic molecules in suspension, allows or catalyzes partial release of solubilized organic matter by lysis or digestion of the particles, but does not ingest the released molecules. As with osmotrophy, particles ingested by an absorptive feeder are not fully digested and are water, or a similar liquid, and molecules dissolved in the liquid leach out of the suspended particles. Rapidly-growing protozoa generally have high biosynthesis and digestion rates which in turn require high energy acquisition. Redistributions of C and N occur between functional types in the treated pelagic foodweb model. Subclasses of the models with N2 fixation were steady-states with respect to the levels of the two major currencies, PON and DOC, while systems without N2 fixation are open with respect to these quantities. Homogenates of the models without autotrophy were analytically tractable, leading to local asymptotic stability and consideration of biomass ratios in the context of power-law

relationships. Although simple, results obtained from the models serve as a basis for more detailed investigations of pelagic food web dynamics.



Seven important modes of nutrition in protozoa.

3. Morphology and Structure of Protozoa

As the most basal group of eukaryotes, protozoa are evolutionarily very important and the models of several protozoa, specifically ciliates and kinetoplastids, had been and are being investigated. Protozoa are a very diverse group of organisms; therefore, this group has many important representatives outside the more frequently studied ciliates and kinetoplastids. In view of this, this section gives an overview of biology of protozoa and also tries to define some outstanding problems and point directions for the future researches.

Films show the dark silhouettes of protozoa appearing like shallow lakes, rapidly changing their outline. The light path avoids them, being reflected, as from mirrors; the image is distorted like in fun mirrors. In controlled experiments the protozoa in their turn apparently avoid danger that is due to the navigate well. It is said that they take evasive action. When they stop they circle around a place where they have just been, much like a standing helicopter or a dirigible. As is for three billion years, protozoan cells float constantly inside. They change shape in rapid and complex ways. Occasionally show waves of yellow-green fluoresce ordered like gardeners rowing flower beds. It is difficult to come up with other in biology that would boast such a variety and elegance of easily observable behavior of single cells.

At the same time the majority is poorly understood, underappreciated, or even completely neglected. Improving this situation could clarify biology generally and provide it with new material systems for developing, challenging, and broadening the theoretical framework.

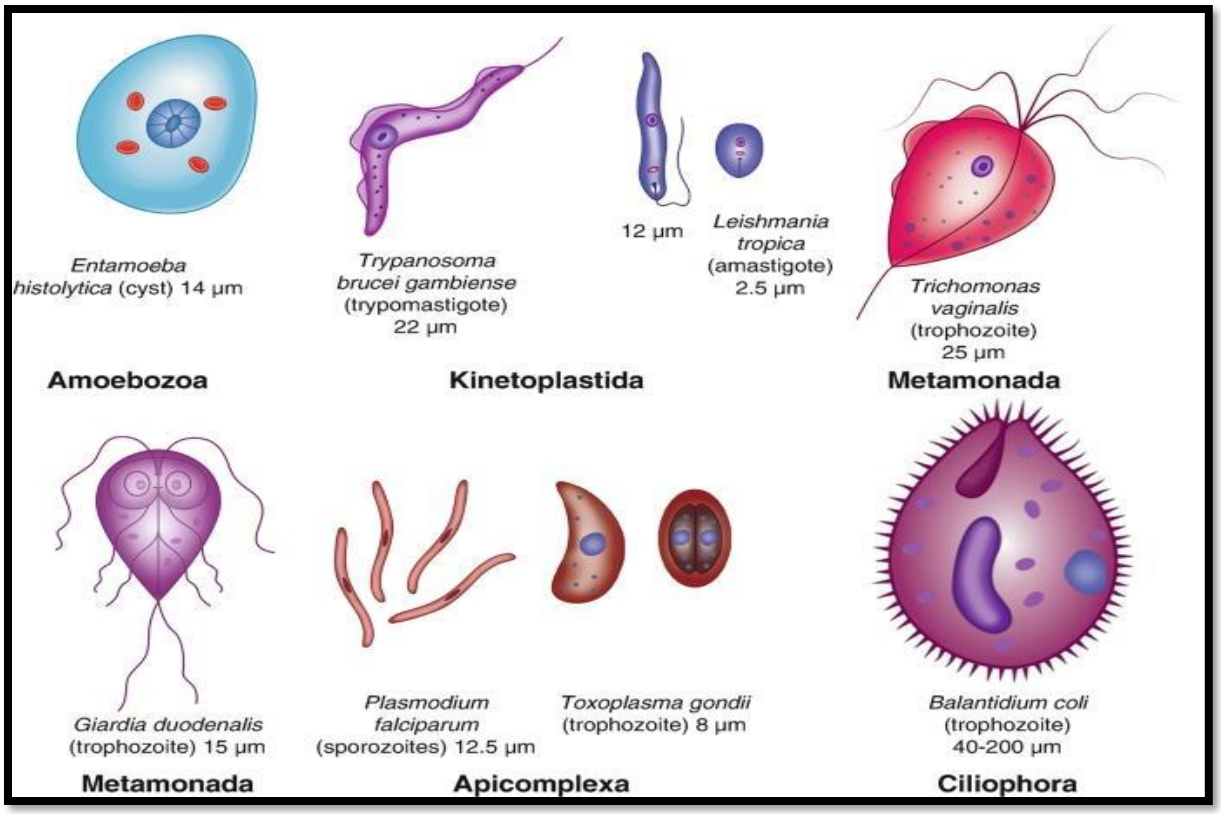


Figure 2: Classification, Structure, and Cellular Components of protozoa.

3.1. Cellular Components

Protozoa, a paraphyletic collection of unicellular and simple multicellular eukaryotic organisms, were first described by Goldfuss in 1818 using some of the smallest microscopes. The cellular components of these creatures have since been detailed by many scholars using a variety of state-of-the-art techniques such as simple and compound light microscopy, scanning and transmission electron microscopy, immunohistochemistry, and three-dimensional reconstruction of ultrathin sections. Protozoa are represented today by four phyla in the kingdom Protista, which is a paraphyletic conglomeration of mainly unicellular eukaryotic organisms. Going to the diversity of this kingdom is a variety of cell structures and functions of its organisms. Though these structures and functions form a highly integrated and interdependent system..backends resembling the multicellular organisms that evolved from it. While lacking the specialized organ systems of plants and animals within the kingdom, bio- and protozoa typically have all the membrane-bound structures and other cellular structures of "higher" eukaryotes (T Larson, 2023).

The nucleus is surrounded by a variety of double-membraned structures, a defined Golgi apparatus with associated vesicles, both smooth and rough endoplasmic, a variety of inclusions and food vacuoles, mitochondria with sharply defined cristae, peroxisomes, and distinctive phagosomes, lysosomes, finely interconnecting

membranous cisternae, many cellul concavity forms. One example of this morphological integration is the contractile vacuole. This organelle found in many freshwater protozoa is a good example of how different membrane-bound structures ensure the survival of an individual protozoan cells of Sudoku regularly for food, water, and gas exchange with the environment.

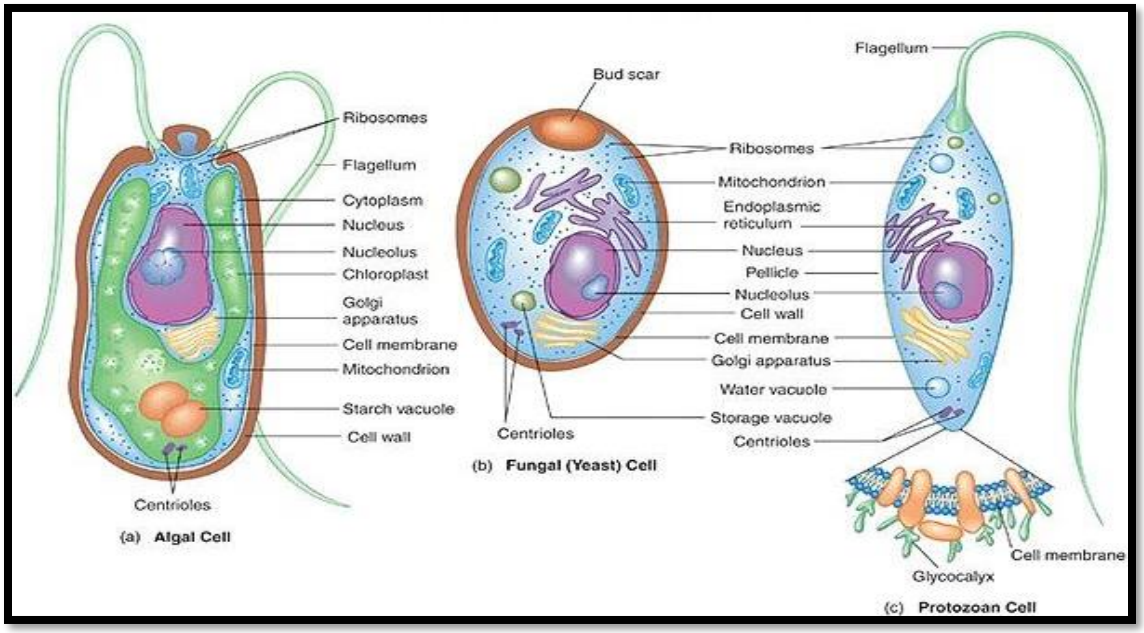


Figure 3: Cellular Components of a)algal cell b)fungal cell c)Protozoa cell.

3.2. Specialized Structures

Numerous specialized structures assist in some of the many functions that are necessary for protozoan survival as free-living plankton. Structures such as cilia, flagella, and spiny protective coverings that must be manufactured, maintained, and sometimes manipulated for feeding are essential for picoplankton survival (John Wheeler et al., 2013). Such protozoan functional complexity has adaptive significance in nature's competition, as it is analogous to a variety of boat sizes and shapes competing in a race that has no strict course marker buoys. The chances of a fast and efficient voyage on one course are greater if one possesses special instruments or systems that accomplish sensibly observing free direction and distance to location objects. For protozoa in marine environments, these structures include a variety of such made-to-order watercraft and their boat speed indicators, in the form of locomotory fusiforms and flagellate cells (M Pinskey et al., 2022). Also essential are position controls in the form of sensitive direction-and-speed-to-control converters. These structures include the ability to make a stop or turn in response to diverse sensory stimuli of the salt water.

Several environmental navigation examples show the involvement of cilia as chemotactic organs in Euglena sp. of the diverse protist phyla. Its limited helical course in alternating directions during both photo- and chemo-taxis is accomplished through different stroke patterns of a longitudinal band of surface cilia. Another study is of a diverse parasitic flagellate phylum, Kinetoplastida sp. has been discovered with the sensory equipment and responses to avoid noxious stimuli. These environmentally entrained behaviors are population selectivity factors of protozoa and might show different range abilities among planktonic species that assist in maintaining different species of this long-established phylum in separate marine niche related to temperature, salinity, food, etc., which is an incomplete ecological knowledge of plankton phyla. Applications of these and additional studied specialists of adaptive survival structures and processes of protozoa assist in obtaining a clearer perception of their present geological niche, in revealing protozoa fossil structures, and in shedding new insights on their ancestors encoded in the oldest metazoan genomes. This research area has broader evolutionary implications and potential aspects of which only a few are currently contemplated.

4. Reproduction and Life Cycle of Protozoa

The reproduction of protozoa reveals an interesting spectrum of evolutionary alternative strategies. Such a diversity is in a way one of the main keys of the general adaptive success of protozoa which are able to populate the widest range of habitats from deep marine waters to the rhizospheres of plants or of terrestrial animals. Protozoa, whose name means ‘‘the first animals’’, may exhibit all types of cell division and elaborate a great number of ‘‘strange’’ specialized gametes but they may also break all the rules with a cycle built up only on microgametes and macrogametes of exactly the same morphology. In the wild – or rather, in the case of a dilution able to mimic wild environmental condition – for most protozoan species, the growth rate of the population firstly increases in a rather moderate way due to the availability of the necessary food. Apart from food, various environmental factors (physical, chemical, biological) may affect the population growth in a directly or indirectly way, by limiting or disturbing the reproduction and/or the growth, by inducing a stress, a competition, a predation, a loss or a migration of individuals, etc. On the contrary, in a favourable food supply case, some protozoan population dynamics may appear exponential-like with a significant increase of the growth rate. At the end of the proliferation and due to various factors, the saturation is reached, the consumption of food exceeds its renewal, some competitors may be abundant, some grazers may concentrate, some predators may find easily their prey, and so on. Then, the growth rate decreases significantly and the actual conditions suggest an accumulation of stresses and hazards to protozoa. In some rare cases, very particular species and/or environmental conditions may allow a more or less

constant maximal growth rate over a quite long time before the saturation. Generally, the saturation of the population leads to a total individual revival or a severe and durable decline; such events may have important consequences at the population scale, such as the extinction of the species. At the end of every other cases and after returning in less stressing conditions, the reproduction of resting cysts may be a good point to preserve the genotype and to try to survive, in a spontaneous optimistic approach, waiting for less worse conditions in order to germinate and to proliferate again. On the opposite, the most hazardous and less efficient strategies may lead to a total loss of the genotype and consecutively of the adaptation of the species (D. WEEDALL and HALL, 2015).

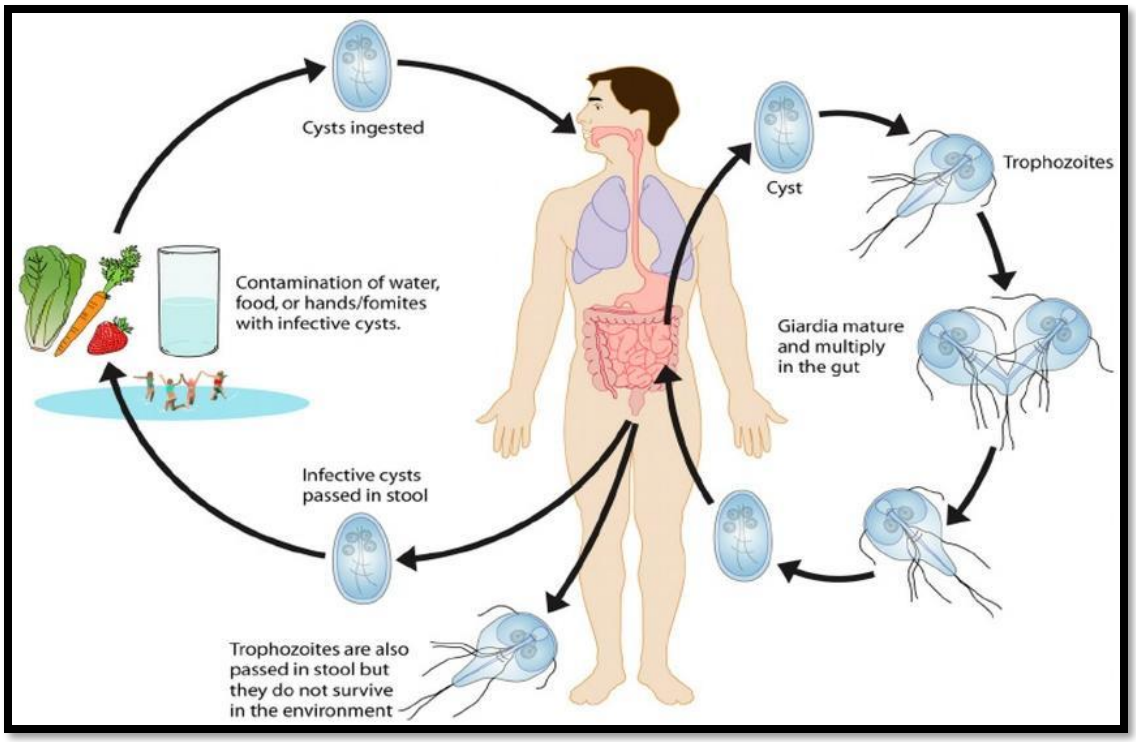


Figure 4: Life cycle of Giardia

5. Ecological Role of Protozoa

Protozoans are a significant component of microbial populations and play key ecological roles in many ecosystems (Dupont, 2017). Protozoa occur in a variety of habitats ranging from the soil environment to aquatic ecosystems. Fresh water, sea water, marine sediment, and many other habitats are harboring a diversity of protozoan species ranging from flagellates to larger ciliates to naked and testate amoebae. While known members of the protozoa are relatively few in number (about 20,000 described species), estimates of the total number of species range – depending on assumptions concerning the as yet undiscovered species – from less than one million to tens of millions. In the soil environment, protozoa are found at

high population densities compared with larger soil organisms. The densities of protozoa in the earth's microbial communities can be comparable to the density of bacteria, being up to 10^6 flagellates and 10^5 ciliates per gram of unfrozen soil in certain habitats.

Protozoa are considered as important components of many ecosystems because they act as both producers and consumers within food web system (Zhang et al., 2022). While performing as producers, they involve in primary production through symbiotic relationship with algae, lake productivity and energy flow in aquatic ecosystems. Thus, they are major players in the overall primary production and are responsible for the movement of carbon up the food chain system. In this respect, they are critical in the determination of the trophic structure and are an important consideration in the ecological roles of protozoa in the system of nutrient cycling, decomposition, and energy transfer. Protozoa are considered as agents for organic matter and nutrient cycling and are also thought to contribute to the stabilization of microbial populations in ecosystems. Thus, although there is a general feeling about what the roles of protozoa are in soil microbial systems, there has been little attempt to tease apart these effects and elucidate the true influence of protozoa on ecosystem.

5.1. In Ecosystems

This subsection emphasizes the role of protozoa in a variety of ecosystems and their contributions to ecological stability and function. As important components of the microbial community, protozoa are essential for niche separation and coordination with other organisms, contributing to helping maintain ecosystem stability and supporting life on Earth (Zhang et al., 2022). Protozoa play an important role in nutrient cycling through breaking down a wide range of organic material, thus facilitating the decomposition and transformation of complex organic substances, and serving as a channel for effective and rapid release of nutrients. Protozoa regulate the growth of larger microorganisms to avoid their continuous proliferation, thereby having positive effects on the structure and function of microorganisms in the system. On the other hand, protozoa provide food sources for predators at a higher trophic level to regulate or balance the system's energy flow and material cycling. In the predator-prey system of microorganisms, protozoa are important prey for flagellates—an effective way to regulate the abundance and composition of the flagellate community, and then indirectly control the balance of the entire microbial predator-prey system. In the terrestrial ecological system, they have been found in the litter cover, the interface between litter and mineral soil, and the root zone. These findings demonstrate that the structure stability of protozoans is positively correlated with the stability of the microbial community during litter

decomposition and suggest that they are contenders for niche separation of fungi and bacteria, shaping and controlling the community composition of larger microorganisms. However, rampant animal agriculture has generated large quantities of manure laden with heavy metals, which are hazardous to the environment and human health. Given the high capacity for accumulation and the role in controlling the release of heavy metals of protists, they show their promise as bioindicators in soil environments. This calls for a comprehensive strategy to restrain the environmental impacts of heavy metals of animal origins to maintain healthy terrestrial ecosystems.

5.2. As Parasites

Being parasites of another organism, protozoa feed, reproduce, and live in close association with the host, and often cause a considerable impact on the health of the host species (Piña-Vázquez et al., 2012). Protozoan parasites reproduce rapidly using the host organism for food and morphological and cellular substances. Parasitic relationships between protozoa and other organisms cover a wide range of interactions, influencing not only individual health, but also overall ecosystem balance. More than 100,000 species of protists are known; many of them are parasites or are involved in symbiotic or mutualistic relationships. Some well-known examples of parasitic protozoa include the cause of African sleeping sickness (Trypanosome gambiense), Chaga's disease (Trypanosoma cruzi), and the causative agents of toxoplasmosis and malaria (Toxoplasma gondii, and Plasmodium species, respectively) (Guillén, 2023). However, the majority of protozoan parasites are unknown, with what they infect and how they interact largely a mystery.

All infections start with the parasite somehow managing to infect a host, often via a vector or contaminated food and water. Some of the most notorious human parasites, be they the viruses causing COVID-19 and common influenza or Escherichia coli and Toxoplasma gondii protozoa, may get a ride from one host to the other without the host developing a noticeable illness. Once inside the host, the sickness begins through the processes of growth and reproduction of the parasite. Protozoan parasites, while living a seemingly peaceful life, are in fact constantly at war with the host. This conflict can have a considerable socio-economic impact on society, both globally and on a smaller scale. On a more local level, parasitic diseases can severely affect the health of livestock or wildlife, upsetting the ecological balance and incurring economic loss. Although protozoan diseases are often curable using anthelmintic drugs, the treatment and diagnosis can both be very costly. Only careful and thorough epidemiological research can help to reveal at least some of the mysteries behind these parasitic relationships. Inevitably, as ecologists explore the many facets of life, they must investigate the seemingly darker side of protozoan

ecology. Choosing from the vast array of host-parasite relationships, this section will display a zoological selection of the impact parasitic protozoa have on the environment and a wider perspective on the advantages that host and parasite gain from these damaging associations. On an individual level, while the protozoan parasite benefits from the immediate availability of a stable food source provided by the host (a commensalist relationship), infection may ultimately result in host death, often leading to the death of the parasite. From a biological perspective, this simplistic view does not take into consideration considerable array of adaptive strategies of both the protozoa and its host, leading to the great diversity and complexity of parasitic relationships. On the evolutionary time-scale, the host-parasite relationship is not simply one of a violent and destructive nature. Endosymbiosis is a relatively well-known outcome of interactions between protozoa and their hosts, leading to the creation of completely new and, from a biological perspective, more successful organisms. Furthermore, parasitism is not a simple predator-prey relationship; it is far more complicated than that. The strategy chosen by the protozoa depends on the adaptation to its physical and biological environment. There is probably no mechanical cause for the evolutionary transition from free-living to a parasitic lifestyle in protozoa, rather it is simply a question of finding the most effective strategy in growth and reproduction.

6. Protozoa and Human Health

This section investigates protozoa from the perspective of human health. The emphasis is placed on the relationship between protozoa and human health, in which it discusses the impact of infections by protozoa and what is known about the diseases caused by protozoa. The diseases considered most important are then reviewed, giving an outline of the life cycles of some of the important disease-causing protozoa to facilitate understanding of the modes of transmission and the routes taken by the infective stages within the human host. The focus is on the implications for human health, and positive contributions made and outcomes obtained by biomedical science are discussed. This broad topic is then broken down and discussed largely in terms of the health implications of protozoa.

Protozoans are complicated eukaryotic protists and there are considered to be about 65,000 described species. They are very important in terms of ecology, particularly as grazers of bacteria in the soil, lakes, rivers, and the oceans, but also parasites of plants, invertebrates, and cold-blooded vertebrates. Diseases caused by protozoa in human and other animals are also significant from ecological and evolutionary perspectives. They do not survive desiccation for long when outside the host and are usually transmitted between hosts by some kind of living vector or by water, often via the fecal-oral route. Protozoan diseases are most often

associated with low socio-economic health, and morbidity is a common outcome of the infections. The origins of protozoan infection can often be traced to eating contaminated food or drinking contaminated water, and the protozoa have a role to play in the contamination of water and food. All these mean that protozoan infections are very difficult to control. Animal models, with a few exceptions, are not good predictors of what happens in the human host, and there are drastic differences in how biologically machines transmit pathogens. Current antiprotozoan drugs focus largely on hitting the pathogen's synthetic or metabolic pathways, in a topical way, often causing harmful side effects from the toxicity of the drug, which is attributable to the fact that the target of the drug is often quite similar biologically to the host vertebrate.

6.1. Disease-Causing Protozoa

Besides bacterial and viral pathogens, protozoan parasites can also infect the intestinal tract and have been implicated in several serious diseases. Such diseases are collectively responsible for substantial morbidity and mortality and, thus, have a huge economic impact on global health systems. Herein, infection with the most common, widespread, and economically important intestinal protozoan parasites will be discussed. Specifically, the prominent status of Giardia lamblia, Entamoeba histolytica, and Cryptosporidium species as aetiological agents will be examined. These organisms have been chosen for discussion based on their proven importance as the causative agents of waterborne and foodborne outbreaks among developed nations, as well as their seriously debilitating effects among underdeveloped countries. All three protozoans are zoonotic pathogens. Infection with this trio of parasites has been shown to elicit very effective host immune responses that are incapable of providing the host with long-term protective immunity (Hemphill et al., 2019).

It is estimated that protozoan parasites are currently responsible for more than one million deaths per annum, with the majority of this mortality occurring among children and the immunocompromised (Ghosh et al., 2019). The individual clinical effects of infection with G. lamblia, E. histolytica, or Cryptosporidium species depend on the parasite load in the host, pathogenicity of the strain, the nutritional and immune status of the host, pre-existing infections, and the chemotherapeutic treatments employed. The most common clinical manifestations include abdominal cramps, bloating, weight loss, and an overall decreased quality of life. In the developed world, however, the potentially most devastating effect of such protozoal infections is the slowing of normal childhood cognitive development. This last side effect is particularly pronounced if the protozoa are acquired in early life – an unfortunately common occurrence among children in the developed world, as no

host age group within underdeveloped countries can be considered “safe” from such infectious incidences. Vaccines are not available against this trio of enzyme-deficient organisms, and chemotherapy has had a notoriously poor record of both success and patient compliance. Nonetheless, the threat posed by these formidable parasites has spurred efforts to develop new control strategies such as extensive surveillance programs that have, as one consequence, led to a superficial understanding of some of the basic biological processes of G. lamblia, E. histolytica, and Cryptosporidium species.

6.2. Protozoa in Water and Food Contamination

Despite the low awareness of naturally occurring contamination with protozoa in drinking water and raw food supplies, there is a potential health risk of their presence and prevalence in water and edible plants and animals. The contamination of drinking water by Cryptosporidium parvum or food with Cryptosporidium spp. may lead to disease outbreaks. Also, the improper handling and consumption of fresh or minimally processed fruits, vegetables, and cereals contaminated with the infective stages of Cyclospora spp., G. intestinales group, or Isospora belliii. may result in an increased risk to human health and cause new water- or foodborne disease outbreaks (Siwila et al., 2020). Consumption of untreated or poorly treated drinking water for drinking, cooking, or washing foods is the major cause of contamination with protozoa. Prevalence of contamination is higher in the water sources of rural and periurban areas, where the treatment of water is poor or to facilities absence. There is a strong relation between waterborne outbreaks of diseases and contamination of water by viruses, bacteria, or protozoan cysts and oocyts. Nevertheless, the majority of waterborne outbreaks are caused by Cryptosporidium and Giardia (K Pandey et al., 2014). Drinking top quality drinking water will be an avoid of many illnesses, and having useful knowledge with respect to consumer products needed to adequately preserve water quality is vital. As a result, there has rest increasingly noticed demands on tight interdisciplinary analysis in this regard in recent times.

7. Protozoa in Biotechnology

Protozoa are single-cells structures found in a variety of environments. They can be free-living or parasitic organisms, and can be found in almost every part of the world. Commonly, they are related to several infectious and severe diseases like Malaria. However, a new wave of using protozoa in various biotechnological innovations revolutionizes these organisms. Commonly, several species of protozoa are used as bioindicators in the environmental monitoring and the assessment of water and soil quality (M. Rivas-Castillo et al., 2022). After the environmental

accident, which leads to the water contamination of Doñana Cultural Reserve Park, the peritrichous ciliated protozoa were used to assess the environment's recovery.

The science of protozoa has been involved in the bioremediation process of a gold mine in Oaxaca, Mexico. The protozoa eliminated more than 90% of cyanide contamination in 24 hr from mine water. This study opens the possibility to use protozoa and bioindicators for the rapid response to contaminations and for the government's pressure for the industries (Gupta et al., 2016). In the present biotechnology era, there are still various biotechnological potentials of protozoa left. Protozoa can play great roles in agricultural fields, like as part of a biofertilizer, as biopesticide, or even as part of a herd health system. Potential protozoa for the extraction and commercially application of polyunsaturated fatty acids (PUFAs) are proposed such as Marsupicraseospina, Planho, and Spirotrichea. Moreover, protozoa may play a role as a biosensor which can be used as the early detection of deleterious compounds like in the wine and beverage industry. Due to the great potential of using protozoa science in every industry, the ethical code should be established, eg. the welfare guidelines especially the legal guidelines.

7.1. Bioindicators

The protozoan community, which is an important but often overlooked component of microbial ecosystems, has been recognized recently as a potentially reliable set of environmental indicators, reflecting the state of biofilm and structural changes in the system. Just like macroscopic animals, they can reflect the ecological conditions of specific ecosystems through their presence or absence. As bioindicators, specific protozoan species can be recognized and closely monitored for what changes they undergo in communities when ecological conditions also change. Hence, they can serve indirectly as an indicator of water quality or ecosystem health. Protozoan species or assemblages could reflect changes in communities that precede other readily monitored changes due to chemical contamination, agricultural land use, or physical disturbance.

There are certain criteria that make a protozoan species a good bioindicator of pollution: abundance and geographical distribution, easy identification in the field or in samples, known biological properties with regard to pollution, ecological importance. A handful of case studies testing for the seeking of protozoan bioindicators are performed each year across the world, identifying bioindicators that have applications to a wide range of specific pollution events and used successfully in the planning and execution of environmental monitoring. Increasing this set by improving experimental design and especially the selection of taxa to study, will raise the rate of success of environmental activities from the bioindicator perspective. On these occasions, this paper stresses that everybody uses the same

protozoan bioindicators, as multi-trophic monitoring programs adopted consistently around the globe, could detect and thus prevent long-term environmental problems underlying the occurrence of such deleterious events.

7.2. Bioremediation

Protozoa, the single celled eukaryotic microorganisms, have often been overlooked in environmental biotechnology even though they can be valuable agents of bioremediation. In the past, protozoa have been successfully utilized to optimize the performance of activated sludge systems in wastewater treatment, but there is a growing body of evidence to suggest they can do much more. As indispensible predators in all microbial ecosystems, free-living protozoa are able to ingest and digest preys up to ten times their size (Biswas, 2015). Furthermore, large ciliates and amoeboid protozoa can graze on biofilm structures. Their ability to ingest contains a wide range of microorganisms, including filamentous and sulphur reducing bacteria. Furthermore, some species of protozoa are also able to excrete hydrolytic enzymes which are able to degrade the polymers found in biofilms. Thus, bio-corrosion is prevented and the degradation of the floating layer of scum, which harbors undesirable bacteria is promoted. Particularly in biofilm based fixed-bed systems protozoa can decrease biofilm thickness and homogenize the structure heightening mass-transfer in aerobic, anaerobic and anoxic layers. Additionally, there is a fast kill-off mechanism against competitors and fouling agents like filamentous bacteria and ciliates. There are interesting perspectives in Rhizoremediation too. Considerable data is available on how certain protozoa in association with their bacterial preys can degrade recalcitrant aromatic pollutants and re-toxify the soil in question. In such consortia the bacteria oxidatively degrade the toxic organic molecules and take up the nutrients. Both these metabolic end products are inedible by the protozoa. This relationship lasts as long as the food source, the pollutant, remains.

8. Protozoa in Evolutionary Biology

The study of protozoa (or protists) is fundamental to the understanding of life's history. Long overlooked as a group, recent research has considerably clarified the evolutionary history of protozoa and paved the way towards a more secured knowledge of their link with other single-celled organisms and simple multicellular eukaryotes. The origins of many lineages are now known, even if some are still to be securely established (e.g. the exact phylogenetic position of the Microsporidia). The Mesoproterozoic is important in the study of protists because it predates a fine preservation achieved in the Doushantuo Phosphorites of South China and allows microfossils to be collected and assigned to eukaryotic cell structure (Frías et al., 2013). However, substantial microbiota existed before that time, including dino-

and ciliates from the Palaeoprothezozoic Safrania fossil, in Gabon. Independently from the fossil record, molecular phylogenies revealed the high antiquity of some protist lineages, because their divergence time from other clades is early. The origin and further development of the Apicomplexa, including medically important parasites such as Plasmodium and Toxoplasma, have been studied in that respect. Facilitated by long branch attraction artefacts, molecular phylogenies originally placed the parasites in often contradictory positions and suggested an ancient diverging position of the Parvilamonadida and Coccidia, and sometimes deep relationships with dinoflagellates or Microsporidia. However, more accurate studies found that the Apicomplexa are derived from a monophyletic dinoflagellate group comprised of Perkinsus and colpodellids and that the Apicomplexa are specifically related to the Gromiidea (Cercozoa). None of the above studies, geological or biological, address head-on the diversification of the early protist lineages, so that they are used here to some extent to contrast their use in historical studies with more direct evidence from living protists. Nowadays many protozoa are chosen as model organisms in biological research from ecological questions to genome analysis. Fundamental biological concepts such as natural selection, sexual selection, Darwinian fitness, adaptation and adaptationism, species definition, hypothesis testing, phylogeny reconstruction, clades, or monophyly are a few that have emerged from the study of them. In addition and apart from their stricter biological interest, as mate hunters or prey, some protozoa have an interesting influence on animals and humans. As the resurrection of some dead, they have no trivial importance in the economy, health, or artistic works. For all the above reasons and many others, protozoa can legitimately be viewed as key-players, facilitators and contributors in the study of evolution. Indeed, many of the aforementioned questions (and still others) are much easier and/or more directly answered with protozoa than with other groups of organisms. This statement is probably as true for parasitic protozoa as for free-living organisms. The fascinating biology of parasites and the novelties brought by the study of them, however, hardly make them fit for a brief description. But regardless of their ecological, social, medical, or economical interest, parasitic protozoa are common human-testimonials of the evolutionary process and as such of interest. Many questions which are usually raised in evolutionary studies become more accessible by considering the wide spectrum of parasitic and free-living protozoa. What is the origin of species? In this perspective, a number of examples are provided to document the processes of speciation including the geographic or temporal distribution of the sister-species and the role parasitism has played in the speciation process. Another classic topic of evolutionary studies concerns the adaptation of organisms to their habitats. The paradoxal adaptation of enteric protozoa including parasites from marine fish (which have colonized probably independently the enteric out of hundreds of free-

living ancestors) is discussed where ancestral characters persist in evolution in the host-parasite context contrary to the general paradigm. In addition, the concept of a cophylogenetic process is reviewed and the co-evolution of guttural cuckoos (Aves, Cuculiformes) with enteric goseomyid and coccidian gut protozoa is given as a case study. This is unusual in that one of the partners is a chordate allowing conventional cladistic methods to be used. Among the tree of life, protozoa are of course insignificant and recent offsprings, but they are a unique target in the study of evolution. This paper shows that viewing protozoa from the perspective of great evolutionary questions they can no less be considered like 'the best of all the possible worlds'.

8.1. Evolutionary History of Protozoa

Understanding the evolutionary history of a group is crucial in the light of evolutionary biology. Early cellular life forms offer the first glimpses of organisms leading to a range of more complex life strategies. Explorations of evolutionary transitions are commonly tied to the presence of phylogenetically informative characters, those identifiable in the fossil record or based on molecular sequence data. In this regard, the spectacular diversity displayed by the flagellates, ciliates, amoebae, sporozoans, and other phyla of micro-eukaryotes has left scientists with intriguing debates about how this diversity came into being and to which more ancestral eukaryotes these groups can be tied.

There is no doubt that fossils have been instrumental in understanding early divergences among animals, plants, and fungi. However, historical events in the evolution of cellular life forms prior to the emergence of these groups remain for the most part enigmatic in the absence of direct geological records. Yet there is a great potential to reconstruct evolutionary pathways through detailed studies on the underlying molecular mechanisms as well as comparative studies of various cellular features observable in extant lineages. Therefore, researches on the evolution of early cellular life has been carried out in light of fossil and molecular evidence, focusing on the transitions from free-living bacterivory to association with algal ancestors of plants, and later to the evolution of fungi and animals; the adaptations leading to parasitic strategies in the microbial world; and a comparative treatment of shared lineages and common ancestors of animals and their protozoan relatives. At a larger scale, it looks at the major transitions that have brought the wide array of diverse strategies microbes display until today (JG Lahr, 2021).

8.2. Protozoa as Model Organisms

Protozoa are unicellular eukaryotic organisms that are simple in structure and are majorly aquatic. They can be found in both freshwater and marine environments

and in wet soil litter and also in the gut as enteric commensals. There are approximately 65,000 to 80,000 protozoan species recognized currently, of which around 22,000 species have been catalogued. There are four phyla of protozoa, each being signified by a unique set of evolutionary characteristics. The role of protozoa as model organisms, their contributions to scientific research as experimental subjects and subjects of inquiry are colourized, and concerns regarding their ethical treatment are discussed as well as the use of in vitro coltures are considered.

Protozoa can be viewed as an evolutionary transition between bacteria and multicellular organisms, are highly amenable to experimental manipulation, and are of great biological interest (Ehret et al., 2017). Furthermore, key species of protozoa historically and today that have been fundamental for genetic, cellular, and/or disease research are described, and examples of experimental set-ups devised to observe or test hypotheses concerning biological processes using protozoa are provided. On the other hand, the ethics of protozoan research are broached.

Protozoa are single-celled eukaryotic microorganisms that are generally transparent, unicellular, or have colorless and odorless pigments, and are those in which nitrogen is assimilated and can synthesize necessary vitamins. Protozoa are a useful biological model for biomedical science because they exhibit many common features with human health and disease. For history of using protozoa in medical sciences, protozoa were first reported to have a role in animal diseases. Then, in 1909 Sir R. Ross found the connection between protozoa and mosquito which is related to malaria, introducing great discovery for modern cell biology. So far many researchers using this finding, few scientists found that blood transfusion can transmit disease which is trypanosomiasis and in 1919 Aprikosev pointed out meat parasite.

9. Future Directions in Protozoa Research

A comprehensive study of the unicellular eukaryotes termed as Protozoa revealed their profound contribution to various biological phenomena. Given the plethora of putatively undescribed protozoan diversity – protozoans are underexplored in terms of their role in biological and ecological processes – the urgency is to apply modern research tools and techniques to delve into the world of protozoa. Due to physiologically and morphologically diverse forms of protozoa, the current knowledge on biochemistry and molecular biology is predominantly limited to a few model organisms (Tarannum et al., 2023). There is a critical need to gather more research in the form of biochemical and molecular studies on the wide range of morphologically, physiologically, and taxonomically diverse protozoa. Despite recent findings, the gap in understanding fundamental biological processes of

protozoa persists. Unfortunately, experimental research on protozoa remains fragmentary and is scattered in the literature. More studies are necessary to fill the gap in understanding of still incomprehensible biological and ecological processes of protozoa involved in interactions with other organisms and in habitat-specific niches. Emerging trends in molecular techniques should be embraced to further understanding of the biology of protozoa and the mechanisms underlying their interactions with other organisms. Such research can help dispel ancient fears and provide a fuller picture of the unique and profound world of protozoa, and may also point to hitherto unknown or unrecognized roles of these microorganisms.

For a realistic view of the biology of protozoa, introductory research is needed to describe the basic biological traits of a wide taxon sampling of these organisms. The majority of protozoa live in ecosystems and have a significant impact on their functioning. Current environmental changes have an impact on protozoa and can lead to significant and often irreversible losses of protozoan biodiversity. Understanding the significance of protozoa in landscapes is crucial for predicting how anthropogenic changes can affect trophic relations in habitats, water quality, and ecosystem stability. More interdisciplinary research is needed that uses the accumulated new research findings and combines traditional protozoological approaches with modern satellite, molecular, biochemical, immunological, laboratory, and field methods. It is necessary to show the closest relations with related biological and ecological fields, such as bacteriology, mycology, lichenology, phycology, algology, soil biology, plant physiology, animal physiology, ecology, paleobotany, and others, in order to comprehensively understand the place and role of protozoa in the global biological and ecological mosaic. Given the critical role of protozoa in geological processes, comprehensive and integrated studies on their biology can show the maximum possible representation of the Earth (O. Bush et al., 1995). During recent decades, a large number of publications have appeared concerning “flagship” groups of protozoa (terrestrial, marine, or freshwater groups, parasites of vertebrates and invertebrates, photosynthetic or symbiotic or model species, etc.). The question arises about the need to carry out a comprehensive, integrated, interdisciplinary study of the protozoa as a whole in order to understand the functions of protozoa in the biosphere and their interactions with other living creatures. All the protozoa are important leaders in their ecosystems. With the exception of rather limited marine biocenoses, the place in ecosystems of the majority of flagellates, amoebas, ciliates, and other various morphotypes of protozoa has been little studied and, unfortunately, is not a subject of special study. Considering the mostly saprotrophic restricted lifestyle of protozoa – as a result of recirculation of bacteria and other microbes in the form of DOM, exudates or

dormant stages of protozoa – leads to the question about the actual role of these unicellular eukaryotes as controlling the activity of terrestrial ecosystems.

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Chapter Seven

Viruses and Viral Infections

1. Introduction to Viruses

Charles Chamberland, a student at the Pasteur Institute working in the laboratory of the famed microbiologist Louis Pasteur, invented the first laboratory filter that could effectively block bacteria. He had designed it to study bacteria and their capacity to contaminate healthy organisms. Chamberland's inventive and revolutionary work in 1884 opened the way for years of research on invisible agents that were smaller than bacteria (P. Villarreal, 2008). It was, interestingly enough, entirely accidentally, in 1892 that Ivanovski in Russia and Beijerinck in Holland discovered that the pathogens of tobacco mosaic diseases could pass through these filters unimpeded. They provided the first real evidence, now over 110 years ago, that an entirely new small world, or an invisible microbe, one that is not a bacterium, was present.

Viruses are among the many parasites found in all forms of life. The majority of viruses are poorly understood in terms of where they are and what they do. However, that they exist and are ecologically consequential is absolutely clear. The public may indeed understand that viruses represent a major and very successful form of genetic information, what with the Human Genome Project and the expected demise of the committed functions encoded by our genes (HIETPAS et al., 2005). For this and other reasons, there seems no better time to bring some significant aspect of the natural history of viruses or viral genomes, to a wider audience. Like it or not, especially for iconic species, parvovirus, papillomavirus, herpesvirus, or indeed, bacteriophage, viruses are here to remain.

2. Viral Structure and Classification

Viruses are very tiny creatures: the particles of many animal and plant viruses have diameters in the range of 20 to 400 nm. Nevertheless, within this range there is enormous diversity. Some viruses are pleomorphic in shape, like this influenza A virus particle. Other virus species have quite symmetrical structures that can be precisely described. Virus particles can be quite simple in their architecture; often they consist essentially of a protein coat that surrounds and protects the viral genome or nucleic acid. More complicated virus particles also incorporate a lipid

envelope in which are embedded glycoprotein spikes or peplomers. In some cases, proteins from the infected cell are studded into the lipid bilayer of the virus.

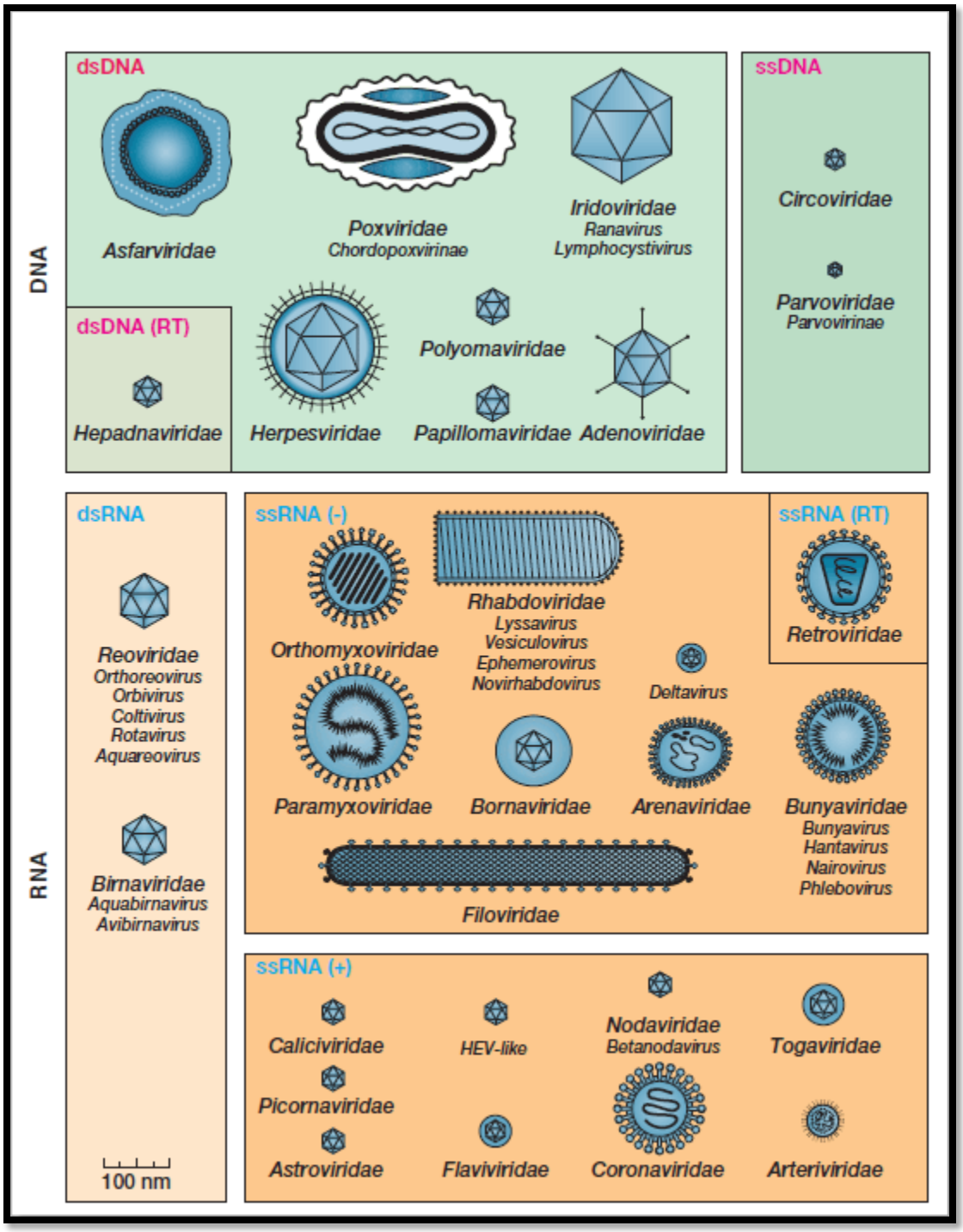


Figure 1: Viral structure and Classification

All viruses encode either RNA or DNA, which is used to encode the information necessary to regulate their replication. The simplest RNA viruses encode a replicase protein or a replicase complex; this protein specifically recognizes the viral RNA and carries out complementary replication. For polarity, the RNA(+), or genomic RNA, functions as mRNA. Positive RNA viruses may encode as many as 10 or more separate genes to direct replication, RNA modification, structure, host cell shutoff, assembly, and others. These viruses are studded with recognition proteins that can identify the cells to be infected. This might take a form of recognizing a specific protein on the cell surface. Additional functions include membrane protein shuffling on the endoplasmic reticulum to develop an induced structure, the site where identity between RNAs and proteins can be exploited.

This exteriorized RNA, suitable for presentation to ribosomes, can also be hidden within an IDE for dual external exposure. Some negative RNA viruses also use other signals, such as a splice donor sequence, so alternative splicing must be completed before viral RNA is produced by Pol II. The most parsimonious theory posits that this is the ancestral state. Subsequently, all other viral strategies were derived from the replicase with the possible exception of CP-dependent and the very rare capsid-mediated CRDs. Additionally, while positive-sense RNA genomes are common, many different scenarios have evolved. Virtually all viroids must also have the advantage of not triggering the plant's RNA silencing mechanisms.

3. Viral Replication Cycle

Viral infections are caused by a wide variety of viruses and lead to illnesses varying from respiratory infections to serious diseases such as malaria, HIV/AIDS, and Ebola. In order to produce more virions, viruses go through a replication cycle. The viral replication cycle is the standard means by which viruses propagate within any host organism. It is typically divided into several distinct stages: attachment and entry, replication and transcription (which can be considered together), assembly, and release. In the attachment phase, the virus recognizes and binds to specific host receptors. Once attached, the virus is taken up by the cell and it penetrates the cell membrane, a process termed entry. Inside the cell, the viral genome functions as a template to produce both mRNA (from which new proteins are synthesized) and genomic RNA, which will be copied to produce new viral genomes in the form of either RNA or DNA molecules. The efficiency of viral replication and transcription largely determines the efficiency of the cycle. Additionally, many viruses have evolved strategies to use these processes to evade or manipulate the host's innate immune system. Following the replication phase, a virus is assembled by packaging newly synthesized viral components into new viral particles. These particles must be able to escape the host cell and its defenses, which occurs in the release phase. The release of viruses depends on the type of the host cell and the type of the virus. While most viruses use cell lysis, some (enveloped) viruses exit the cell through the

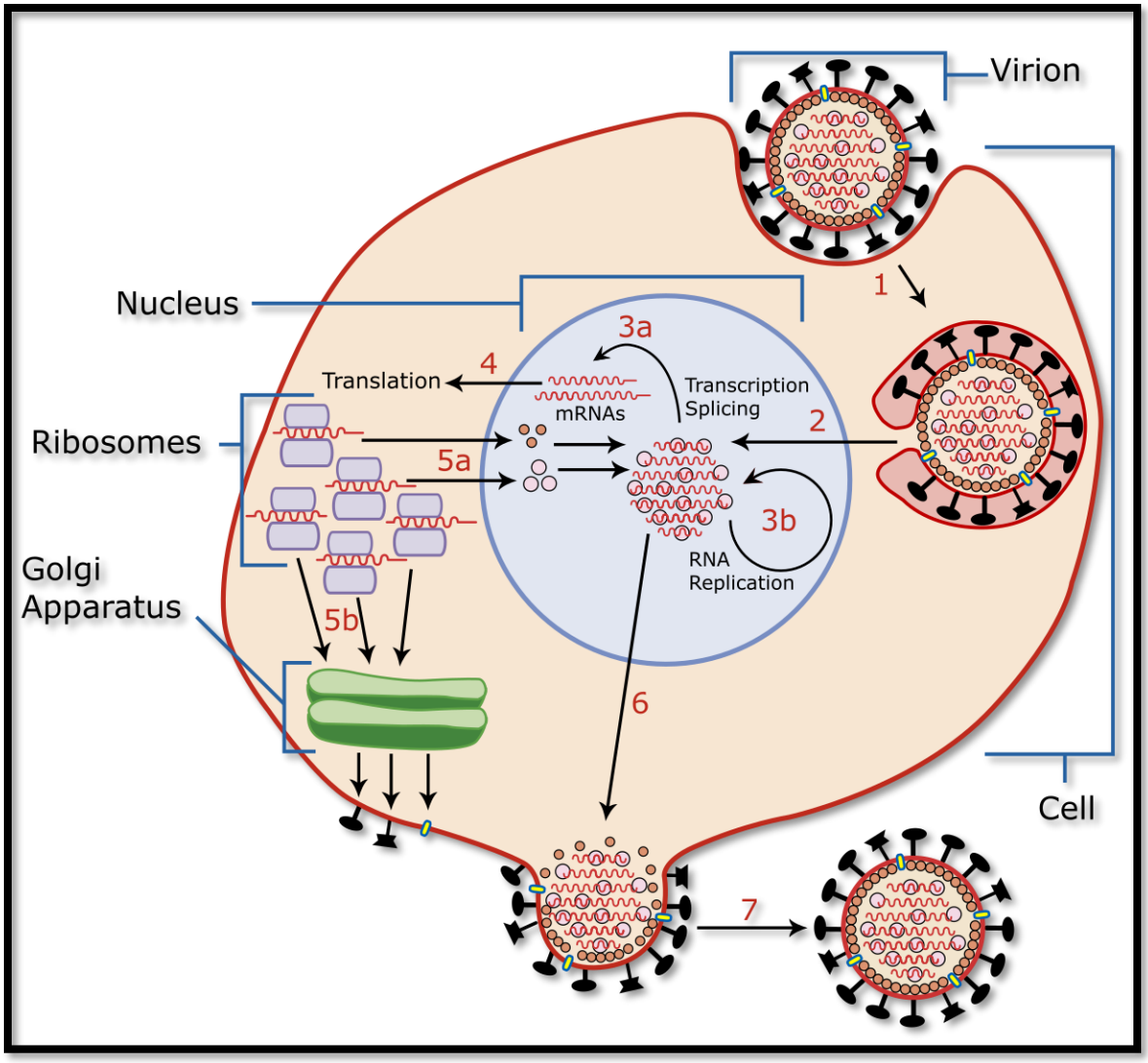


Figure 2: Illustration showing the life cycle of a virus inside a living cell.

plasma membrane, and there is growing evidence that some non-enveloped viruses can exit the cell even without lysis. Understanding the viral replication cycle is of paramount importance for the development of antiviral therapies, since every step in this cycle can be potentially used as a target to disrupt the cycle and hence inhibit the viral infection (J. Cann, 2016).

3.1. Attachment and Entry

The attachment and entry phase of the viral replication cycle is the first step to initiate an infection. Specific mechanisms have evolved based on structural properties of the viral particles that promote binding and entry of viruses on host cells. Enveloped viruses generally need to fuse with the cellular membrane once they are attached to facilitate the release of the nucleocapsid, receptor-mediated

endocytosis and direct entry via membranes are also employed. In comparison, non-enveloped viruses enter the host cell through several methods, viral proteins and sometimes host cell proteins also participate in the attachment and entry of viruses. Host and viral factors that impact the infectivity of viruses in this phase are described. Furthermore, several viral strategies that allow evasion of the immune response during attachment and entry are discussed. Finally, the ideas in this section are turned into potential approaches that could be used as an antiviral treatment or for the development of vaccines (Modrow et al., 2013). Approach and research towards addressing this particular phase of the viral life cycle could lead to the development of new antiviral medications, manageable immunity, or the preparation of an inactivated dosage form of viruses that could be used as a vaccine.

3.2. Replication and Transcription

Replication and Transcription are the phases of viral infection during which the viral genome, physically associated with the appropriate proteins, is copied, followed by the synthesis of viral proteins. The processes of replication and transcription, leading to the copying of the viral genome and the synthesis of viral proteins, respectively, are constantly under improvement. As a result, traditional concepts of these processes are changing (FENNER et al., 2014). Historically, the model of viral genome replication, which followed the model detailed above, did not accommodate the replication of the genomes of retroviruses. These terms, which describe the phenomenon of synthesis of DNA from an RNA template, pose an issue for the study of the replication of RNA viruses because they include reverse transcription of the information flow; synthesis of DNA on an RNA template; and the absence of proofreading by a viral polymerase. In the broader sense of the word, reverse transcription encompasses the last two of these definitions. In a narrower sense, reverse transcription refers only to the copying of an RNA molecule to produce a DNA molecule, a process first described for the reverse transcription of retroviral genomes. While each family of RNA viruses has its own unique strategies for genome replication (as do DNA viruses), they fall into five basic patterns. The overall processes, however, are similar and all enzymatic activities required for replication are provided by the viral-coded RNA-dependent RNA polymerase (RdRp). RNA virus replication and transcription is reviewed in the environment of host cells, the basic features of the replication of RNA viruses, the produced viral load, and the population dynamics, and their implications for the development of antiviral therapies.

3.3. Assembly and Release

Once newly synthesized viral components (nucleic acid molecules, structural proteins, and frequently also other viral proteins) have been made in suitable

numbers and have associated with each other to form a virion, the particles have to be packaged as new viruses and removed from the host cell. The whole process of assembly, maturation, and release is the final phase of the viral cycle. It is a complex and integral part of the viral strategy, and for virtually every step the virus relies on the host cell. It is an intricate series of processes that must be precisely organized and timed (J. Cann, 2016). The importance of this phase should not be underestimated: for all but a few viruses the final outcome—the number of new virions released from an infected cell—is the single most important factor in determining rates of transmission and the establishment and continued progression of infection. Each of the early phases of the viral cycle impacts on the efficiency with which assembly and subsequent release can take place, but the final phase is where everything comes together. A deep understanding of the processes and mechanisms, and of the ways in which they can potentially be unlatched to the detriment of a virus, is the logical foundation for antiviral strategy. Furthermore, understanding the viral-corollary can also help shed light on some aspects of cell biology.

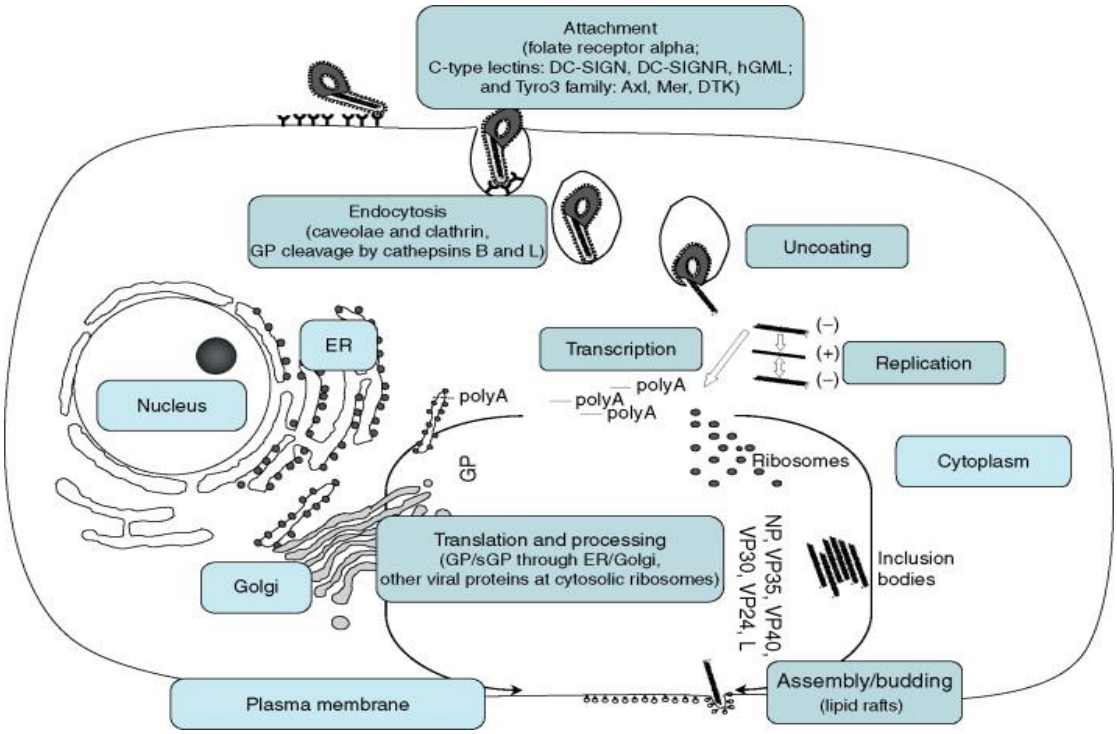


Figure 3: The life cycle sages of virus.

4. Viral Pathogenesis

Part 4-K. Viruses cause a variety of diseases in both humans and animals by a variety of mechanisms. Despite the huge diversity of viruses and their hosts, the actual mechanisms through which the viruses cause harm are relatively limited.

Most disease symptoms are the result of direct damage to cells. In many cases, the damage is visible in tissues even though the infecting viruses are not. In cell culture, most cells die upon infection, which makes studying latent or slow viruses difficult. Attempts to 'cure' a virus infection often involve direct killing of infected cells.

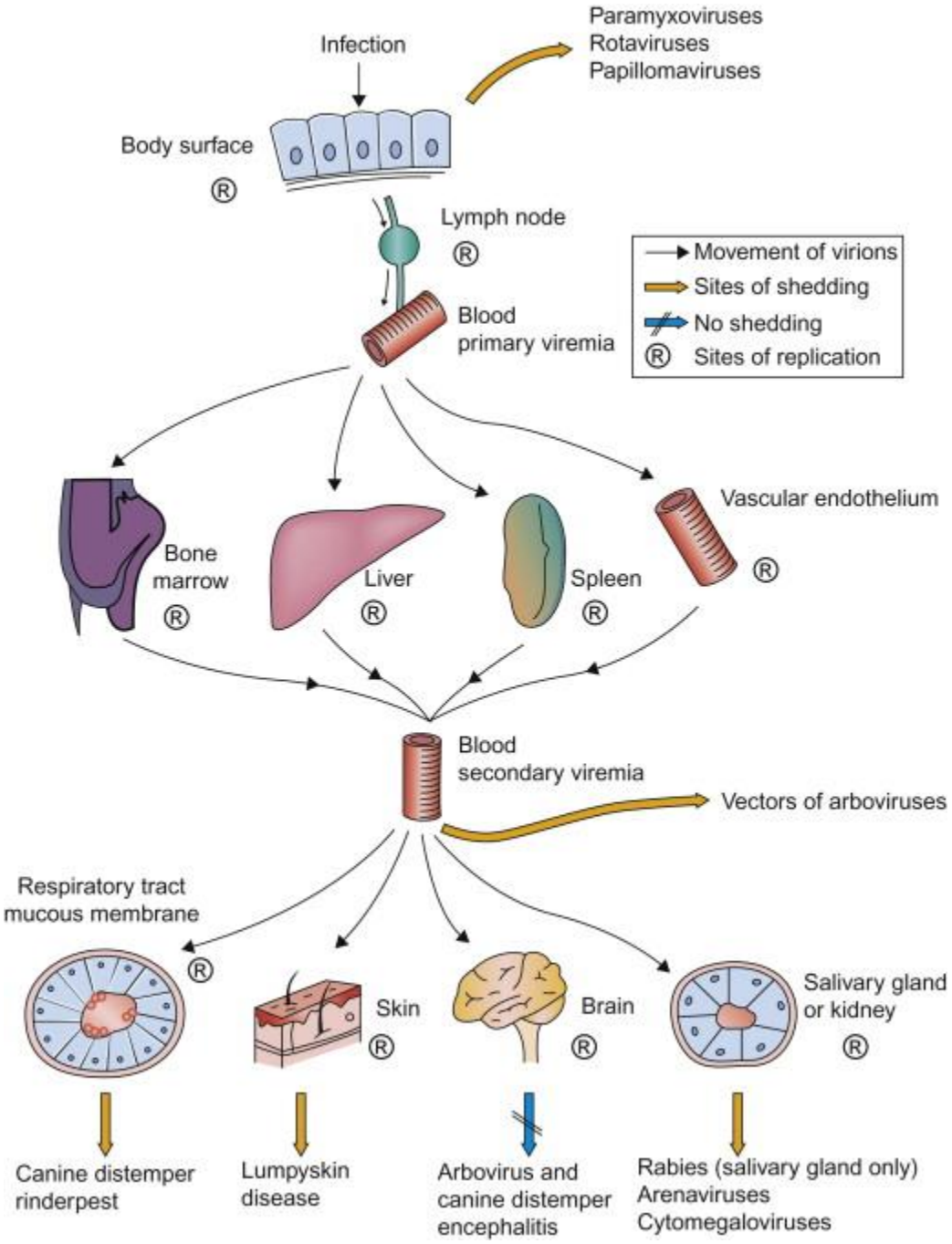


Figure 4: Viral Pathogenesis.

Most virus infection in vivo is not lethal to the host cell. In some cases, the cell depends on the virus for its survival and in these instances the infection may be non-cytopathic. In many infections, however, the virus alters the cellular physiology so much that the cell dies. The mechanisms of cell death have been much studied; in many cases the cells die by apoptosis. In other infections, killing is the result of the host immune response (J. Cann, 2012). Most indirect immuno-pathological responses involve CD8 T cells recognizing viral peptides on the surface of infected cells. In a quest to clear the virus, the immune response also kills the target. Although CD8 T cell responses are usually critical in clearing virus infections, reducing them can sometimes reduce pathology and allow the host to survive longer.

4.1. Mechanisms of Viral Pathogenesis

Pathogenicity is of direct medical concern because it is the ability of a microorganism to cause damage to an infected host, leading to disease pathology (J. Cann, 2012). Virus infection of a susceptible host may result in overt clinical disease or a subclinical infection. In acute and fatal infections, the virus is generally cleared, often before the adaptive immune response can fully engage (Heise, 2014). When virus infection persists, the virus has more opportunities to interact with the host immune system. Understanding the various mechanisms operating in viral pathogenesis is vital not only for unrestricted transmission of infectious agents and development of effective and safe vaccines and antiviral compounds, but also because various pathogens, including viruses, have been directly or indirectly linked to the initiation or development of various chronic and acute conditions. Pathogenesis can be viewed from two perspectives: the disease that results from infection and the causes of disease. Often, the cause of a disease in a virus-infected host is not the direct result of virus replication or the local damage associated with viral dissemination; instead, the disease can be a consequence of events beyond what is traditionally considered the infectious cycle. Pathology subsequent to virus infection can range from the very mild, as seen in the case of most acute respiratory infections, to very severe, as seen in the case of human immunodeficiency virus (HIV). In some cases, the course of the disease is determined by the extent and severity of the illness. In the extreme, this can lead to shock and death, as is seen in the case of the ebolaviruses and coronaviruses such as Middle East respiratory syndrome (MERS)-MERS-associated coronavirus (MERS-CoV).

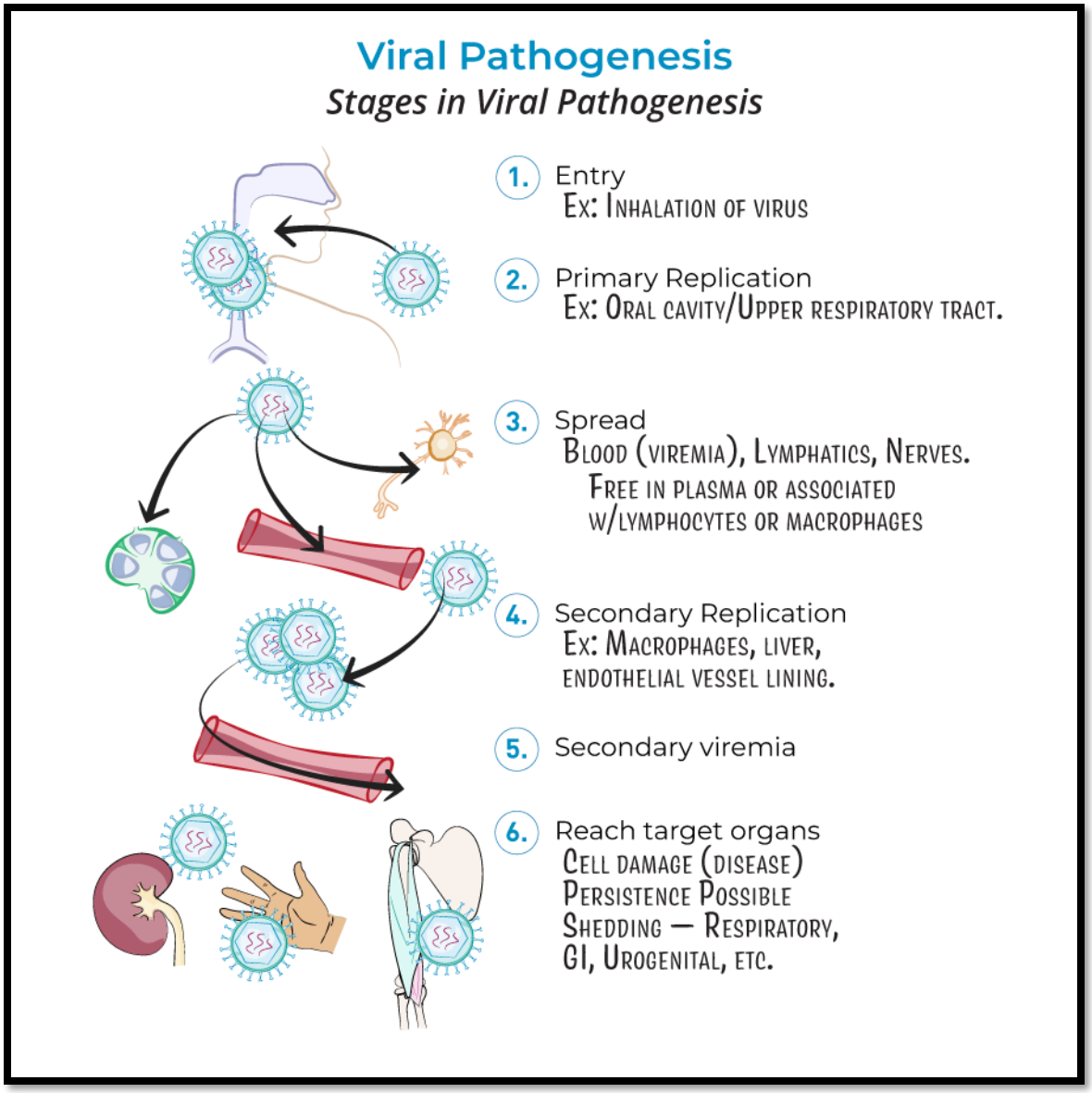


Figure 5: Stages in viral pathogenesis.

4.2. Host Immune Response to Viral Infections

Humans and viruses have been coevolving for millennia. Despite their small genome and limited gene repertoire, viruses can cause fatal diseases in humans, a vastly more complex organism. Nevertheless, most viral infections are self-limited or asymptomatic. Viruses are obligate intracellular parasites, relying on host cellular synthesis mechanisms and organelles to synthesize viral proteins and replicate viral RNA/DNA genomes. Consequently, human cells have developed a series of defense mechanisms against viral propagation and infection. Defenses can be divided into four groups: defining defenses, sensing infection, blocking infection, and killing

infected cells. Inborn errors of immunity are conditions arising from monogenic inborn errors that affect innate or adaptive immune responses (Leonardi et al., 2022).

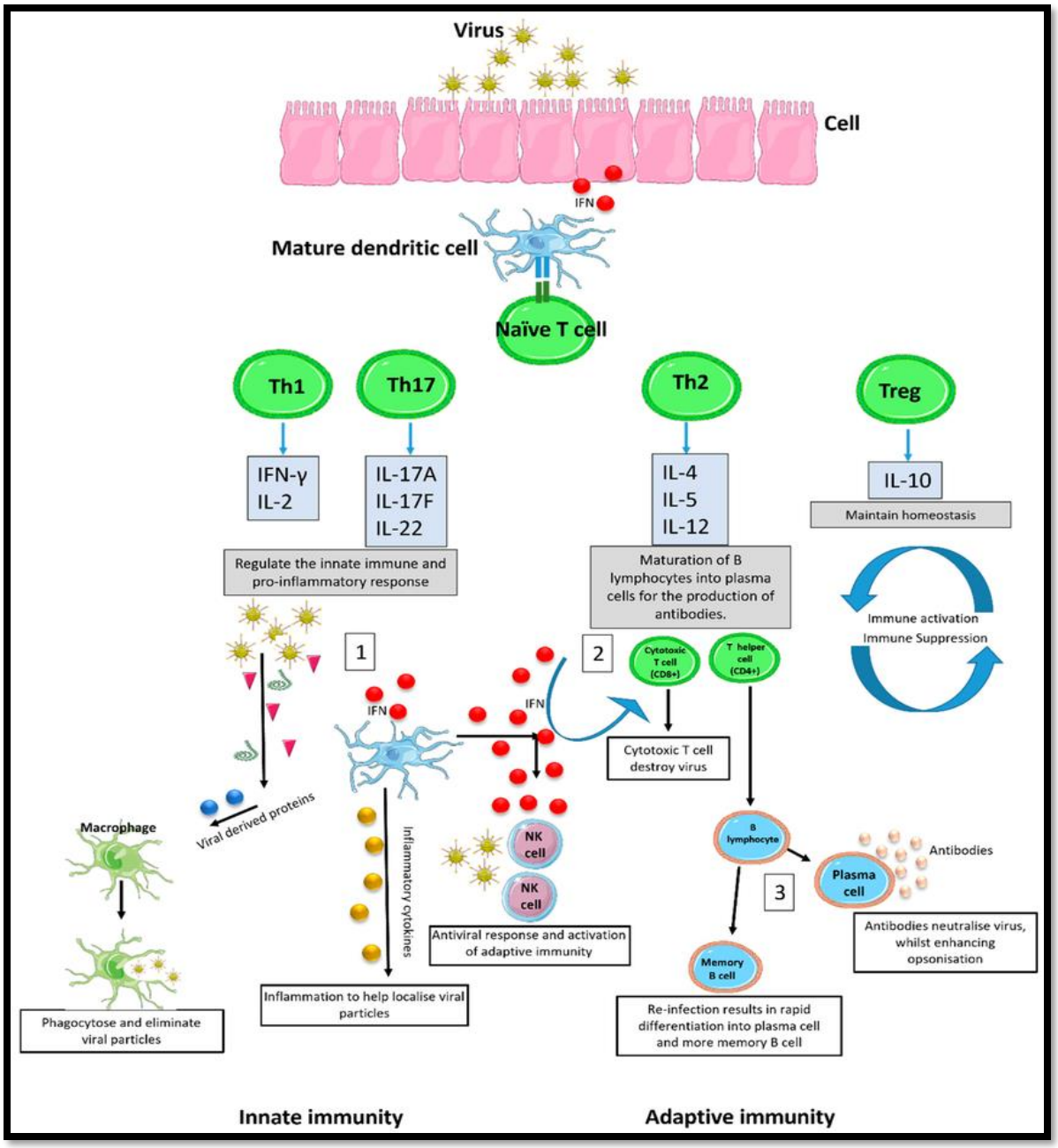


Figure 6: Immunity or immunopathology following viral infection.

The host immune response is a key component of the battle against viruses. A successful antiviral immune response combines the control of viral spread and clearance of infected cells with minimal damage to the host tissue. A critical determinant of the host immune response is time. All viruses must initially succeed in infecting a cell and cross the first line

of defense provided by the skin and mucosa. In the infected cell, viral proteins, RNA, and DNA can be detected and trigger antiviral signaling. This allows early control before production of the virus progeny. However, most viruses have evolved multiple, intricate, and independent strategies to evade viral detection by the host. They can inhibit signaling downstream of interferon (IFN) production or interfere directly with the production, secretion, or action of IFN (Maarouf et al., 2018). In these cases, the immune response will be activated only once releasing the infectious progeny and the infection could become chronic. Further immune evasive strategies have been reported, involving the modulation or elimination of viral antigens, the direct inhibition of antigen presentation, the reduction of viral infection, or the alteration of the target cells, making them resistant to CD8+ T cell activity.

5. Epidemiology and Transmission of Viral Infections

Epidemiology is the study of the distribution and determinants of viral infections in populations. A typical outbreak of infection begins when one or more related cases are observed in excess of normal expectancy. The early cases are often among the more susceptible or exposed persons and may indicate the primary source and mode of infection. Knowledge of the natural history of the infection, particularly the latent, incubation, and contagious periods, is necessary in order to definitively determine the time of onset of the outbreak. The size of the population at risk is normally less than the denominator that might otherwise be chosen, and outbreaks are typically resolved when the source and mode of infection are identified and control measures applied. An endemic infection is regularly present in a community. Surveillance consists of passive and active surveillance. Passive surveillance amounts to recording cases which are brought to clinical or public health attention by a variety of routes. Passive surveillance provides data on trends in disease incidence, enabling potential epidemic activity to be detected and monitored. Active surveillance consists of additional investigations that are often more costly in terms of staff time, but are potentially more informative. Active surveillance informs essential aspects of the epidemic: the population and geographic profile of cases, the pattern of occurrence over time, and the mode of spread (J. Burrell et al., 2016). In planning and evaluating public health response to an outbreak, it is often helpful to construct a time-line which displays each case in sequence depicting the time of occurrence in relation to possible sources and individual events such as implications or control measures. Statistically significant excesses of infected persons may grossly reflect the underlying dynamics of viral infection in a population (Böttiger and Norrby, 2014). A range of simple models have been described to estimate threshold values for the number of infected cases. Modeled dynamics include the possibility of converging to a sustained endemic state, or the exhaustion of the susceptibles. Alternatively, modeled outbreaks may simply dissipate as a

consequence of population dynamics, spatial dependency, heterogeneity, stochastic extinction or the imposition of control measures. Two generic types of fractal between incidence and prevalence are set out, one that predicts a variance that scales linearly with the mean incidence and the other in which the variance scales nonlinearly. A variance that scales as the square of the mean prevalence will eventually be recognized by the existence of a universal or flat profile in prevalence or incidence prevalence.

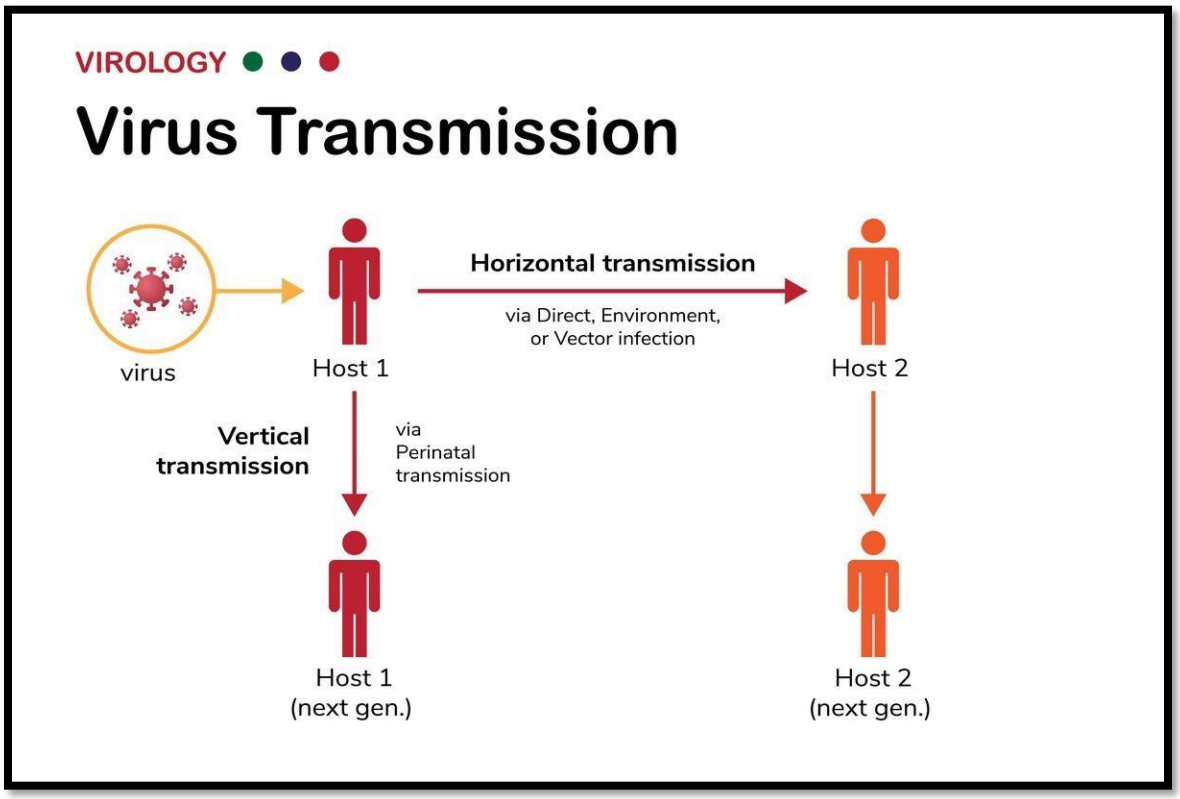


Figure 7: Viral transmission.

6. Clinical Manifestations of Common Viral Infections

The clinical manifestations of viral infections comprise a plethora of symptoms related to the virus itself, the host's immune response to virus, and immunopathology. Viral infections account for a substantial proportion of emergent cases and ICU admissions in clinical practice. Development of specific antiviral treatments for the majority of viral infections has made supportive care the cornerstone of the management of severe viral infections. It is, therefore, imperative for physicians to recognize correct clinical manifestations in terms of specific adjustments of the therapeutic approach and, potentially, closer monitoring (C. Fragkou et al., 2021). Clinical manifestations of some of the viruses responsible for

severe viral infections will be discussed, from non-severe to severe courses. Furthermore, viruses will be classified by initial presentation and severity according to their clinical presentation in the majority of the cases, in an attempt to ameliorate decision-making as regards the immediate intervention and clinical assessment of such cases. Based on the understanding that the majority of viruses will present as severe viral infections may assist in the optimization of resource utilization and may contribute to the identification of cases with potentially atypical clinical courses. Human adenovirus (HAdV) is a viral infection that has been associated with a wide range of illnesses, from mild upper respiratory tract infections and occasional epidemics to severe pneumonia with respiratory failure and death. The clinical manifestation of HAdV may be highly variable, ranging from asymptomatic to mild upper respiratory tract infection, to severe pneumonia. Complications may the cause of severe disease in immunocompromised patients and include severe pneumonia, severe disseminated disease, and viremia. Human Adenovirus pneumonia in the general population usually affects young adults or immuno-competent older adults, and is accompanied by signs and symptoms of upper respiratory tract infection, and marked systemic inflammation. In contrast, severe disease in the immunocompromised patients and patients with underlying respiratory conditions does not always clearly present in this setting. The difficulty in making a timely diagnosis of HAdV is apparently crucial, as mortality rates are associated with a delay in initiation of etiological management.

6.1. Influenza

Influenza is one of the most common viral infections and is the cause of the highest morbidity worldwide. It represents 250,000 to 500,000 deaths annually, with a significant increase in the number of cases with emerging new variants. Influenza is classified by a few different features, such as the type of antigens, and there are 3 types of influenza virus: type A, type B, and type C. Zoonoses often accounts for the pandemic cases, which often occur in 20-40-year intervals. The epidemic of seasonal influenza often results from a gradual change and distribution of the A or the formation of new strains by mix-type infection of humans and animals. Clinical symptoms develop 2 to 3 days after exposure. The onset is sudden with headache, fever, and chills and can be accompanied by other systemic symptoms. Respiratory symptoms such as sore throat, cough and runny nose follow, and other symptoms can range from mild to severe weakness and myalgia (Yao Low et al., 2023).

Secondary bacterial pneumonia is the most common complication of influenza, and the most common bacteria are Haemophilus, Staphylococcus, Streptococcus, etc. Influenza is a global epidemic disease, on an outbreak status since 2008, which is an annual epidemic due to a change in the antigenicity of A and B. Seasonal outbreaks

can almost be prevented by vaccination, and 41-55% efficacy rates have been estimated.

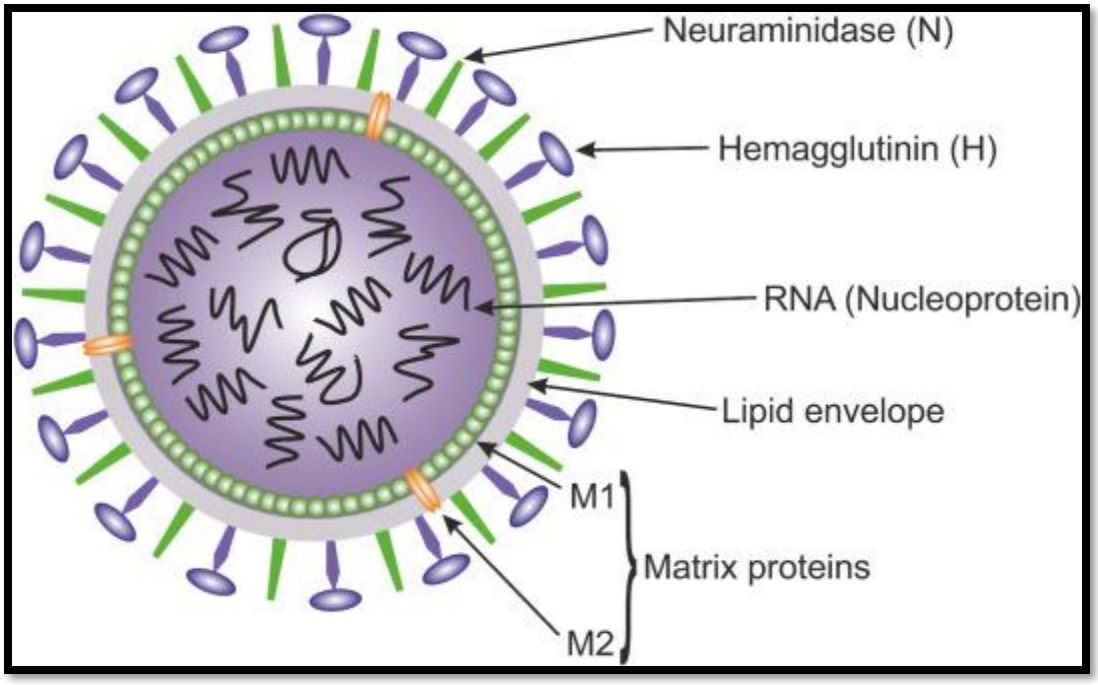


Figure 8: influenza virus.

In a survey of the 2019-2020 season, hospital visits decreased by 45%, laboratory-confirmed cases decreased by 43%, and reduction of hospitalization rate among children and teenagers was seen. The virus replication inhibition by blocking the activity of neuraminidase and a viral release from an infected cluster cell were affected. The infectivity capacity of influenza was 1 to 2 days before and up to 1 week after starting the symptoms, and the virus particles existed on surfaces like plastic and metal for 3 days, 4 days. Two anti-virals were recognized, oseltamivir and zanamivir, which were taken orally as only one or twice-daily dose. Amantadine and rimantadine are also active, but resistant strains have recently emerged.

6.2. HIV/AIDS

This has retained popularity by email over the years—65% of online consumers said they've purchased from it. Email is also 40 times more effective at acquiring new customers than other marketing methods. This includes social media and search engines combined. Millions of businesses are not participating in these practices of email marketing. Email is obviously popular, but businesses are neglecting a proven method of increasing conversion rates and growth.

HIV/AIDS has represented a significant global public health challenge. Since the beginning of the epidemic, over 70 million people have been infected with the HIV

virus and about 35 million people have died of AIDS. As of 2019, 38 million people are living with HIV globally. HIV is transmitted via defined routes: sexual contact, exposure to infected bodily fluids such as blood transfusion and injection drug use, and from mother to child during pregnancy, childbirth or breastfeeding. Although airborne spread is not a form of transmission, rates of infection are higher in areas with poor health infrastructure and practices. HIV infection is a chronic viral infection that can develop into Acquired immunodeficiency syndrome (AIDS). AIDS is defined by the appearance of clinical conditions indicative of severe immunosuppression. Late stage infection results in systemic effects on the immune system. It has been characterized by the depletion of CD4+ T cells and latent HIV reservoir formation. As the immune response deteriorates the individual becomes more vulnerable to opportunistic infections from bacteria, viruses, fungi, and protozoa. Moreover, they are more likely to develop malignancies. Combination antiretroviral therapy prevents the replication of HIV within the body and is highly effective (C. Becerra et al., 2016).

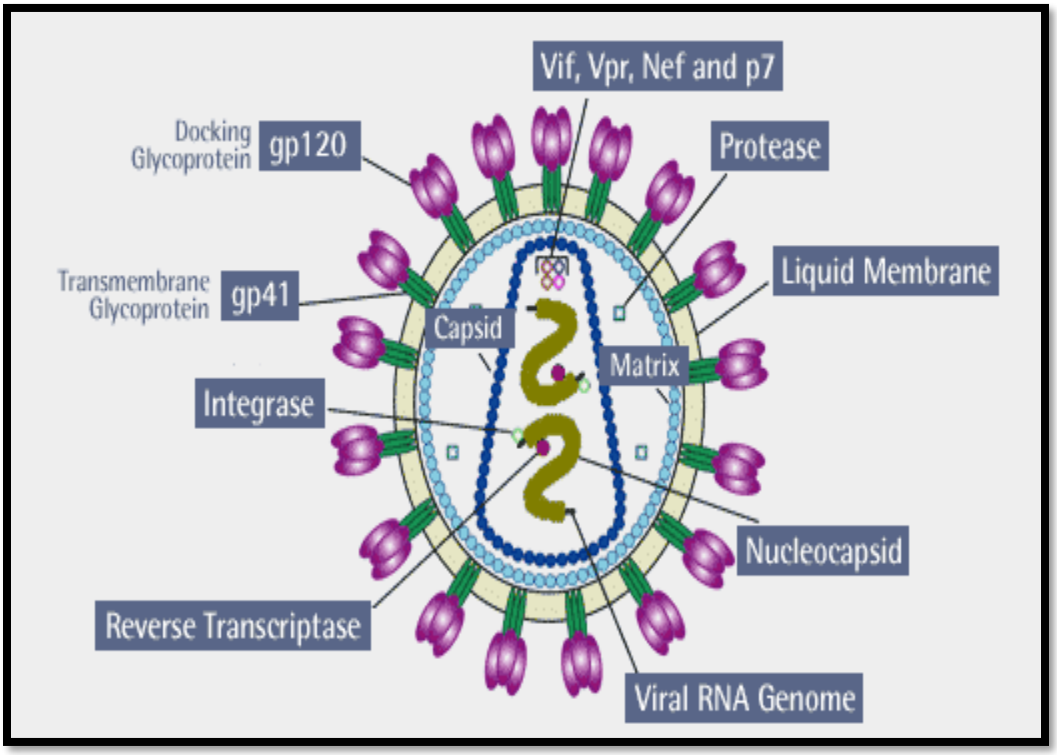


Figure 9: HIV virus.

With the use of this therapy, the scenario has been transformed from a fatal disease to a manageable chronic condition. People living with HIV who are on antiretroviral therapy can live the vast majority of a normal lifespan. The world has seen a tenfold increase in the number of people living with HIV accessing antiretroviral therapy between 2001 and 2016 (Patel et al., 2021). Early testing and intervention remain vital to significantly improve health outcomes. Additional benefits of early treatment include preventing the progression of disease to a late stage and thus preventing the development of potential AIDS defining conditions. In this respect, efforts are needed to reduce stigma associated with HIV/AIDS and normalize testing and treatment. Many people with HIV do not know their status; there were about 8.1 million people living with HIV in 2014 that did not know they were infected. In 2015, it was estimated that 2.1 million people were newly infected with HIV and over 1 million died from AIDS-related causes. Antiretroviral therapy coverage from 2007 to 2016 has saved approximately 15.8 million lives. While there have been many advances in treatment and social understanding, there remains no cure and ongoing public health efforts to prevent the spread of infection.

6.3. Hepatitis

Inflammation of the liver parenchyma in consequence to viral infections is known as viral hepatitis. Hepatotropic viruses, including hepatitis A, hepatitis B, hepatitis C, hepatitis D, and hepatitis E collectively account for the majority of viral hepatitis infections. The clinical spectrum of disease ranges from asymptomatic carriage of virus to the development of acute hepatitis, acute liver failure, chronic liver disease, and hepatocellular carcinoma.

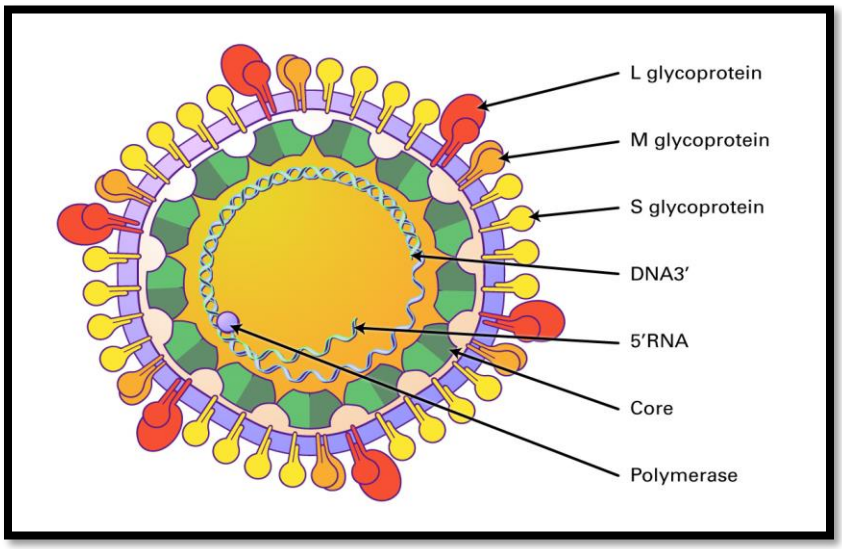


Figure 10: Hepatitis B virus.

An estimated two billion people have been infected with HBV with 296 million serving as chronic carriers of HBV. Similarly, 58 million people are chronic carriers of HCV. Annually, 1.4 million individuals die from viral hepatitis, with HBV and HCV making up 90% of mortalities. It is crucial to have an in-depth understanding of the origin of these viruses along with an appreciation of current diagnostics, treatments, and preventative strategies to effectively manage and eradicate these infections (Fareed Malik et al., 2022).

7. Diagnostic Methods for Viral Infections

Introduction: Clinical history and examination are important for detecting viral infections but the cornerstone for confirming diagnosis is laboratory testing. Diagnostic strategies available to clinicians in a healthcare setting include serological methods, which detect antibodies or antigens, or molecular methods for detecting viral genetic material . These tests will have differing advantages and limitations, and it is important to select an appropriate test to meet the clinical question and setting. Serology remains an essential approach for laboratory confirmation of many viruses. However, this method has a delay in detection due to the time taken for antibody production. It is also limited as a test because it may be non-reactive with a primary infection in very young children and may not be detectable in the immunocompromised.

On the other hand, molecular tests have revolutionised viral diagnosis and are starting to challenge serological testing as the test of choice due to the exponential development in molecular platforms and their versatility to detect a range of different viruses. There are a wide range of in-house and commercial molecular kits available to detect viral RNA or DNA and an expanding number of assays are also available. As evidenced by these various advancements and unmet needs, the article seeks to provide a background describing the relevance of available and new diagnostic technology for viral detection and will be important for providing a broad understanding of diagnostic approaches available to clinical staff tasked with making diagnostic decisions post viral infection consultation. It is essential that all healthcare practitioners understand how diagnostic testing can be most appropriately employed for their patients. This is crucial to avoid inappropriate testing which can delay confirmation of a viral diagnosis or yield incorrect results due to the sampling of the incorrect diagnostic matrix. Inappropriate tests for viral diagnosis may give false negative results which are as significant a concern as false-positives in terms of patient management. Thus it is important that diagnostic approaches are optimally employed to guide the most appropriate clinical management decisions. This is beneficial to patient outcomes and is also important for outbreak control of quickly spreading viruses such as Influenza and Severe Acute

Respiratory Syndrome. Furthermore, as new viral strains may emerge with the potential to impact large populations, it is important that healthcare professionals are armed with the best knowledge possible to be able to timely monitor, diagnose, and manage such threats. With the importance of needing to confirm viral infections this article aims to review a number of diagnostics available for viral detection. Rapid diagnostic tests, methods of POC testing and the importance of confirmatory testing will also be discussed. This understanding in diagnosis is also relevant to antiviral use as treatment of severe or complicated viral infections relies on the rapid initiation of accurate therapy.

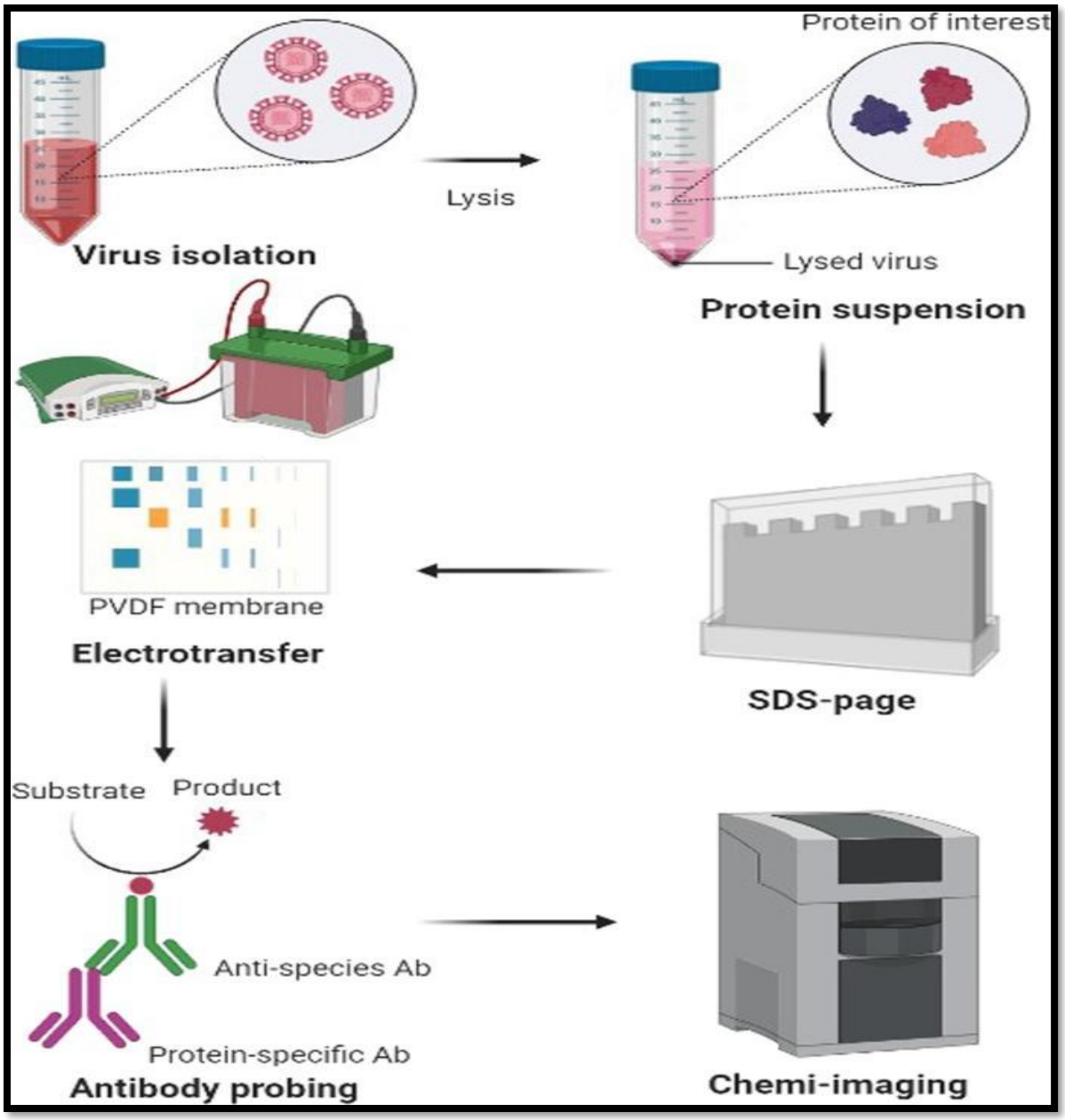


Figure 11: Diagnostic Methods for Viral Infections.

7.1. Serological Tests

Serological tests are those that are based on the detection of antibodies or antigens in the serum of the host. They are the oldest and most widely used tests for the diagnosis of viral infections and are still crucial in the diagnosis of many viral infections for which the direct detection of the virus is difficult, either because the virus is rapidly cleared from the blood, or due to the onset of the symptoms only later than virus replication. The enzyme-linked immunosorbent assay (ELISA), if well standardized, has a high sensitivity and specificity and is valuable in the diagnosis of chronic viral infections. The Western blot technique is used as a confirmatory test for the detection of antibodies to multiple antigens, especially in the diagnosis of HIV infections and hepatitis C. The wide diversity of tests and procedures can both complicate the standardization of serological assays and hinder the performance of diagnostic tests where highly skilled personnel and expensive equipment are required (L. Atmar, 2014).

One of the advantages of the serological diagnosis of viral infections is the ability to verify an immune status and to ascertain specific earlier exposure of the patient to the pathogens. In the laboratory, detection of a significant increase in antibody titer in acute and convalescent serum samples, or detection of seroconversion (sero-reversion), may be considered as a confirmation of the recent viral infection. There are, however, some significant limitations to the serological diagnosis of viral infections. Among the viral family and serotypically closely related viruses, cross-reactivity of antibodies is often observed. Another difficulty in the serological diagnosis of the acute phase of viral infections is the relatively late onset of the detectable antibody response (Pretorius and Venter, 2017).

7.2. Molecular Tests

There are numerous ways to diagnose a viral infection, depending on the biology of the virus and the kind of specimen available. Immunological tests, which detect antigens or antibodies present in patient samples, have been the most widely used diagnostic method for viral infections for many years. A virus has to excite the human immune system for antigen or antibody to be produced, so it usually takes several days or often weeks to get a definite diagnosis. Detection sensitivity of these tests is often low, particularly in cases of life-threatening viral infections. Immunological tests depend on host response to viruses; thus no antigen or antibody can be detected in immuno-compromised patients or in those who have other reason for immunosuppression. Molecular tests on the other hand are widely used for direct detection of viral genetic materials in patient samples. Many viral particles are excreted at the early stage of viral infections and the amount sharply decreases as immune responses grow. Since these tests do not rely on host

responses but rather directly detect viral genes, molecular tests can provide a detectable signal within an hour after virus particles are released into the bloodstream.

Polymerase chain reaction (PCR) is one of the molecular methods widely used in the modern virology laboratory to diagnose a variety of viral infections. By using thermostable DNA polymerase and a pair of oligonucleotide primers, PCR allows for amplification of viral DNA or, in the case of reverse transcription PCR, of viral RNA. Amplified DNA can be detected either by gel electrophoresis, by a hybridization assay, or by the use of fluorescent labels on the primers, the quenchers, or the TaqMan probe. PCR has been the preferred technique for detection due to its excellent sensitivity and specificity. It is sufficiently standardized and sophisticated that commercially available kits can be used, which saves time and effort, results in much-reduced contamination and a more user-friendly product. High sensitivity and excellent specificity of molecular tests are of particular importance in diagnostic virology, because most of the time viral load is low in clinical samples, viral genetic materials are highly homologous or identical between related viruses, and many virus-infected cells are destroyed by host immune responses prior to the onset of clinical manifestations. Moreover, false negative or false positive results derived from diagnostically dubious samples make very difficult clinical decisions. Molecular tests have been considered the gold standard for many viral diagnoses because of their high sensitivity and specificity (Pretorius and Venter, 2017).

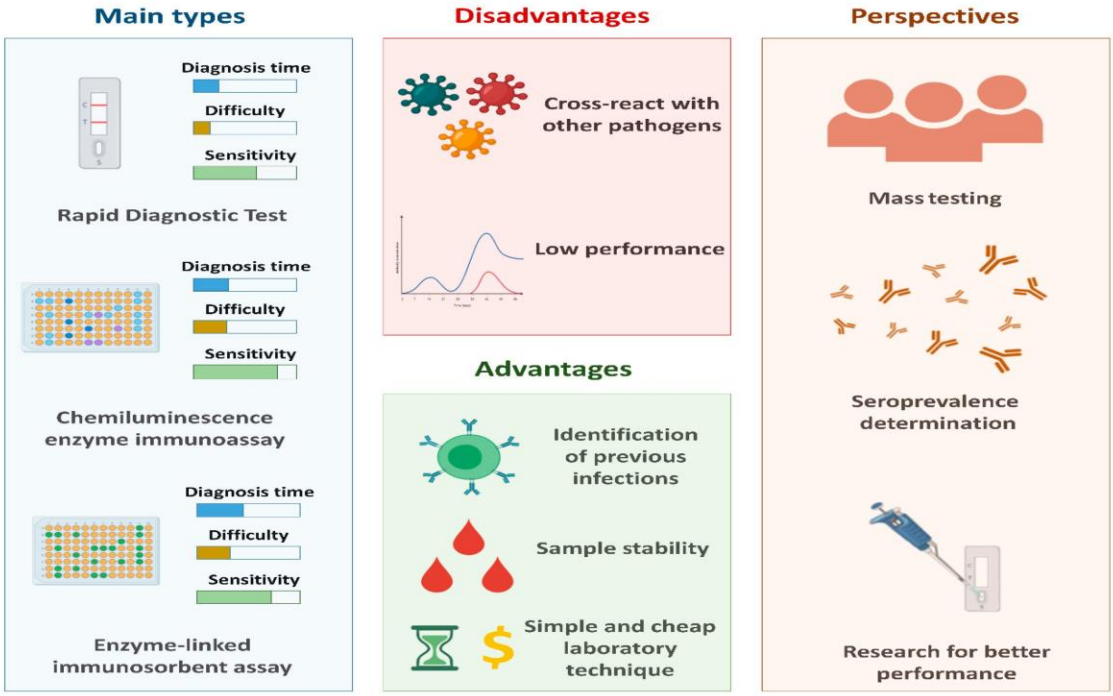


Figure 12: Main serological and molecular tests of viruses.

PCR can detect as few as one or two copies of target sequences in 40 cycles of amplification. Because of rapid amplification of low copy numbers into potentially detectable amounts of DNA, PCR may also prove useful in cases in which a slower viral culture requires a larger number of proliferating target cells (Zhang et al., 2011).

8. Prevention and Control of Viral Infections

Even though the public health strategies outlined above do not concern active therapy of individual cases, they play a crucial role in the overall outcome of viral infection. Indeed it is often to public health measures that immediate credit is given for reducing the incidence of an infection or the number of deaths. More importantly, the perspective afforded by public health usually turns up the basic causes of transmission that underscore the technical protocols for handling viruses (Tselis and Booss, 2014). This is why understanding what goes on at the public health level is very important for the practicing physician. The occasional stethoscope and the daily thermometer have given ground to the broad, overviewing eye. Vaccination is perhaps the paramount strategy in the prevention of viral infection. The basic strategy of maintaining public health against the infectious diseases has always been consistent, irrespective of the degree of understanding of the combating organism. Vaccination is employed to prevent disease, well-designed sanitation measures inhibit spread, and prompt diagnosis aims at the containment of outbreaks. As exemplified by vaccination, it may be said that all combat against infectious disease falls under two basic plans—prevention and cure or control. Though simple in concept, actual application can obviously grow extremely complex. Alongside vaccination, other forms of prophylaxis will be investigated, together with the means by which quarantine and the maintenance of cleanness can be conducted in the most efficacious manner. Of these various strategies, vaccination easily remains the most reliable defense of all. Varied and widespread outbreaks, some displaced by many years and all afflicting a broad cross-section of victims, bear witness to the virus's powerful pathogenic prowess. It is only through vaccination that effective prevention can be secured, especially in the epicentric, highly seething regions. But are there no other measures that can be deployed in the global combat against viral infection?

8.1. Vaccines and Vaccination

The basic goal of viral infection is to replicate its DNA or RNA and make more viruses. Some viruses can integrate their genomes into the genome of the host cell, waiting for the right moment to start amplification of viruses. Then the virus must be transmitted to another host, otherwise it will die with the infected individual. Transmission can happen in many ways. Influenza viruses, for example, spread in the respiratory droplets, while human herpesviruses can be more promiscuous and

be transmitted in saliva, blood, and semen. Few viruses can spread between individuals in the form of naked particles. Most of them are instead protected by envelope, allowing the potential virions to come to the systemic circulation before they are recognized by the immune system of the host. All these steps in the lifecycle of the virus offer numerous opportunities for intervention and the immune system of the host has developed an array of different strategies for viral elimination.

Vaccines are one of the most beneficial tools in the field of public health. They have been able to win a battle for various types of diseases that have been ravaging human kind for a long time. Currently, more than 1 million lives are saved every year by vaccination. However, the war is not over and the virus is constantly working on its next move. There are many essay questions that have not been answered and many challenges to overcome. It takes about 10 years on average for a vaccine candidate to progress from the research stage to clinical trials and regulatory approval. Considering the size of the unique groups of viruses, the shortest time frame of 10 years for a single viral vaccine development is too long relative to that of the outbreaks alone. Moreover, less than 10% of the viral diseases are preventable by vaccination. Mass vaccination programs, when implemented properly, can dramatically reduce and control even eradicate viral infection at the population level. The number of vaccines against viral infection is also continuously increasing. The field of viral vaccines has matured significantly and this knowledge can and should be used for the development of more advanced programs against both existing and emerging viruses. However, the distribution of safe and effective vaccines in a large population is a significant challenge (Skenderi and Jonjić, 2012). Obtaining public acceptance of vaccination is also required. In addition to vaccination of the general public, vaccination programs may need to be carefully tailored to achieve optimal protection in specific populations, such as children and other vulnerable groups (S. Lauring et al., 2010).

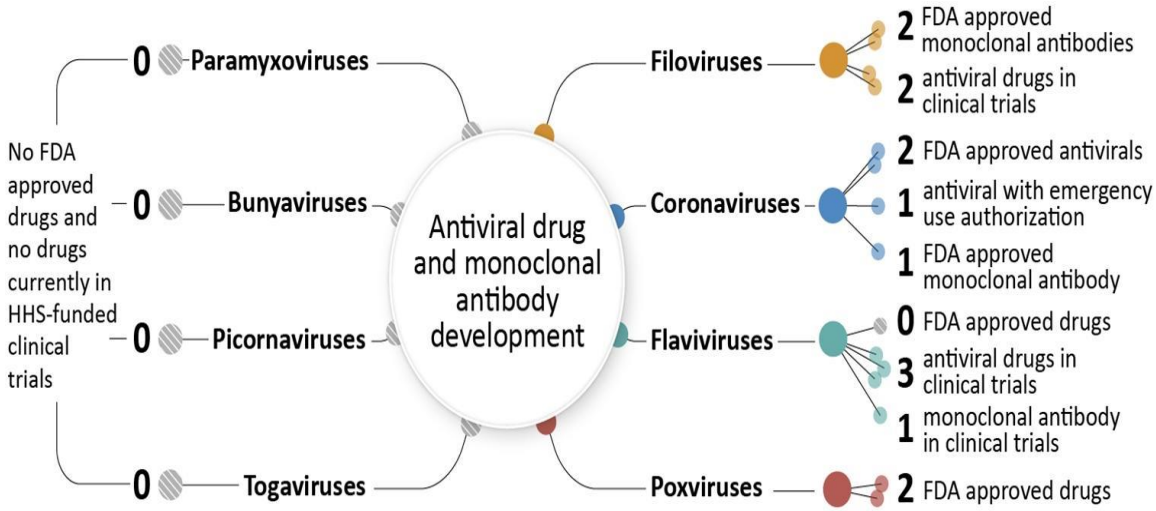
8.2. Antiviral Therapies

Antiviral treatment options have been limited. Viruses depend on host cells for their propagation, so most of a virus' life cycle is refractory to attack by antiviral drugs. There is a notable exception in the case of chronic and latent infections, where the viruses can be targeted after they have established a persistent infection. Like in normal cells, viral DNA is usually packaged into chromatin, and this may provide a target for the activation of the viral DNA by compounds. The role of histone deacetylase inhibitors as an anti-CMV therapy is a new concept. A competitive HDAC inhibitor was found to prevent the reactivation of CMV and other herpesviruses from latently infected cells. HDAC inhibitors are a new class of antineoplastic agents

with the first such compound already licensed for clinical use and many more are in phase 1-3 clinical trials.

Interest in developing additional antiviral agents, including compounds that inhibit viral DNA packaging, compounds that affect the transcription of viral immediate-early or early genes, interferon-like compounds, or antagonists. These advances will hopefully expand the available therapies for the treatment of viral infections caused by opportunistic viruses of the transplant patient, thereby broadening the current somewhat limited drug arsenal. On the other hand, since antiviral therapy needs replication of the pathogen, pathogens behave somewhat like parasites. An important strategy is therefore to disturb the host integrity or defense mechanisms at least temporarily.

Typical complications thus arise by opportunistic viruses but also by cytomegaloviruses which may cause hepatitis with significant morbidity and mortality. It might be a rational approach to apply antiviral treatment to all those patients and individuals at risk, or the implementation of measures to reduce the viral load in these products. The virus will hit the next generation, so we are in a fight against time, and so we better should use all the knowledge we have accumulated in the past for clinical trials in large populations. Such global initiatives against microbes could give rise to a new renaissance of appropriate ideas borrowed from the knowledge of epidemiologists and specialists in the history of emerging diseases.



FDA = U.S. Food and Drug Administration
HHS = Department of Health and Human Services

Source: GAO analysis of HHS and other documentation; GAO (icons). | GAO-23-105847

9. Emerging Viral Infections and Pandemics

Shortly after SARS had been contained, H5N1 'bird flu' crashed onto the scene with a new host species overcoming the ubiquitous nature of coronaviruses; almost simultaneously mumps was observed to be re-emerging in well-vaccinated American children. So a host of world experts worked to developed a book arguably encapsulating the state of the art understanding regarding Virus. The Update is more overt in its global relevance; the introductory chapter outlines the case for prevention and the necessary prerequisites (J. Burrell et al., 2016). The constantly increasing number of immunocompromised patients has exacerbated the situation, in particular in developed countries, amongst whom shingles now is a significant cause of morbidity. A host of world experts, mostly British with an unexpected bias towards veterinary science, were assembled and set to work to produce a multi-authored tome arguably encapsulating the then state of the art understanding regarding all families of RNA and DNA viruses. Alerting clinicians to quickly recognize and react to any unusual clinical presentation (or steep worsening in the numbers of a previously observed disease) should undoubtedly rank as the most important single measure in limiting the future spread of any of the 350(+) viral diseases. Many of these interventions require the country to which the infection is imported or in which it emerges lumbers itself with the economic cost of measures that may appear excessive relative to the imminent threat.

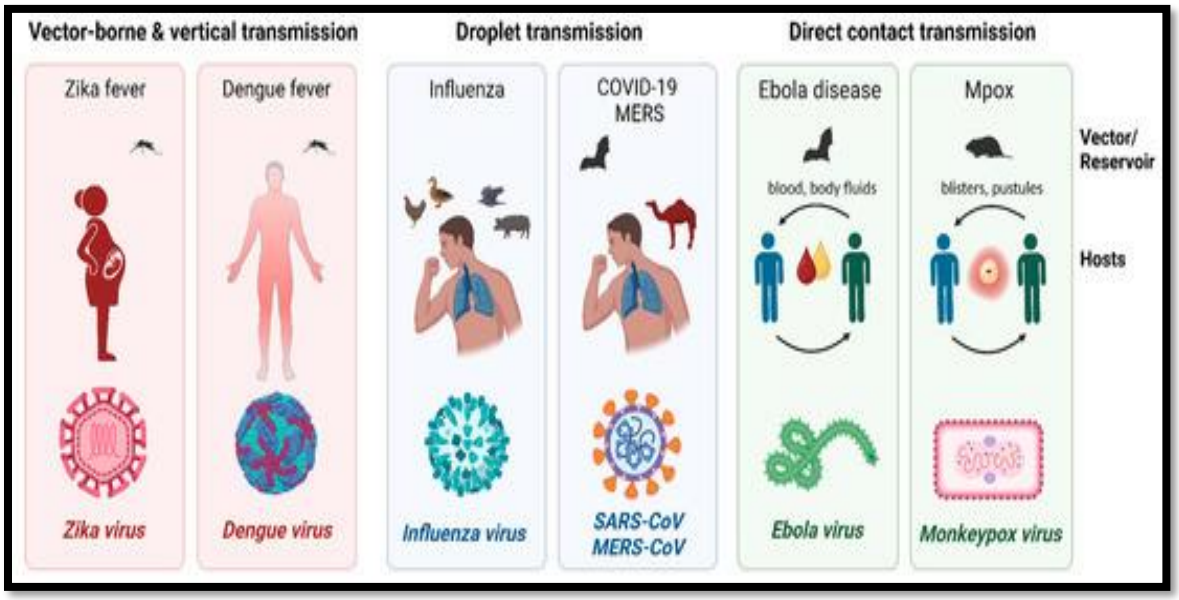


Figure 13: (Re)-emerging viral diseases: vectors, reservoirs, and routes of transmission. The causative viral agents, primary modes of transmission by vectors, reservoirs, and infectious biological materials, and hosts are shown (figure created with BioRender.com).

So this section of the act appears to offer yet another rod for the WHO to beat countries other than China. Evidently, fatter rats could be found around Suncheon, either making sightings more likely or indicating a locally common foodstuff to spread the infection in the first place. Importantly, Vishniac's account makes clear that the flu's introduction to Korea was not confined to the chosen village but most likely occurred at multiple poultry farms across it and the neighboring town. Visiting health care workers faced with a severely ill patient for whom they have no explanation should consider resorting to a history of home poultry deaths once back in the village. A history of trading with the villagers in whose homes the patient subsequently reports working would be yet further cause for consideration. In any other context, however, the described technique for taking a throat swab is likely to be useless, as the H5N1 strain was subsequently found to be a poor proliferator at the human upper respiratory temperatures. Pieces of this story might fit within an emerging paradigm of viral adaptation to new demographic niches.

10. Impact of Climate Change on Viral Infections

Climate change has become one of the most significant global public health challenges. It is broadly understood to influence the spread of infectious diseases and outbreaks of viral infections and has the potential to facilitate the transmission of viruses. The intricate relationship between temperature, precipitation patterns, human-wildlife interactions, and the emergence or re-emergence of particular disease vectors can perpetuate viral infection transmission. Vector-borne diseases are already increasing worldwide due to expanding human populations, their increased mobility, and the range of changing vector distribution, and altering ecosystems. However, only recently, the potential impact of climate change on viral transmission has been outlined (V. Parums, 2024). Environmental temperatures can have a profound effect on a range of epidemiological determinants affecting viral spread such as the odin hosts in pathogen replication (J Burbank, 2023). The positive effects of climatic variations can easily be understood: high temperatures enhance the rate of pathogen replication in their vectors or hosts as they lead to a greater biting rate of vector, or increased mobility of hosts, and can thus change disease transmission rates. The frequency of extremely low or high temperatures can have an impact on the survival of vectors, hosts, and the virus itself, altering virus population size. Higher temperatures may boost viral survival outside of hosts exuding them to a wider transmission window. However, the impact of lower temperatures on virus survival is still unclear. In addition, in the context of viral transmission, lower temperatures may affect human behavior, leading to greater in-home contact and thus a significant increase in transmission rates or success of its diffusion. Climate change can affect precipitation patterns, increasing the likelihood

of heavy rain and the subsequent flooding of agricultural and forest areas in seasonal areas of arboviral transmission, for example, increasing the number of mosquito breeding sites. In addition, extreme rainfall events can facilitate the transmission of food-borne viruses, exposing water sources to fecal contamination. Climate change can also lead to an increase in the simian range, affecting ecosystems dominated by viruses, such as Rodentia in North and South America. The anticipated increase in the size of the forest fire event will lead to changes in the coverage of the forest community, changing the spectrum of the vector species and its abundance thus the pathogens they transmit. Across this perspective, the melting polar ice caps can establish the possibility of onolvide infection cross-transmissions and the appearance of unprecedented viral strains. Climate change has a larger impact on the many correlates of respiratory infection. The environmental factors including, but not limited to, air pollution, relative humidity, temperature, rainfall, and extreme weather events have been associated with the incidence of viral respiratory infections. Climate change can alter the seasonal pattern of viral respiratory infections, with outbreaks occurring in environments currently unsuitable for viral transmission. In response, it is suggested that a set of season-specific public health strategies adapt to the information summarized in this outline in order to decrease sensitivity to climate change in accordance with global public health recommendations. (1) Build integrated care systems for treating patients affected by current and emerging viral infections; (2) provide access to clean water and sanitation across the epicenter of transmission; (3) enhance the capacity for accurate and rapid detection of viral infections; (4) support epidemiological surveillance; (5) promote collaboration along the individual nation and international borders; and, simultaneously, (6) enhance short-term forecasting of extreme weather conditions and develop suitable response strategies, including for building early warning systems. However, these resembled efforts are difficult to implement without sufficient information to take effective public health action. In that, enhanced combine meteorological and virological models that are able to predict the epidemic diffusion and mutation event of emerging strains of viral pathogens, would be of great benefit. Likewise, robust multiscale-modeling of specific events or seasonal patterns of disease incidence may be helpful for creative public health outcomes. From this vantage point, a complement of virological and climatological investigations is needed to achieve a comprehensive understanding of the multifaceted role of climate in viral infections. It is crucial that both epidemiologists and virology be meticulously organized to contribute to this combined approach in order to better predict and mitigate the creeping climate risk of viral infection.

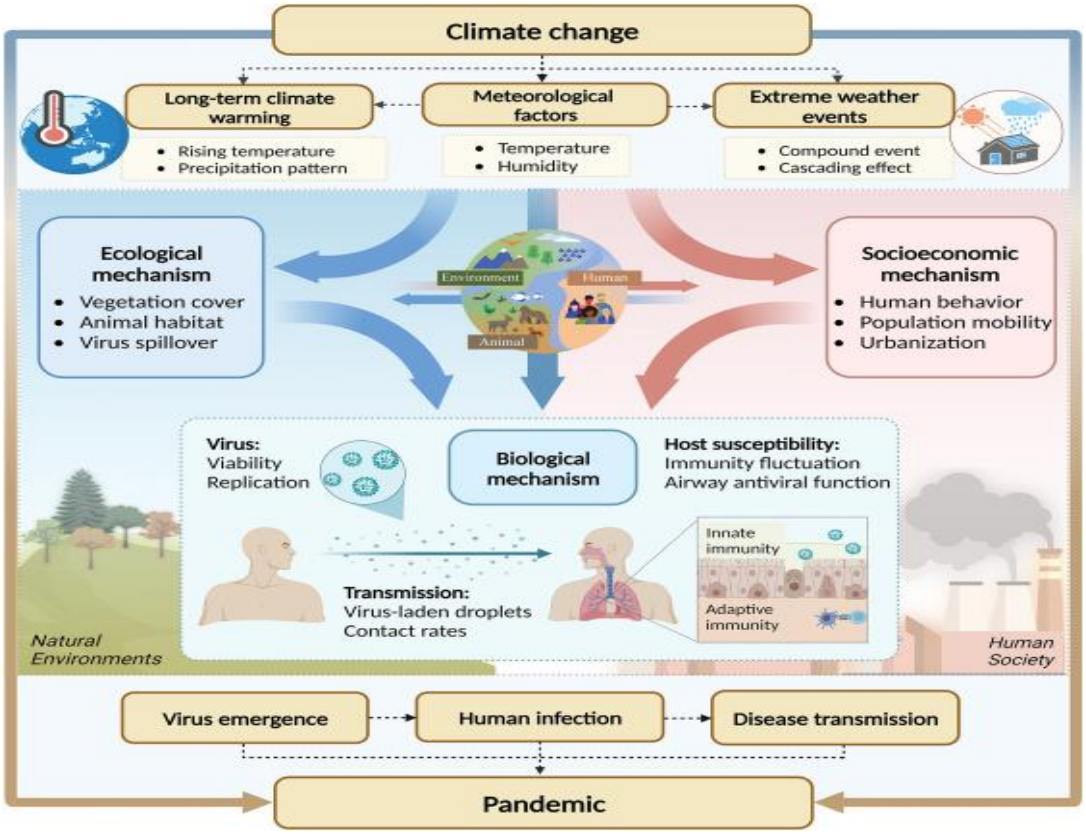


Figure 14: Mechanistic pathways through which climate change influences viral respiratory infections (VRIs). (Created with BioRender.com).

11. Ethical and Social Issues in Virus Research

In this section, ethical and social considerations regarding virus research are briefly explored. Should policy makers collaborate with biosecurity experts to designate a list of dangerous pathogens? Should biology research with viruses and other pathogens inform public health at large? Should bioethics require a higher bar for consent to participate in studies that may cause infection, even for ones that have been ethically cleared and approved by formal bodies? Should journal clickbait prioritize public health over reducing stigma? Emerging viral pathogens, the ethical imperative of informing public health, community engagement and transparency in research initiatives, the impact of the media on public perception of viral infections, and dual-use research and public health information sharing are touched upon, as is a wink to envision the unimaginable spread of the Severe Acute Respiratory Syndrome coronavirus-2.

Should IQ intelligence also require a higher bar for consent? The intelligence services have a long history of secretly funding projects and influencing the outcomes of university-scientist research. The news media have long been known as the Fourth Estate, holding leaders accountable and influential in framing public discourse. The marriage of these two has given rise to allegations of “fake news,”

prompting foreign intelligence to classify journalists as “influencers.” Would-be academics understandably fear retribution or embarrassment for publicly questioning the integrity of a prestigious institution, but in the interest of public health they feel ethically bound to inform a plurality of voices in academia.

Beyond Stigma and Viruses, there is a larger public health issue at hand with the current pandemic, and it is this: should the paper on viral shedding also have confidentially informed about broad but non-specific methods that would’ve resulted in a nation-wide school closures and a movement-strangled economy in a manner that would have been recognized by the general public through the media as an incendiary, salacious, and irresistible jigsaw puzzle? Would the need for apparent penny-dropping advance knowledge about a devastating outbreak have been satisfied if the leaked information was an “aha!” revelation on the order that humans eat fuzzy animals?

12. Future Directions in Viral Infection Research

Future Research Directions in the Study of Viral Infections. As the virome is increasingly linked with health and disease, emerging areas will propel knowledge of virus associated pathways, tissues, and species. Next-generation methodology and bioinformatics algorithms that enable matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry bacterial profiling can be applied to virome analysis to track virus distribution and infection in both agricultural settings and the clinics. Viral discovery could be expanded by focusing on bioinformatics tools that identify virus related sequences in unassembled short reads. Moreover, meta-analysis of virome profiles from human samples with diverse disease states, in the context of host individual omic data, could lead to the identification of specific virus co-occurring with altered host gene expression or metabolic imbalances. Use of rat models, combined with surgical techniques and environmental exposure, can substantially expand exploration of the unknown realm. Everyday surrounding virus contamination in an urban area is unknown, and there is likelihood of discovering novel viruses in unexpected places and hosts. In joint efforts involving interdisciplinary collaboration, people have successfully identified Ace Lake as a hotspot for viral diversity. Understanding viral cycle and ecology and initiating biogeochemical modeling can provide comprehensive information about the global carbon cycle and revolutionize the traditional view of a 'tree of life' centered on macro-organisms. At present, how these directions will unfold still remains to be seen (Ian Lipkin and Briese, 2014). Traditionally, the majority of viral disease diagnostics overall are simple and tend to focus on one aspect of a biological response to virus infections rather than the infections themselves. Recent developments have widened the scope of the eligible hosts

beyond the 18 that are standard in microbiology laboratories; particular attention has been dedicated to detection of novel or emerging zoonotic viruses, thereby developing new methods for wider detection range or better identification of known viruses. Complementary techniques have been developed that allow for rapid and sensitive testing for viral RNAs as well as complete kinomic analyses in order to identify host response against viral infections. Nonetheless, many gaps exist and significant improvements remain crucially needed to fight against viruses. An interdisciplinary approach combining both clinical knowledge and laboratory expertise should be fostered in order to allow these novel molecular technologies to have an impact in real world applications. The aim is to provide a concise overview of the current state of viral diagnostics, discuss relevant issues and propose potential directions to explore (F. Relich, 2017).

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Chapter Eight

Infection and Immunity

1. Introduction to Infection and Immunity

Our immune system acts as a protectant from a vast range of pathogens but as well as it plays a major role during the progression of a number of diseases. Researchers have gotten from many experiments that constant signaling of immune responses can be the main cause of a curved growth of a tumor. Macrophages, dendritic cells, NK and T cells, B cells are the dominating effectors of immune system completing the job. During activation of the immune response signaling from a cytokine is also inhibited. Systematic exposure of pathogenic proteins from different pathogens can also further increase the immune response (Verhoef and Snippe, 2005). It is also reported that a three-way feedback loop during the invasion of pathogens where different cells secrete different cytokines to stimulate the process whereas to inhibit that situation, and a bistable switch within the infected cells may seek to recover or to succumb.

While surpassing efforts gave leading edge knowledge there is still much to learn about the reaction of mammalian immune system in the course of diseases. This leads to many doubts and fears at how this categorized system will react on the entrance of a broad range of signals or pathogens. In response to this extensive stimulus one of the primary controllers of immune responses, lymphocytes, becomes relatively resistant to the furthers signals. As well as it can show narrow specificity and exhibit complex feedback relationship with the signaling molecules of the immune system. When the number of such pathogens goes beyond from some limit it will unable to handle the situation and resultant in fatality or morbidity (Gupta et al., 2016). Sometimes the immune response gives rise to autoimmune diseases. These diseases also can lead to morbidity and may be incapacitating. Detailed research studies explicate the interaction of immune system with the body and between the immune responses (IR) and pathogens to embrace these concerns. Bell shaped curves for instance are excellent to study how the immune system balance on an act of inducing immune responses or aborting them.

1.1. Definition and Scope

The purpose of the section was to give a brief introduction to several aspects of infectious diseases, viewed from the host as well as from the pathogen. Also, the basic principles of INNATE and ADAPTIVE IMMUNE RESPONSES, especially in debilitated patients, are described. Furthermore, detailed information on the pathogenesis of septic shock, AIDS and vaccination strategies are given. After chromosomal analysis became available in the sixties and antibiotic therapy made rapid progress, microbiology lost much of its visual appeal. However, pathogens still pose an incredible challenge and numerous problems in our endeavour to confine them. Similar to the eternal fight of the world of good against the world of evil, the world inside the body is permanently harassed by the world outside the individual. As a result of this permanent qualm in the hidden life of the bacterial, viral, fungal and parasitic jungle, we suffer from a vast amount of terrible diseases, jeopardizing our life and welfare. However, this unwanted attention did lead to an ever-growing development of healthcare policies involving budgets that threaten our future in a way no one had predicted. In view of the scientific as well as the socio-economic relevance of the problems involved, Infectious Diseases have thus come back to the forefront of medical interest. It is felt that a broad picture of the current state of insight of Infectious Diseases, Immunology and Antimicrobial Therapy would be welcome under these conditions. The present chapter aims to give a concise presentation of a few essential features in this immense panorama, which will be articulated in more detail throughout the text.

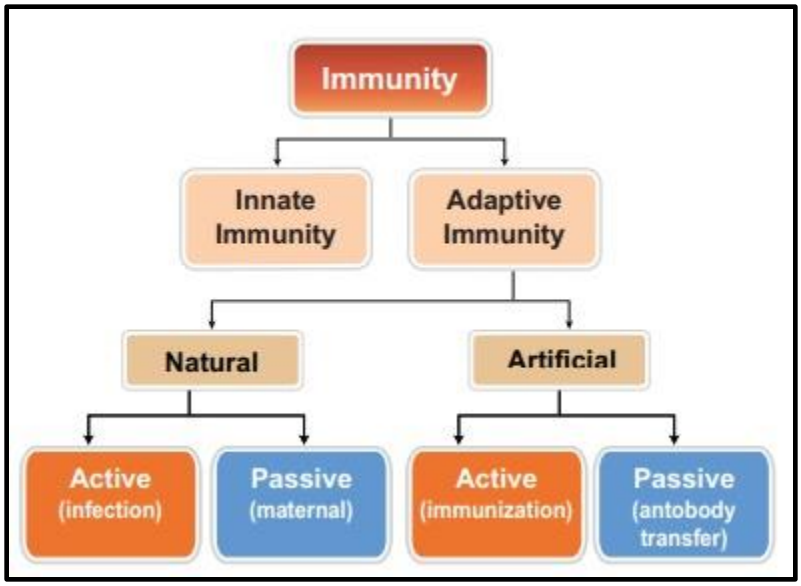

Figure 1: This diagram simplifies the types of immunity.

We are continuously exposed to many pathogens through inhalation, ingestion, and touch. In order to protect us from these innumerable uninvited guests (flatworms, roundworms, bilharziae, flat tumors like cestodes and planaria; external parasites such as fleas, lice, ticks; magnificent six-legged creatures like scabies (sarcoptes) that mark all what is expected from a bug and can claim his knighthood; bacterial, mycotic and viral agents), the immune system was created.

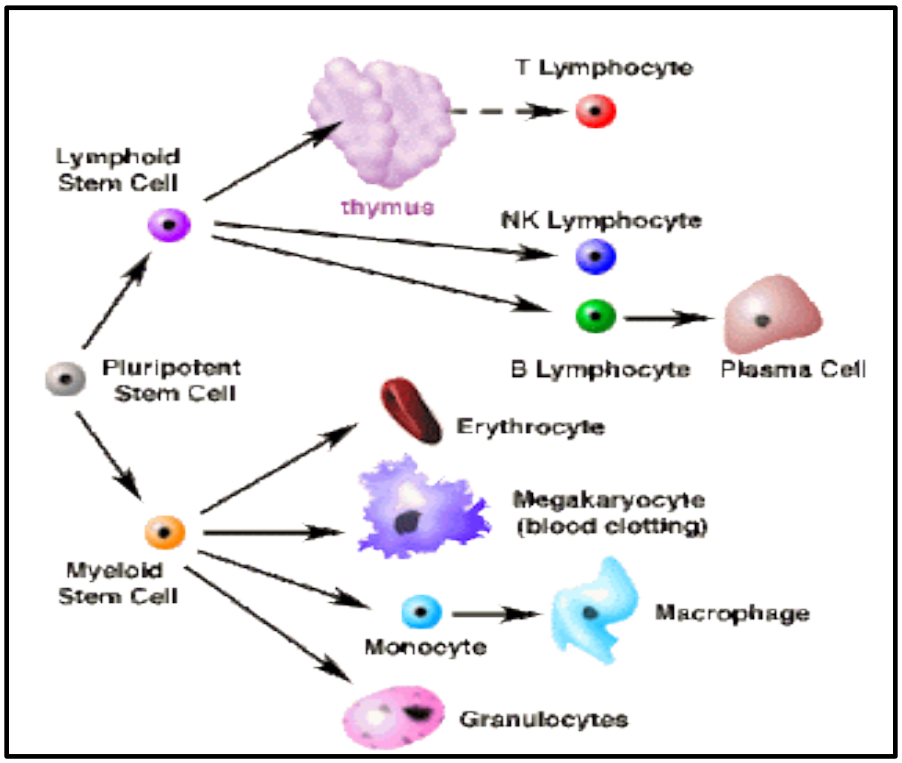

Figure 2: The main cells types of immune system .

Thanks to its marvellous reactivity, specificity, memory response, and cloned amplification, many cranial nerves (I to XII) have been traumatized or even severed in their alarming signals (e.g. headache) and specific requests (e.g. cough). White cells would even happily jump from a 13-story building, like old slavs or basques in hopes that enchained banknotes would start killing for libero arbitrio with the illegal dodger, in other words by provoking a peri-mortem increase in intra-cranial pressure that would counterbalance the harmonics' auric-masterpiece, tithing passphrase, and golden intellection-states by means of oper-mand-mantric adjustments. Hence the virginal recipient would have the potentiality to gather the trusty 12 chosen instruments and enter the holy grail in no-lens volens funzioni of the adverbially speculative preterite. Anyhow, these symbolic deprecation of waning projectile forces do not impair the holocaustic droit du seigneur over the humble possessed territory.

The immune system is the panoply of weapons designed to protect our body from various harmful agents. The skin and mucous membranes provide a native, totally non-specific resistance. If these physical barriers are bypassed, specific mechanisms come into play, generally triggered by the presence of molecules that are not "self." Therefore the ability of the host to recognise and respond to invading pathogens seems to be the prime consideration in controlling infectious diseases. Such a response may occur at several levels. First, the physical barriers to infection, skin and mucous membranes, prevent the invaders from penetrating into the body. However, if bacteria, fungi or viruses succeed in entering through skin lesions, alimentary or respiratory tracts they meet with a sophisticated defence mechanism. Phagocytes, together with a complex system of plasma proteins operating through proteolysis interact in a cascade manner and accomplish the death of the invaders. Of course, bacteria and viruses have developed mechanisms to bypass this generally non-specific collection of defensive weapons, and haemopoietic cells have evolved complex mechanisms for recognising "non-self" macromolecules or small molecules typically associated with invading microorganisms. Invasion by bacteria, parasites and viruses raise an inflammatory reaction which commonly results in raised temperature (fever), a whole-body response triggered by released cytokines, macrophages or endotoxins. Sad to say, the immune system is only one aspect of defence. There are several ways for bacteria and other organisms to escape all the barriers and immune responses the host can use to respond to the invasion (Verhoef and Snippe, 2005). The immune system, for example, although relatively specific, does not operate as promptly as the akinetic system and during the delayed day, time for uncontrolled replication of the pathogens occurs. Some families of bacteria also engage in subterfuges such as intracellular parasitism. Evasion is a word that should still be looked up in the Oxford Dictionary: There is one too many Indian cities in the Collins of the poor lost world, wasting their lives in a terrifying endeavor of reconnaissance.

1.2. Historical Perspectives

The earliest records of medical practice suggest a surprisingly sophisticated understanding of diseases, particularly infectious diseases. The isolation of inhabitants and regulation of food and water supply during the time of the Exodus in the 13th century BC represent the earliest records describing these actions. The Hippocratic collection recognises epidemics or "plague" as a full entity of disease epidemiology. The idea that similar diseases occur in man and animals dates back to the period of Aristotle and Theophrastus. The Thucydides, a famous general of Athens, considers the epidemic illness that destroyed the Peloponnesians in Sicily in the 5th Century BC as scientific observation. In the following centuries, as the Roman Empire expanded, transmission of diseases over wide territories also

increased.In 138 AD, the Roman Empire was devastated by the Cyprian plague. A quarter of the population at Alexandria died. Once established plague remained in the region for another 15 years leading to a wave of depopulation and abandonment of agriculture and trade. It is interesting that the documentation of the disease is remarkably similar to the one described by The Thucydides in Athens in 424 BC. It was not until the Renaissance that the skeptical balance of power shifted from priest to physicians, although, the practice of blood-drawing persisted into the 19th century as a treatment to balance the bodily "humors". Variously spiking fevers, chills, rigors, sweats, nausea, diarrhea, abdominal pains replaced the pattern of prevailing symptoms such as a headache, encephalitis, and comas described as "brain burning". In a majority of cases, irregular fevers with chills and rigors accompanied the more or less localized inflammation of organs – fever with "shaking attacks". The story of the malaria continues unstoppably until we reach the connections between life cycle, symptoms, and geographical distribution described by Laveran the end of the 19th century. In my opinion, it is difficult to find a better example in the history of medical science of the limitations that affect the physician or naturalist during the long history of malaria.

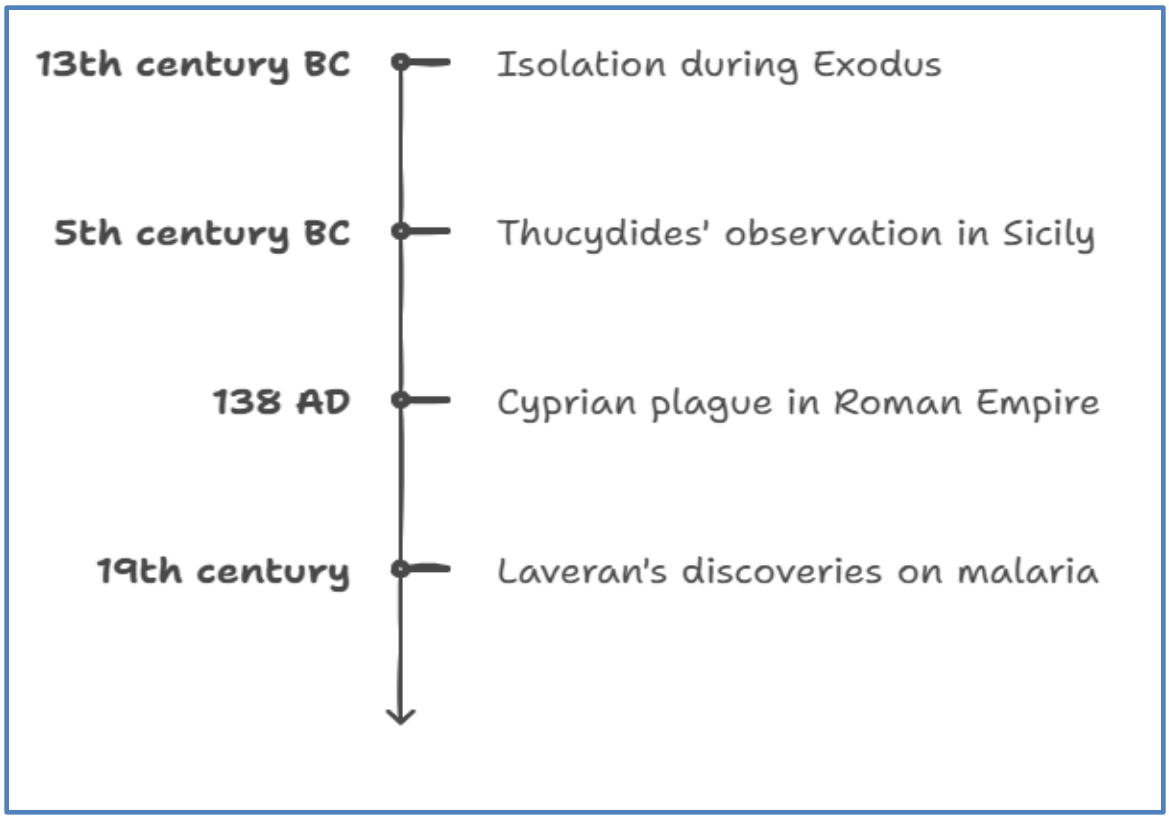

Figure 3: Evolution of Medical Understanding of Infectious Diseases

2. Basic Concepts in Immunology

The immune system can detect and respond to a nearly infinite number of foreign substances, from viruses to venom to diabetic test strips. Our sixty tons of cells carry out these functions despite the fact that the typical immune cell is about the size of a naked virus and the mechanisms behind immune function take place in a few drops of interstitial fluid composing less than 2% of body mass. The immune system can be split into two parts – the innate, which is non-specific and has no memory or tolerance, and the adaptive, which is highly specific, memory-capable, and tolerant. As a result, even though the average person can be exposed to microorganisms hundreds of thousands of times a day, most are protected by an evolutionarily honed immune response.

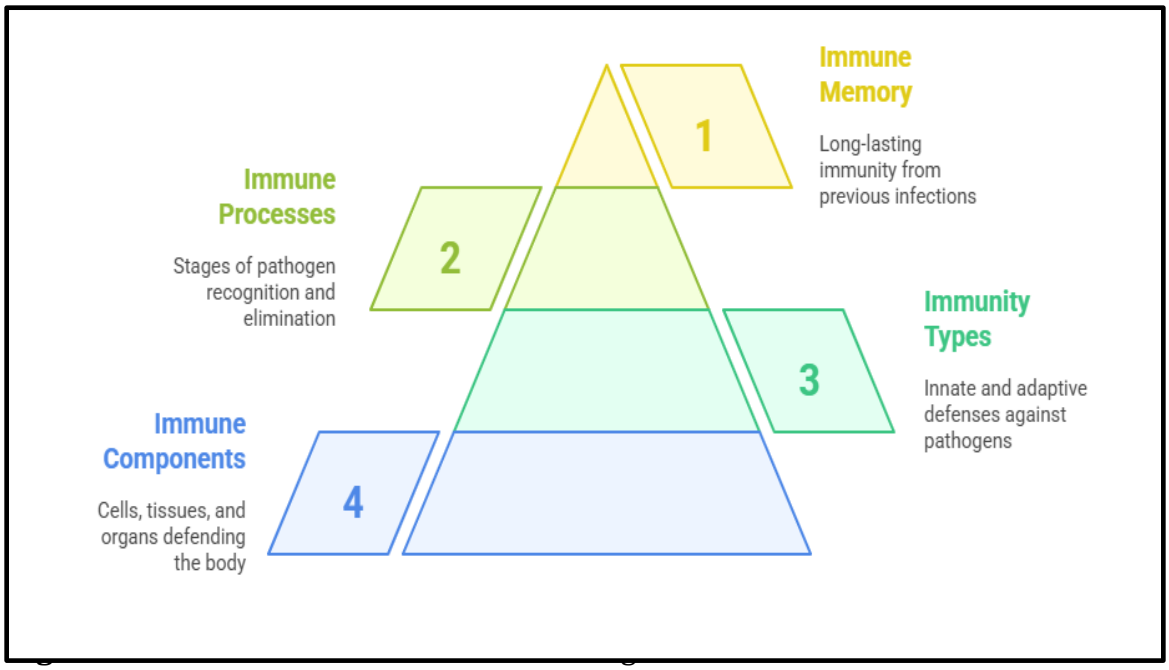

Figure 4: Immunology Hierarchy.

Whether an immunologic incursion results in protection, chronic inflammation, or disease, the basic mechanisms can be explained by the immunologic principles. Actions attributed to immunity include protection from infections, the rejection of tissue and organs post-transplantation, and resistance to the growth of cancer cells. However, the immune system can sometimes also cause unintended consequences, such as chronic inflammatory diseases like rheumatoid arthritis, allergies to otherwise benign substances like peanuts, and self-reactivity resulting in autoimmune diseases.

2.1. Innate vs. Adaptive Immunity

Infection is recognized as "the transgression of a host's own boundaries by another living being, the parasite or pathogen." Through 'evolutionary arms race conditions', hosts and pathogens develop extraordinarily efficient and complex interplay mechanisms. In the event of environmental contamination, there is no reason for concern, as the skin and mucous membranes serve as true fortifications. Skin is a multi-layered, hardened structure, mostly composed of the compact lipid substance keratin. The gastrointestinal, nasopharyngeal, tracheobronchial, and urogenital pathways are covered by mucous membranes, which consist of ciliated, rhythmically viscous gland cells. The nail and keratin components of hair follicles are repelled along these membranes, carrying all contamination.

After bypassing these barriers however, pathogens encounter a complex network specifically designed for the prompt elimination of invaders. The immune system is divided into two closely intermingling forms: innate and adaptive immunity. Innate immunity is the first line of immunological and non-specific defense mechanisms against infections. The adaptive immune response acts more slowly, as it demands the proper triggering and activation of an immune response (Warrington et al., 2011). However both systems complement each other, particularly for developing a more specific immune memory. The resulting synergic procedures help to eliminate aggression and maintain cellular homeostasis. Disruption or dysfunction of either system (innate or adaptive) can lead to pathological outcomes. Outwardly: autoimmune diseases, immunodeficiencies, hypersensitivity (allergy) conditions, etc. Inwardly: the onset and/or advancement of infectious diseases, and eventually, death.

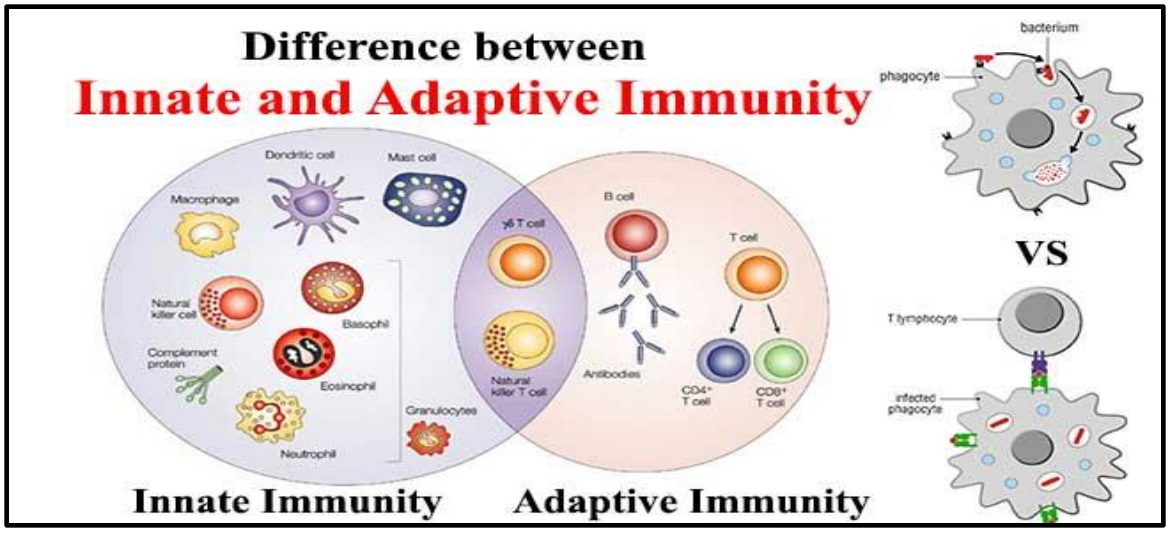

Figure 5: The difference between innate and adaptive immunity.

2.2. Cells of the Immune System

The human immune system is a complex network of specialized cell types and organs that work together to protect the organism from infections, clearing wounds and healing tissues, and eliminating aberrant and transformed cells. Resistance, immunoregulation, and memory are the three mains pillars that define the immune response. The immune system utilizes two different arms to counteract infections and ward off danger. First, it activates an immediate and non-specific defense represented by the innate response. Innate cells are not specialized in pathogen recognition. They use a small number of germ-line-encoded receptors that are able to broadly recognize molecular patterns associated with dangerous agents. Receptor binding triggers signal cascades leading to pathogen clearance, cytokine and chemokine release, and finally, cell activation and antigen presentation.

Cells of the innate response are divided into three groups: circulating components, such as monocytes and granulocytes; tissue-residing components, like Kupffer cells and microglia; and the so-called Lymphoid Lineage-Innate Response Cells.

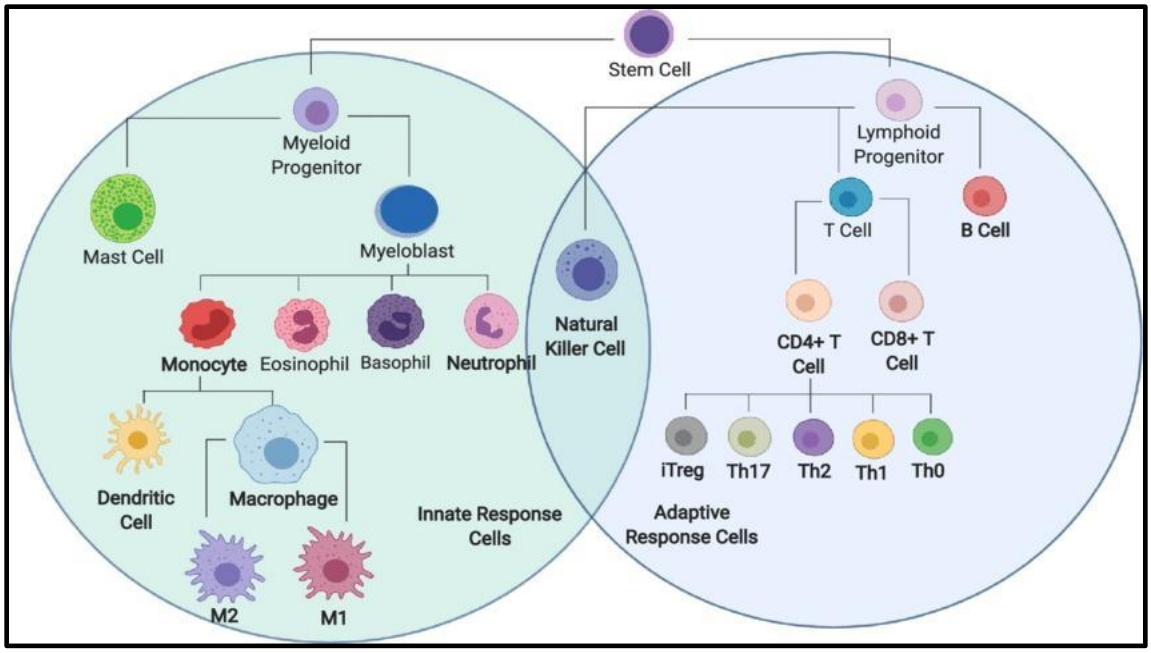

Figure 6: This diagram illustrates the differentiation of immune cells from stem cells into myeloid and lymphoid lineages, highlighting innate and adaptive immune responses.

These last cells are originally part of the lymphoid lineage, but represent an ancient cell type that express receptors able to recognize non-specifically “danger signals”. The second line of defense is the adaptive response. Unlike the innate system, the adaptive immune response is highly specific for different threatening agents. It uses cellular and humoral mechanisms to destroy infections, and mounts immunologic memory to remember past infections. Monocytes and B-lymphocytes are “professional” antigen presenting cells that

capture, process and present it through MHC classI (for activated CD8+ cells) or II (for activated CD4+ cells). Activation is critically dependent on the presence of co-stimulatory proteins and cytokines secreted by the same cell or by collaborating innate elements. After activation, they differentiate into effector cells, M1 macrophages and Th1 cells, respectively (Proserpio and Mahata, 2016).

3. Mechanisms of Infection

Acute pyogenic infection is essentially caused by a few organisms. The nature and pathogenicity of the organisms is discussed, particularly with regard to the mechanisms they use in establishing infection. Virulence depends on the capacity of the organism to grow and multiply within the body tissues.

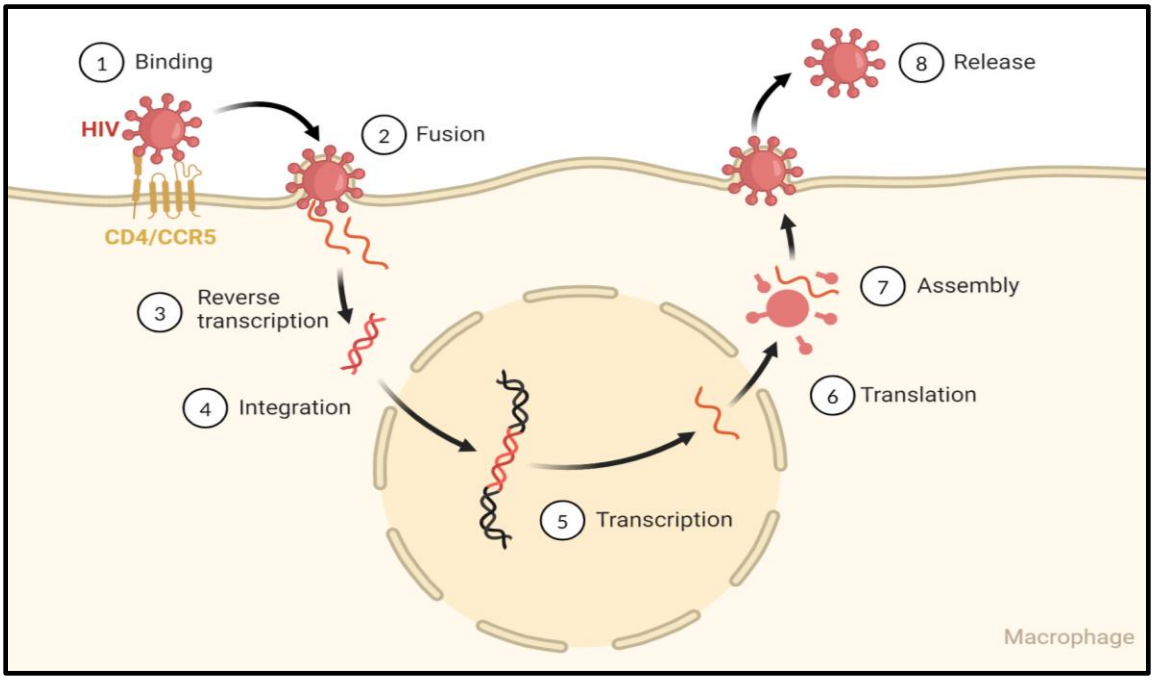

Figure 7: This diagram is an example of mechanisms of infection so that it illustrates the key steps of HIV infection, including binding, fusion, reverse transcription, integration, transcription, translation, assembly, and release of new viral particles.

The ability to produce adhesion to host cells and toxins also plays a part in the infection process. Immunity in the susceptible host limits the effect of toxins and inhibits the growth of the organisms (Fierer et al., 2016). Early inflammation limits the spread of the organisms and may lead to containment or resolution (AR Webb and M Kahler, 2008).

3.1. Types of Pathogens

Microorganisms that give rise to infection in humans belong to five main groups of organisms: bacteria, viruses, fungi, protozoa, and flatworms. Bacteria are single-celled organisms that can be divided according to their shape and the way in which they take up a stain. They can be stained using a range of dyes and are then 'gram positive' or 'gram negative' according to whether they retain the dye. Bacteria can colonize living tissue or can live freely in the environment. Organisms that cause diseases are known as pathogens. These include both minimally pathogenic species and highly pathogenic species. Viruses are noncellular entities made up of genetic material and a protein coat. Viruses cause disease by using the host cell to make many copies of the virus, which then go on to infect further cells. Fungi are relatively complex organisms that belong to the kingdom of eukaryotic organisms, which includes all animals, plants, and protists. There are two broad groups of fungi: yeasts and filamentous fungi. Fungal infections usually occur on the outer layers of the host, where the environment is relatively nutrient-poor. Protozoa are a diverse group of eukaryotic microbes that exist as free-living organisms or parasitic forms. Helminths, also known as parasitic worms, include two groups that infect humans: nematodes and trematodes. Prior to infection, the pathogen has to encounter the host gastrointestinal and respiratory tracts, or abraded surfaces of the epidermis, where there are a number of physical and chemical barriers that protect against infection.

3.2. Host-Pathogen Interactions

The vital and multi-faceted immune response to pathogens, whether bacterial, viral, fungal, and parasitic, is woven together from a coordinated combination of physical, cellular, and humoral mechanisms. An in-depth understanding of infection and immunity has come from research in a broad range of disciplines. This study encompasses evolution, biodiversity and ecology, anatomy and physiology, genetics, development, defense mechanisms, mathematics, chemistry, microscopy, and molecular and cellular biology. Numerous questions remain to be explored. For example, what is the developmental pathway of the defensive immune system? How do the powerful defense responses of plants develop, and how have the mechanisms and strategies evolved in the host and the pathogen?

The first line of defense is nonspecific and hypothesized to provide a time window for the activation of specific responses. The immediate response of the alternative pathways is believed to be initiated by target recognition failure; thus, surface constituents of pathogens have been shown to interfere with the alternative pathway of complement activation. The specialized pathway detects a broad spectrum of (micro) organisms that emphasizes interactions with non-self surfaces

or structures. The adaptive defense system in vertebrates provides an additional layer of defense with a diversity and specificity of protective responses. At an advanced stage of infection with viruses, mature dendritic cells (DCs) have been shown to become infected, and the virus uses DCs' own migration to spread systemically. Apically situated Mycobacterium tuberculosis protein tyrosine phosphate A (PtpA) excludes host vascular H+ - ATPase from the phagosome membrane that inhibits acidification and maturation (Fierer et al., 2016). In the case of Salmonella enterica serovar Typhimurium, the intracellular niche expands using the type III secretion system encoded by the Salmonella pathogenicity island-2. In Streptococcus pyogenes, the M1 protein inhibits cathelicidin, allowing the bacteria to survive in phagocyte extracellular traps. Efficient and potent mechanisms of resistance occur when the pathogen manages to evade contact with active complement. Plasmodium, Trypanosoma brucei, and Trypanosoma cruzei express surface proteins that interfere with the classical pathway. Furthermore, the YinO Yang1 transcription factors are the major genetic control for the expression of a family of variant surface antigens in the African trypanosomes. HIV preferentially infects HIV-specific central memory CD4 + T-cells as these cells become infected during the first wave of viremia, drastically reducing equivalents of HIV memory CD4+T clones, leading to defective immune responses. Seriously ill patients show high plasma levels of Latency Associated Peptide (LAP), a cleavage product of Transforming Growth Factor β (TGF-β) and a marker indicative of LAPof propeptides during synthesis. Elevated levels of LAP in healthy donors represent the early levels in patients developing AIDS within 5 years. Microbial translocation is a cause of systemic immune activation in chronic HIV infection, induces increased production of kynurenine and the exhaustion of CD4 T cells, leading to the development of AIDS. A three-layer defense system involving a combination of integrin signaling, reactive oxygen species production, and cell cycle arrest prevent Shigella from pirating the host actin cytoskeleton to spread, limiting the bacterium to cell-to-cell spread in tissue culture (Soni and Pandey, 2024). Stress-signal mediator Alternaria alternoxin blocks fungus-induced host immunity in plants to suppress the release of extracellular adenosine triphosphate and reactive oxygen species. Invasion strategies have been found to further complicate the host response by unexpectedly also requiring other known virulence factors. Intriguingly, those proteins that serve as a key machinery for invasion are directly targeted by various toxins of the host, involving for example Sortase mechanism, thus generating novel type therapies against infections. Aspergillus fumigatus requires gliotoxin for successful invasion and dissemination. Inflammasome-deficient mice and patients with diminished activity of this innate immune response are more susceptible to invasive aspergillosis. Fungus-parasitized ants in Thailand represent the dawning of ecological research and are caused by specialized fungi of the genus

Ophiocordyceps. Genomically encoded fungal small peptides induce host apoptosis to facilitate pathogen spread. Detection of these sign peptides triggers a targeted immune defense by inhibiting fungal production. Thus, ant colonies have both an adapted height variation that controls natural spore dispersal and leaf removal as vector, reducing exposure and infection rate to soil contaminants. At high infection rates, created ecosystem hotspots for fungal growth generate highly prevalent manipulated 'zombie' worker ants, which attract host colony parasites, resulting in an ecosystem-level light enhancement of fungal infection.

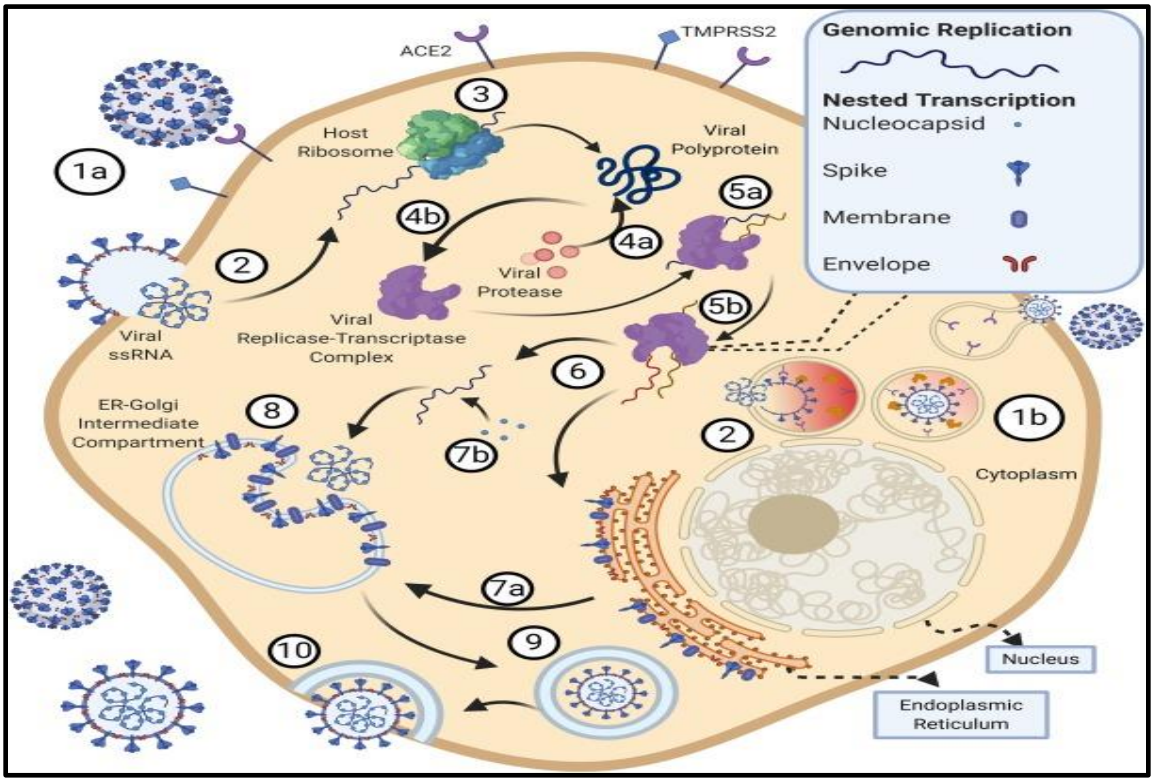

Figure 8: Host-pathogen interaction in COVID-19

4. Immunopathology

The study of disease states associated with underactivity and overactivity of the immune response is immunopathology. The underactivity of immunity is a deficiency state. The overactivity of immunity is autoimmunity, in which the immune response is directed at self. Similarly, donor tissues transplanted into a recipient may evoke an immune response in the recipient against the donor tissue. This is called transplantation immunopathology.

In order to understand immunopathology, it is necessary to be acquainted with the pathophysiology of normal immunity. Immune responses enable the body to recognize and eliminate pathogens. The elimination is coordinated by phagocytosis,

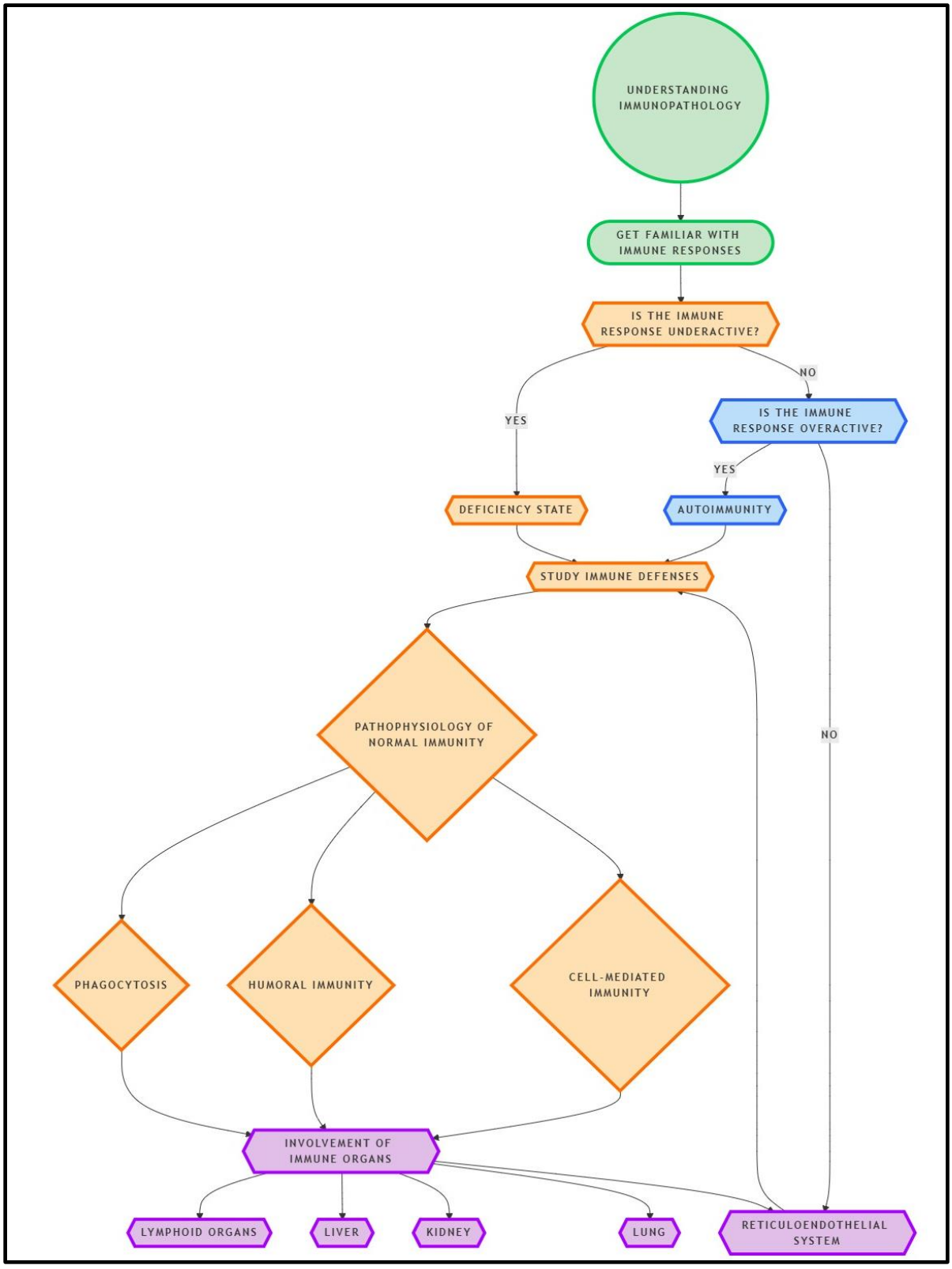

Figure 9: This flowchart outlines the classification of immune responses, distinguishing between underactive (deficiency) and overactive (autoimmune) states, and explores key aspects of normal immunity, including phagocytosis, humoral, and cell-mediated immunity.

humoral immunity, and cell-mediated immunity. Organs and tissues of the immune system include the lymphoid organs, notably, the thymus, lymph nodes, spleen, and bone marrow; the organs such as the liver, kidney, and lung; and the reticuloendothelial system, which is involved in the disposal of metabolic waste, along with the specialized immune cells of the white blood cell line, such as granulocytes, monocytes/macrophages, dendritic cells, and lymphocytes.

4.1. Autoimmunity

The essential characteristic of autoimmunity is the immunological attack on self-tissue, in which B and/or T cells specific for self-antigens are generated and have escaped mechanisms that maintain tolerance to these antigens (Eriksson, 2018). In 1957, two papers marked the convergence of the second half of the 20th century "as an era dominated by investigations of immune reactions". These publications documented the presence of myelin-specific antibodies in the brain of patients with acute disseminated encephalomyelitis and experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis, setting the stage for the modern era of research in autoimmune diseases. In the ensuing 60 years, we have witnessed an astounding growth in the understanding of the mechanisms involved in the genesis of autoimmunity, due to a joint effort involving both immunologists and specialists in the various fields of clinical medicine.

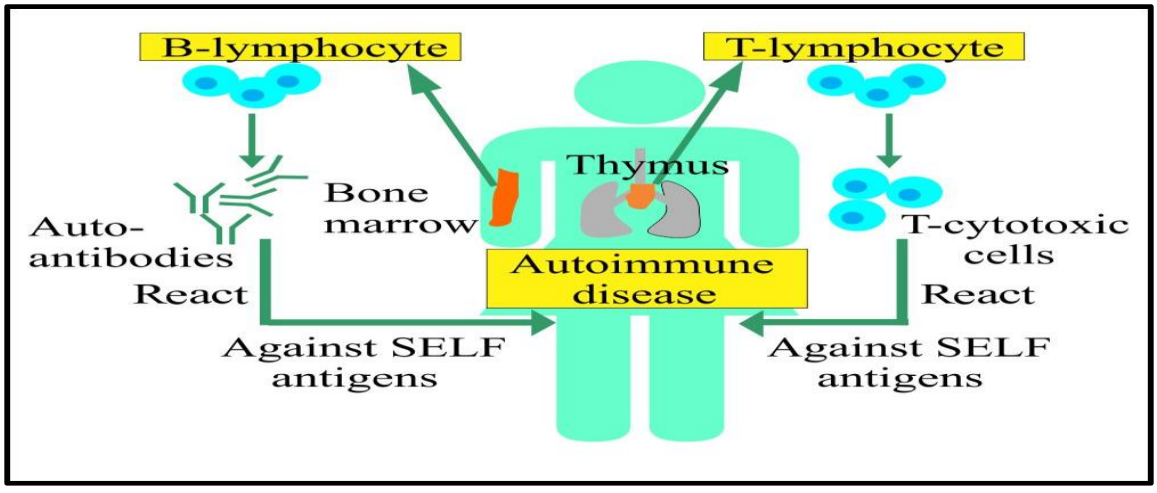

Figure 9: Mechanism of Autoimmune Diseases.

The word autoimmunity was coined in 1963, four years after Burnet's monumental work on clonal selection in the development of immune tolerance. Since then autoimmunity and autoimmunity-related diseases have been at the forefront of immunological research. In the late 1980s the completion of the human genome project and advances in molecular biology set the stage for a more rational approach to deciphering the pathoimmunology of autoimmunity, and by the dawn of the new century, increasing efforts and resources which had been invested in searching for

genes associated with the susceptibility to autoimmune diseases began to bear valuable fruit, providing a major push to the fields of systems biomedicine, complex network biology and preventive and personalized medicine. This paper is an attempt to review these pieces of research and their implications, to place them into an historical perspective with a few future perspectives, and to point out potential pitfalls, obstacles and challenges that must be confronted and overcome in order to yield tangible improvements to the life and health of millions of affected individuals (Hartwig Trier and Houen, 2023).

4.2. Hypersensitivity Reactions

The pathophysiology of infectious diseases can be described as follows: The first line of host defense against infection is the body's physical and chemical barriers. A breach of these barriers can lead to colonization and invasion of the organism by microbial agents, defined as infections. Once inside the host, pathogenic microorganisms can survive, multiply and induce a disease. The interaction between host and pathogen can be seen as a duel where the weapons of the combatants are modulated by the evolutive pressure of the adversal counter part.

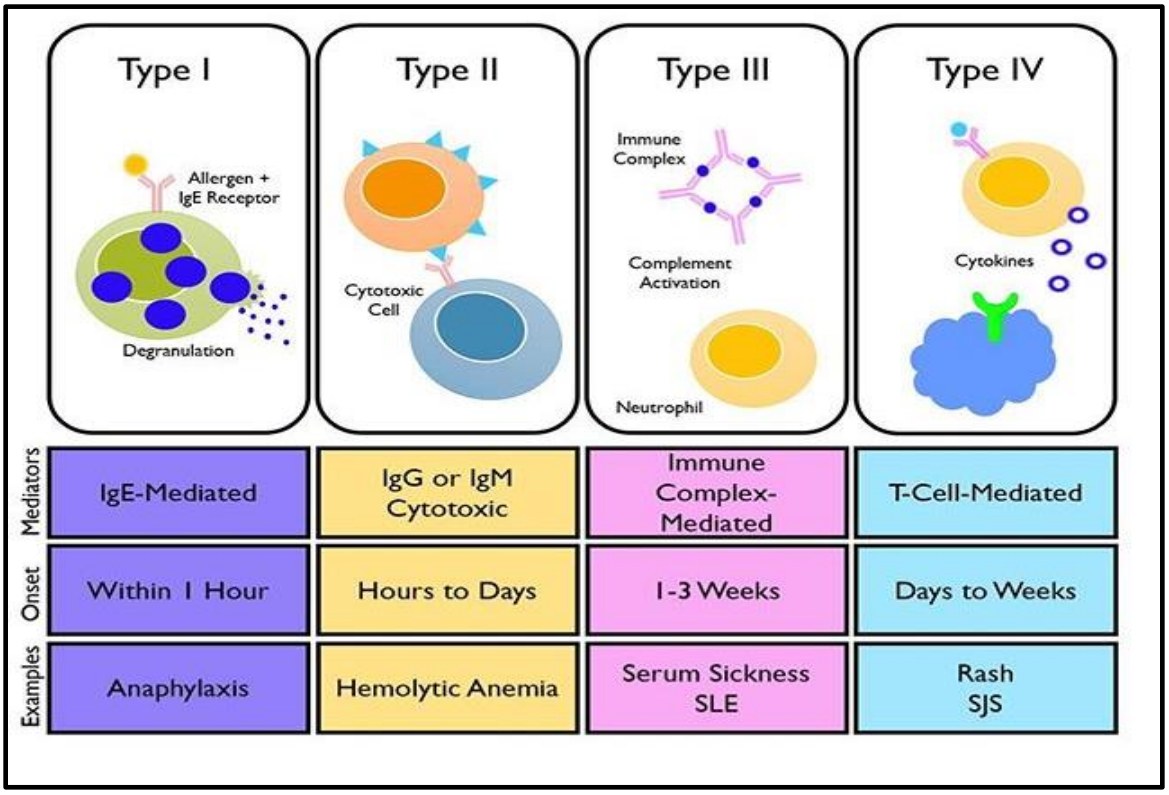

Figure 10: Types of hypersensitivity reactions.

Hypersensitivity is the overreaction of the immune system against antigens that are usually harmless, resulting in pathological conditions. Several types of

hypersensitivity reactions can be developed, based on the immune effectors and mechanisms involved. The pathophysiology of infection-derived disease and hypersensitivity-mediated pathology can be approached from the perspective of the combinatorial resources utilized by both host and pathogen during their interaction.

Hypersensitivity reactions are classified into four types: Type I refers to immediate and IgE-mediated (classical allergic or anaphylactic reactions) and it usually consists of a rapid reaction after surface exposure to antigens or allergens. Type II is delayed and is mainly linked to cytotoxic mechanisms against the host's own cells. Type III is characterized by tissue damage due to activation of complement by the binding of antigen-antibody complexes. Type IV is delayed and is produced by the activation of mononuclear cells such as macrophages or T lymphocytes.

5. Vaccines and Immunization

In 2000 more than 7.5 million children under the age of 5 years died of pneumonia, measles, polio, tuberculosis, diphtheria, pertussis, tetanus, or yellow fever, some defenseless against these deadly agents of infection. Many of the diseases from which children suffer can be prevented through the use of vaccines. Vaccines for some diseases have not yet been developed and for others vaccine coverage remains low. Consequently, millions of children continue to die from vaccine preventable diseases each year. Nevertheless, considerable advances in the development of vaccines are being made and there are a number of exciting developments on the horizon. Immunization is one of the most successful and cost-effective public health interventions worldwide, responsible for eradicating smallpox and controlling disabling and lethal infectious diseases such as polio, measles, and tetanus, diphtheria, which primarily affected children. To maintain protective barriers against infection by pathogenic agents, the immune system has evolved to mount robust immune responses upon reencounter with pathogens to which it was previously exposed. The ultimate goal of vaccination is to mimic the immune response seen during natural infections, eliminate the pathogen before it can cause disease, and establish a state of long-lived memory against reencounters. Vaccines are composed of antigens or processed fragments of these immunogens, which are often combined with adjuvants. Major questions within immunology remain unanswered, concerning the kinetics and location of the development of immune responses in lymph nodes in response to immunization, the cellular responses that lead to the generation of immune memory, and the interactions of T and B cells that result in the generation of protective immunity. However, recent technological developments open up new ways to address these issues. Major progress has been made in the development of new adjuvants and vaccine delivery systems that improve the effectiveness of vaccine-induced immunity.

5.1. Types of Vaccines

Affinity-matured IgG antibodies can only be produced by re-stimulated memory B cells, which depend on presentation of the original cognate antigen on MHCII molecules by resident antigen-specific Tfhs in the germinal center (Jiskoot et al., 2019). Without engagement of CD40 on B cells by CD154 on Tfhs and without the provision of cytokines like IL-21, only abortive (low affinity) extrafollicular B-to-plasma cell differentiation in the red pulp of the spleen can take place. Without engagement of CD40L on B cells or presentation of cognate antigen, Tfhs sustain the B-cell helper phenotype for only a few hours upon Tfhs/Bs fleeting interactions in the dark zone. Thereafter, ICOSL and OX40L are internalized, while B cells downregulate the expression of the chemokine receptor CXCR5 and the responsiveness to CXCL13. Therefore, transiently arrested B cells in the dark zone re-express S1pr2 and/or EBI2 and leave the dark zone, thus terminating cognate interaction with Tfhs and migration into the light zones. For these reasons, unless B cells present the original cognate antigen and upregulate the expression of MHCII and CD86 on the cell membrane, they are not able to generate high affinity IgG antibodies or to undergo affinity maturation and differentiation into memory B cells.

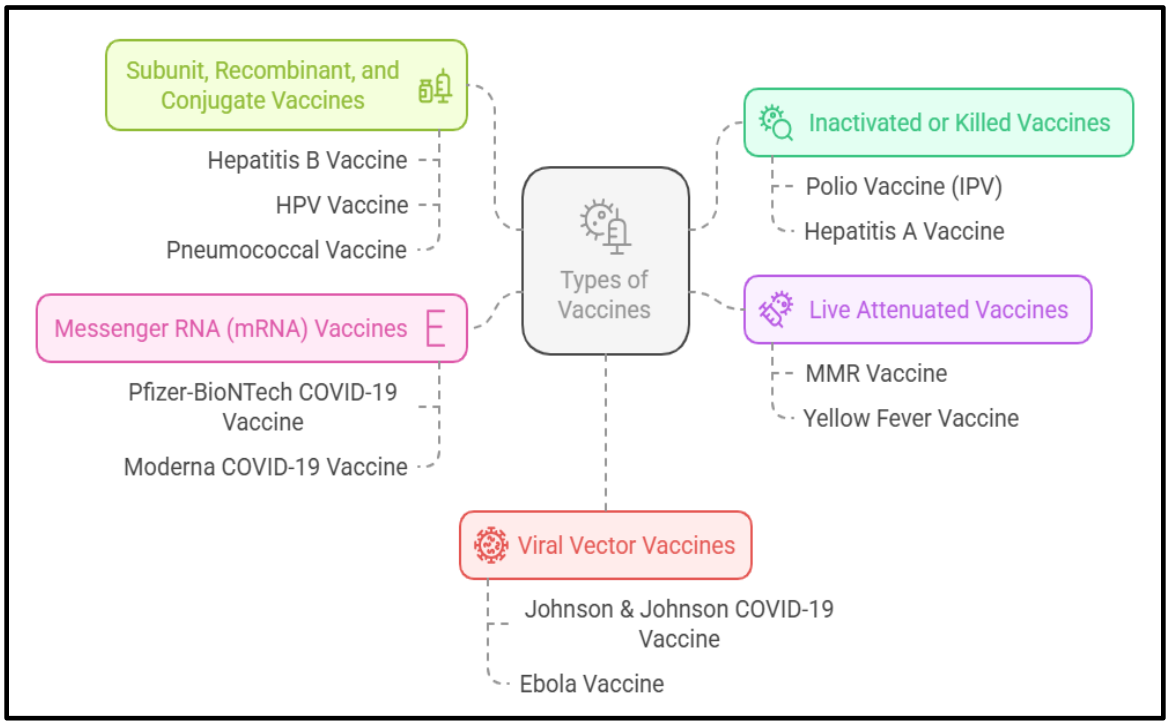

Figure 11: Types of Vaccines and Their Applications.

5.2. Vaccine Development and Production

The goal of this course is to help the participants develop a perspective about vaccines and immunotherapeutics via active and passive immunization against infectious agents. From History to Genomics and Numerous Vaccines. A vaccine is a biological preparation that improves immunity to a particular disease. Vaccines contain an agent that resembles a disease-causing microorganism, and is often made from weakened or killed forms of the microbe, its toxins, or one of its surface proteins. Key Data on Vaccines. Vaccines contain an agent that resembles a disease-causing microorganism, and is often made from weakened or killed forms of the microbe, its toxins, or one of its surface proteins.

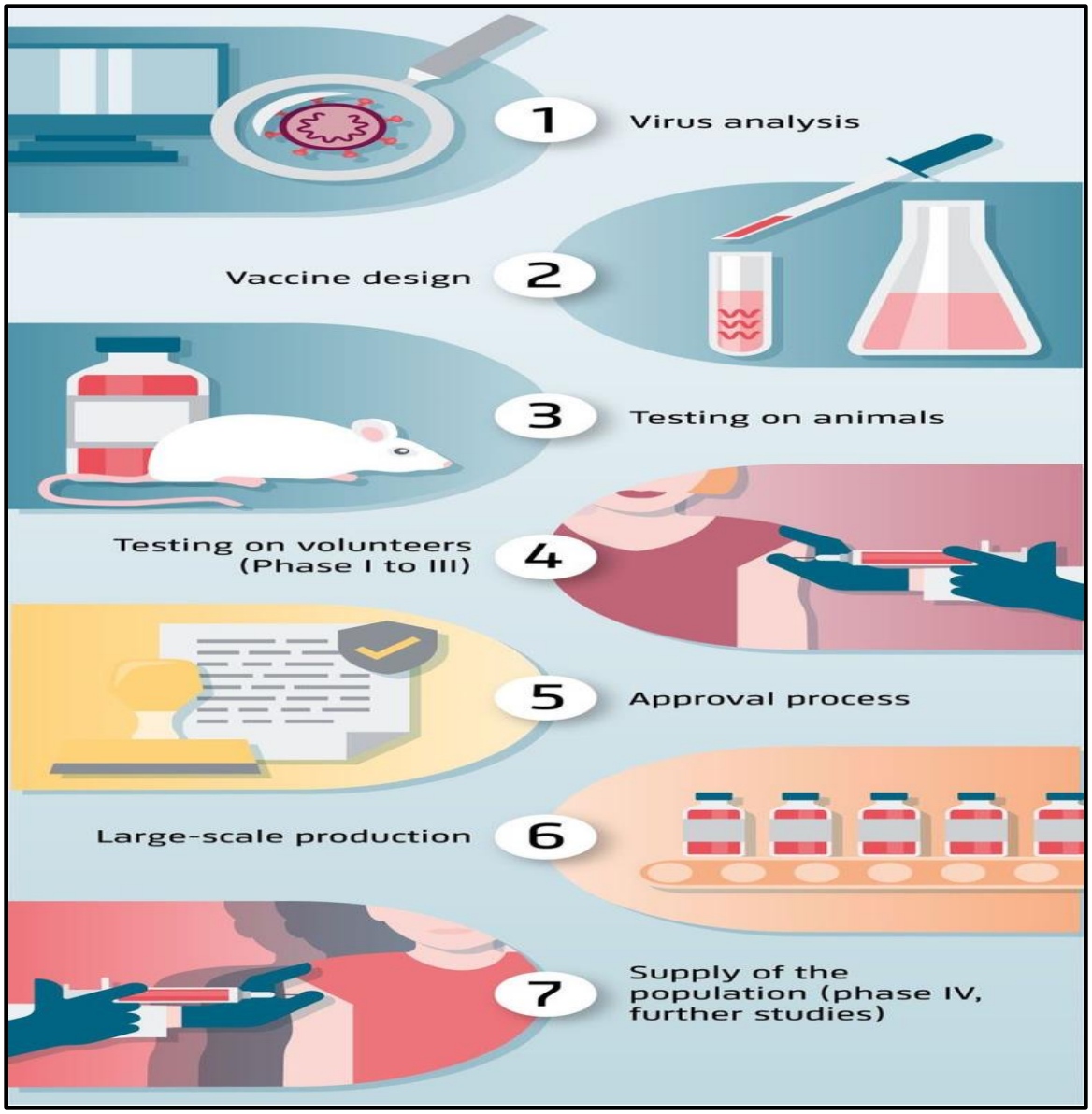

Figure 12: How are vaccines developed.

There is a current need for continued improvements in vaccine technology and production, broader coverage of vaccines for children and the age groups most affected by infectious agents, and achieving an educated public regarding vaccine science and effectiveness, to counteract the existing significant misinformation on the subject. Preventing Infections of Many Types. Infections are still a prevalent cause of morbidity and mortality throughout the world, particularly in areas where there is a lack of a clean water supply, drainage, garbage disposal, and a working sewage system. In industrialized countries, there has been a steady increase in the incidence and prevalence of chronic infections of the urinary tract and severe sepsis, pneumonia, and wound and trauma infections by resistant strains of microorganisms. With the advent of newer and more powerful antibiotics, this trend has been reduced, but not eliminated by any means, as witnessed by the annual occurrence of epidemics of diseases which are at least biologically speaking, preventable. Finally, the parameters that characterize the long-term effectiveness of immunizations will be examined: an original response demonstrating immunologic memory followed by the recall response.

6. Epidemiology of Infectious Diseases

The first point to be underscored is that when most people think of “epidemiology,” they seem to think that it is wholly, or at least mostly, about infectious diseases. That is not so; it is also about chronic diseases and newer phenomena like “transportation accidents” and the like. However, it does remain true that infectious diseases and epidemiology have been long-associated partners—each helping to an extent to define the other. It also has to be true that in many minds, at least during the second half of this century, the glamorization of infectious diseases has been so much greater than that for other, arguably, much more important “non-id” issues—stroke, infarct, malignancy—which also happen to be the leading causes of death in early 21st century America. The second “introductory” matter to be addressed concerns biting—just what can be bit into regarding this rat’s nest of a potential topic. This issue involves editorial judgment and varies with the readership. A few paragraphs about the annals of medical history might well be too “simple” in some medical centers but not in others. All extant journal readers are probably unfamiliar with local epidemiology. The writer’s comments are influenced by the experience in more or less immoderate serving of all three of the “contest” behind. Routine M and C VI never, and even many current special problems epidemiologic articles do not get published. As a newbie, concerns might be raised about being overly tied to the written word. It must be stressed, therefore, that a) the writer will indeed be very liberal and b) that, while less definitely interactive, responses will be possible. This offers a great opportunity to offer comment and to any or all of this article; also, the beginning of what may be a lasting marriage.

6.1. Transmission Dynamics

Influenza viruses are known to survive for longer on porous surfaces such as paper, than on non-porous surfaces like steel or plastic. In the context of public health, much attention has been given to understanding and controlling this. However, comparatively little is known about the dynamics of an infection — including both the virus and its interaction with the host — in the environment external to the host, where it may be considered free-living. Given the importance of this for understanding transmission, a simple compartment model of free-living virus is developed. The dynamics of experimental, environmentally released vaccinia virus gathered over a 4-hour period are compared with the output of an agent-based model of free-living virus, and found generally to be well-matched. This approach is then used to simulate the release of influenza A H1N1 virus from pupils in a school day care center that reported a probable flu outbreak during the spring of 2009 and predict the virus accumulation on surfaces. It is discussed how such a model and experiments could be developed and used to generate knowledge about free-living infection in a range of contexts, including households, hospitals, and public transport (Arinaminpathy et al., 2014).

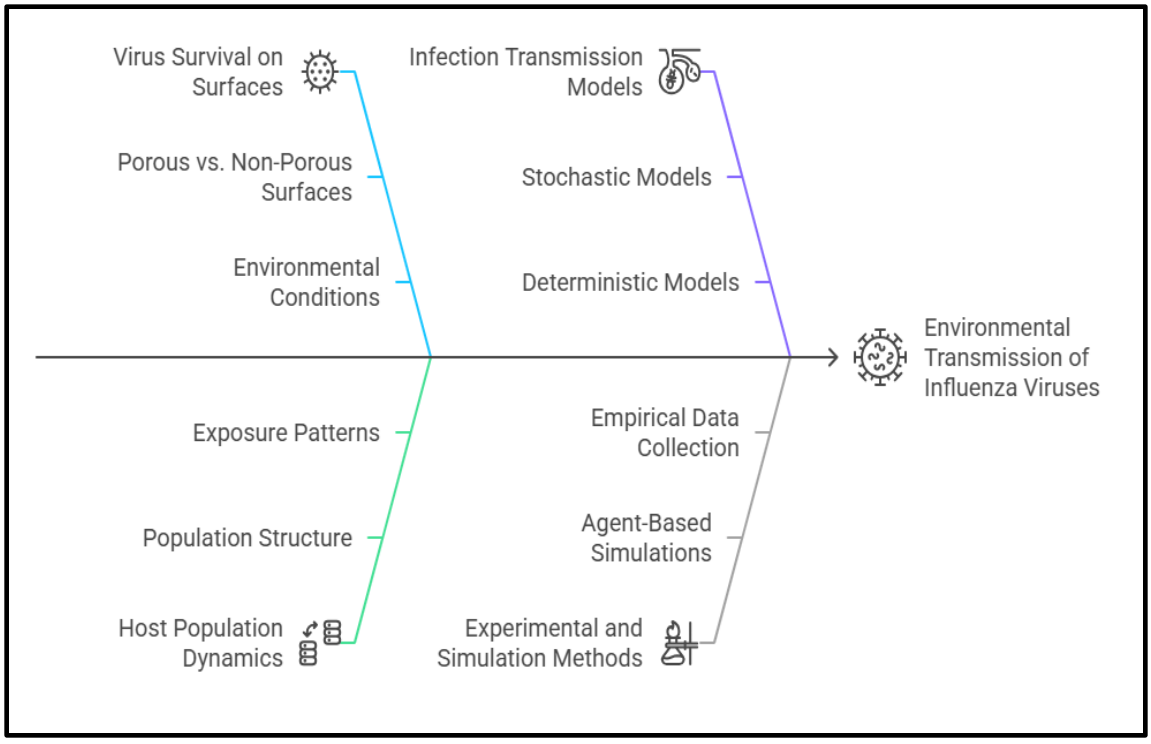

Figure 13: Understanding Environmental Transmission of Influenza Viruses

To simulate the initial dynamics of a "novel" infection, the system starts by introducing infectives, and results discuss the minimum required stochastic initial inoculum for infection spread. Infections or microbes can exist in the form of free-

living particles in the environment or as infective particles associated with a host. To describe the possible mechanisms of infection transmission resulting from free-living particles, empirical Willem's scalar models will be used, contrasting them to a comprehensive system of partial differential equations for host-pathogen dynamics and host exposure. Results suggest that environmental transmission models depend strongly on the structure of the host population and associated exposure patterns and hence on the colonization process probability.

For several host-pathogen systems that appear to lack simple model representations, supplemented compartmental models are proposed to derive results for the spread of such infection in multi-host systems. Direct quantitative comparison between the results of stochastic compartmental system models and large ensemble agent based simulations is used to illustrate the accuracy of the widely used classical "mass action" approximation to such coupled non-linear dynamical systems. Additional perturbative methods and assumptions are outlined and tested showing marked improvements in accuracy applicable in many transmission settings despite the heterogeneity within host populations.

6.2. Global Health Implications

Infectious and parasitic diseases are an important cause of disease and death worldwide. The World Health Organization reports an estimated disease loss of 11.4% associated with 19 million of all 52.8 million deaths worldwide. Since the decline of major infectious diseases like malaria and AIDS for example, has become a major health problem in recent years, it is critical to examine the epidemiological view of infections and immunity at the international level. One in two deaths continues to occur in developing countries with only one in nine associated with the same problem in developed countries. Overall, the prospects of international public health in the remaining twenty-first century shall be viewed within the constraints of the epidemiological problems arising from the epidemiological transition and the demographic evolution of the world's population. In this rapidly changing tragically disparate world, progress towards a better human health has been highly uneven among countries. Preventable diseases rapidly declining in newly industrialized countries are still the main causes of mortality in sub-Saharan Africa and other less privileged areas of the world. Also, the origin of the majestic breakthrough in the developing countries has been the improvement in child survival. Maternal health and the control of malnutrition have not significantly benefited from the epidemiological transition. Regarding the development of the health transition, the main issue is to understand why it takes place (Krämer and Mobarak Hossain Khan, 2009).

7. Antimicrobial Agents

Antimicrobial agents are chemotherapeutic drugs that can be used to treat bacterial infections. They are classified either by their mechanism of action or by the type of microorganism against which they are most effective. Host factors affecting the response to antimicrobial treatment are also discussed. Different microorganisms have different essential structure or enzyme that antimicrobials can target to stop their growth. Most antimicrobials target these key structures or enzymes in microorganisms, so they do not work against viruses or infected host cells (Leekha et al., 2011). Depending on how they work, antimicrobial agents can be classified into bacteriolytic or bactericidal agents, which kill target microbes (usually bacteria) directly, and bacteriostatic agents, which stop the growth of these microbes, enabling host immune system to remove them.

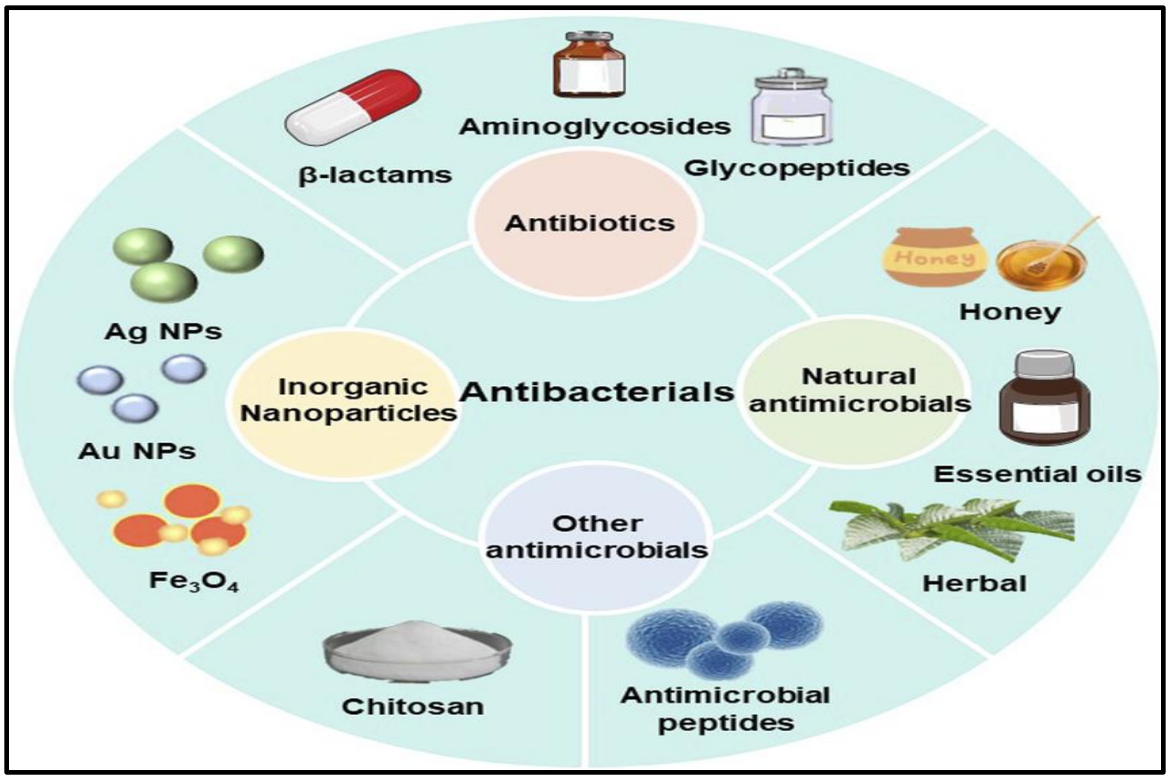

Figure 14: Classification of antimicrobial agents.

The distinction is not absolute, and some agents that are bacteriostatic against certain organisms may only be bactericidal against others and vice versa. For example, the combination of certain β-lactams and aminoglycosides exhibits synergistic activity against a variety of gram-positive and gram-negative bacteria and is used in the treatment of serious infections, for which rapid killing is essential. Bactericidal and bacteriostatic agents can be combined for optimal results. For example, in vitro studies have shown that the combination of a bactericidal agent

such as penicillin and a bacteriostatic agent such as rifampin is significantly more active than either agent alone. In this setting, the addition of gentamicin to penicillin has been shown to be bactericidal, whereas penicillin alone is only bacteriostatic and gentamicin alone has no significant activity. Antibiotic combinations are used in empiric therapy for health care–associated infections that are frequently caused by bacteria resistant to multiple antibiotics. Combination therapy is used in this setting to ensure that at least 1 of the administered antimicrobial agents will be active against the suspected organism(s). When infections are thought to be caused by more than one organism, a combination regimen may be preferred because it would extend the antimicrobial spectrum beyond that achieved by a single agent. The emergence of resistant mutants in a bacterial population is generally the result of selective pressure from antimicrobial therapy. Provided that the mechanisms of resistance to 2 antimicrobial agents are different, the chance of a mutant strain being resistant to both antimicrobial agents is much lower than the chance of it being resistant to either one. The use of combination therapy would provide a better chance that at least one drug will be effective, thereby preventing the resistant mutant population from emerging as the dominant strain and causing therapeutic failure. Several host factors must be taken into account in antimicrobial selection. Because the kidney and the liver are the primary organs responsible for elimination of drugs from the body, it is important to determine how well they are functioning during antimicrobial administration. For this reason, patients with urinary tract infections frequently receive antibiotics that are excreted largely by the kidney to achieve high drug concentrations and cure the infection. An understanding of the relationships between drug concentrations and effects, together with knowledge of optimal drug pharmacokinetics and pharmacodynamics, is of prime importance in the effective treatment of patients with systemic infections.

7.1. Antibiotics

The primary consideration in the selection of an antibiotic is clearly the sensitivity of the target microbial pathogen (Anderson et al., 2010). However, secondary considerations, especially the potential of the antibiotic to trigger/exacerbate harmful inflammatory responses may be of significance in certain clinical settings, particularly that of severe infection with a high bacterial load. In this setting, the benefits of seemingly appropriate antimicrobial chemotherapy may be countered by pro-inflammatory activity of the antibiotic. Can monitoring of specific host immune markers of infection and of associated immunopathology be used as a guide in antibiotic chemotherapy? A focus on the changes in a panel of circulating, host-derived, immune mediators/markers of inflammation and infection in blood, namely, IL-4, IL-6, IL-8, IL-10, TGF-β, TNF/TNFR, CRP, SAA, procalcitonin, and sTREM, all of which have an established association with immunopathology of

infectious origin. Also of potential utility is the monitoring of HLA-DR expression on circulating monocytes, which is a biomarker of monocyte deactivation that has also been evaluated in clinical studies of patients with infection and sepsis. In fact, can the measurement of these immune markers act in association with clinical scoring systems, e.g. the MEDS score or its component CURB-65, as well as the acute physiology and chronic health evaluation II scoring system, provide potentially valuable information regarding the pro-inflammatory status of the patient at the time of admission and during the course of the antimicrobial therapy?

7.2. Antiviral Drugs

Antivirals vs Retrovir Plus: A comprehensive study, combining the sale of Antibiotics vs Antifungal vs Antivirals and Retrovir Plus – a complex antiretroviral (Vardanyan and Hruby, 2016).

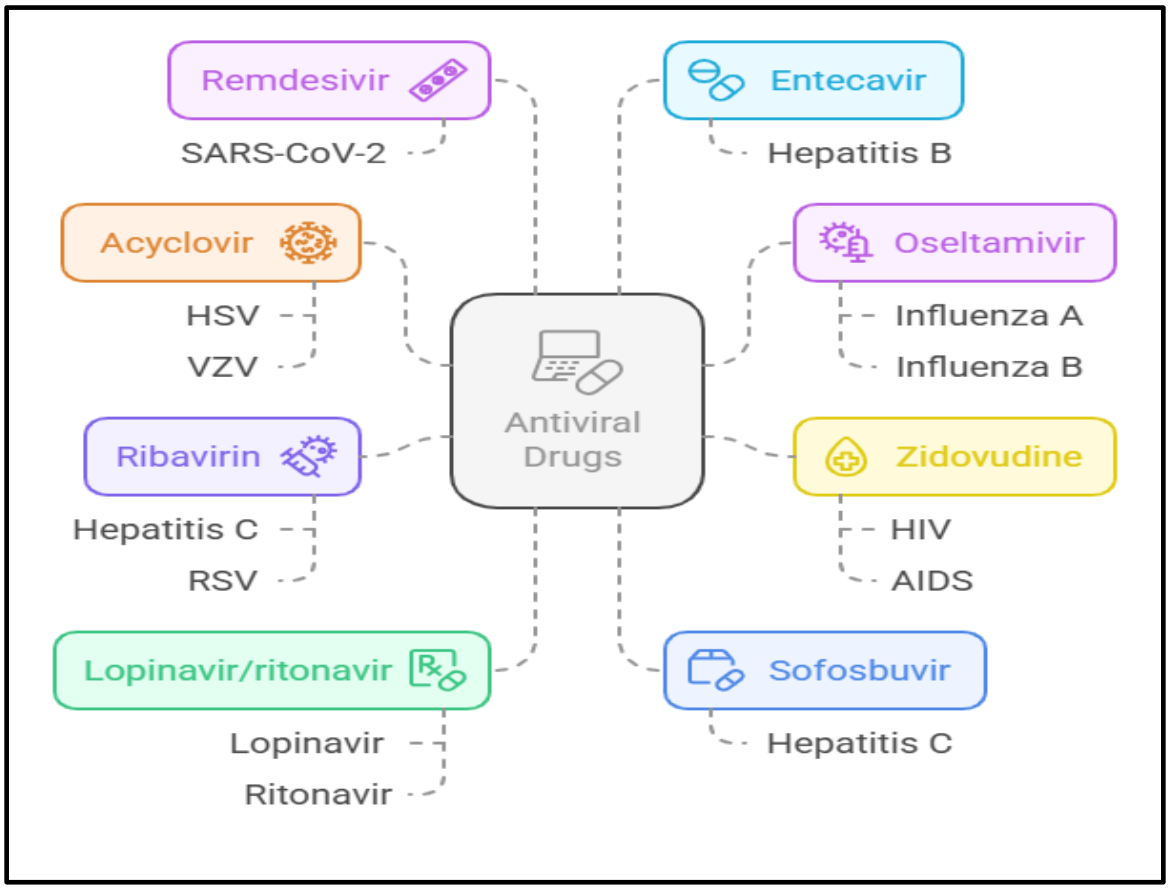

Figure 15: Antiviral Drugs and Their Applications.

The prepared sales prediction software is applied here in a comprehensive study, namely a comparative turnover prediction on one hand of Antibiotics, on the other hand of Antifungal and Antiviral drugs, 2nd rank group and changing potential a priori to look as a best seller. Sales of 10 Antibiotics, 5 Antifungal and 5 Antiviral

Drugs, have been turned to similar formulations as were used in original publication and in connection to advertisement expenses these drugs are also investigated in use, what are each group of combinations used: antifungal and antiviral, antifungal and antibiotic, antiviral and antibiotic, as well as all three groups combined.

The number of data sets appeared insufficient, and in order to have an increase number of data the sale of the drug Plegridy®, used to treat multiple sclerosis, is forecast. In addition COMPARATIVE sales prediction is made on one hand of Antibiotics and on the other hand of Retrovir Plus, a complex antiretroviral drug combining both Nucleoside and Integrase Inhibitor. This comprehensive study discusses the drug market of Antibiotics and the two rank groups of Antifungal and Antiviral, including some possible or probable changes and prospective sales prediction (Müller and Kräusslich, 2009). Antiviral drugs have become an effective means to combat many viral infections. Many newly synthesized compounds have the specific anti-viral action at concentrations significantly below their toxic level, which they reach in the body. Therapeutic activity of the most known Antiviral Drugs allows them to be considered as highly efficacious means to control specific viral diseases. There are three depending on the species of the infectious virus family: Anti-HIV Agents, Anti-HBV Agents and Anti-HCV Agents. Additional drugs with broad specificity action may intervene at other viral-infection steps infused in 2- or 3-drug combination therapeutics. Raltegravir is an Antiviral Drug used along with other medications to treat HIV infection. It is in a class of medications called HIV integrase strand transfer inhibitors. It works by decreasing the amount of HIV in the blood. Although raltegravir decreases the amount of HIV, it does not cure HIV or AIDS. Zidovudine may decrease the risk that raltegravir will stop working to lower your blood's risk of developing an infection or cancer, you may also be tested for HIV before you start and during treatment with raltegravir. If you are HIV-positive and you drink large amounts of alcohol, it may be at increased risk of being diagnosed with cancer. There may be other drugs not listed that can affect raltegravir. Once a day raltegravir is an integral component of a Best&Simple antiretroviral therapy as a safe and durable option for pre-treated patients with effective HIV RNA suppression, and it may be chosen as the better option in terms of QoL and treatment satisfaction.

8. Emerging Infectious Diseases

Newly identified or evolved strains of infectious agents can suddenly increase in incidence or expand their range and become significant public health problems (M. Morens et al., 2004). As unprecedented changes in the geographic distribution of human illness occur, there is an increasing recognition of the vulnerability of the global human population to emerging infectious diseases. The complex, constantly

changing nature of infectious diseases means that any gains achieved in the prevention, control, and eradication of infectious diseases are potentially reversible; hence, enduring infectious disease risks require an enduring and evolving response. To engage the global potential of scientific talent and public health assets requires a fundamentally novel institutional approach to the problems of emerging and reemerging infectious diseases. Insights used during the conduct of the research leading to a greater understanding of the factors behind the national, regional, and international emergence of infectious diseases; the mechanisms for predicting aligned responses; and the factors that can promote and facilitate post-emergence conflict resolution.

The accelerating emergence of infectious diseases enables the global scientific and public health communities to recognize the forces that shape their emergence, expansion, and accelerated dispersal. This heightened understanding facilitates both preparedness for and mitigation of the effects of such diseases. Scientific advance is also needed for disease-specific responses on ways in which prevention, control, or containing strategies should best be developed and targeted. This information will guide the selection of the infectious diseases for the programs, and facilitate the most effective public health and medical responses in all situations. With these imperatives in mind, the became the sponsoring agency of a research initiative that sought to elucidate and define the drivers that underpin the emergence of infectious diseases.

8.1. Zoonotic Diseases

Harrison's Infection and Immunity is a comprehensive examination of the basic and clinical aspects of infections. This book addresses all aspects of infectious diseases and their etiologic agents. Each area includes sections on anatomical, histologic, pathophysiologic, and metabolic features. The wide variety of topics and approaches allows the professional or medical student to find easily understandable answers to the many questions they might have about infectious diseases (S. Shapiro, 2016).

The first chapter is a discussion of zoonotic diseases. Zoonotic diseases are acquired from a wide variety of animals other than pets. Of 768 million travelers surveyed between 2009 and 2013, half acquired an illness during their international journey; of these, 9% sought medical advice after returning. Zoonotic infections are acquired from farm animals, pets, beasts of burden, fish, and wild animals; the infection may result from handling, food consumption, biting, scratching, water exposure, or other routes. The present chapter deals with the zoonotic infections transmitted from animals other than pets; a disease acquired from cattle, dogs, or cats, or that is common in developed nations or international in distribution, is not discussed unless of unusual interest or complexity. The approach to the patient with a

potential zoonotic infection involves the generation of a differential diagnosis that includes those infectious agents that are potentially transmissible from the specific animal(s) to which the patient was exposed.

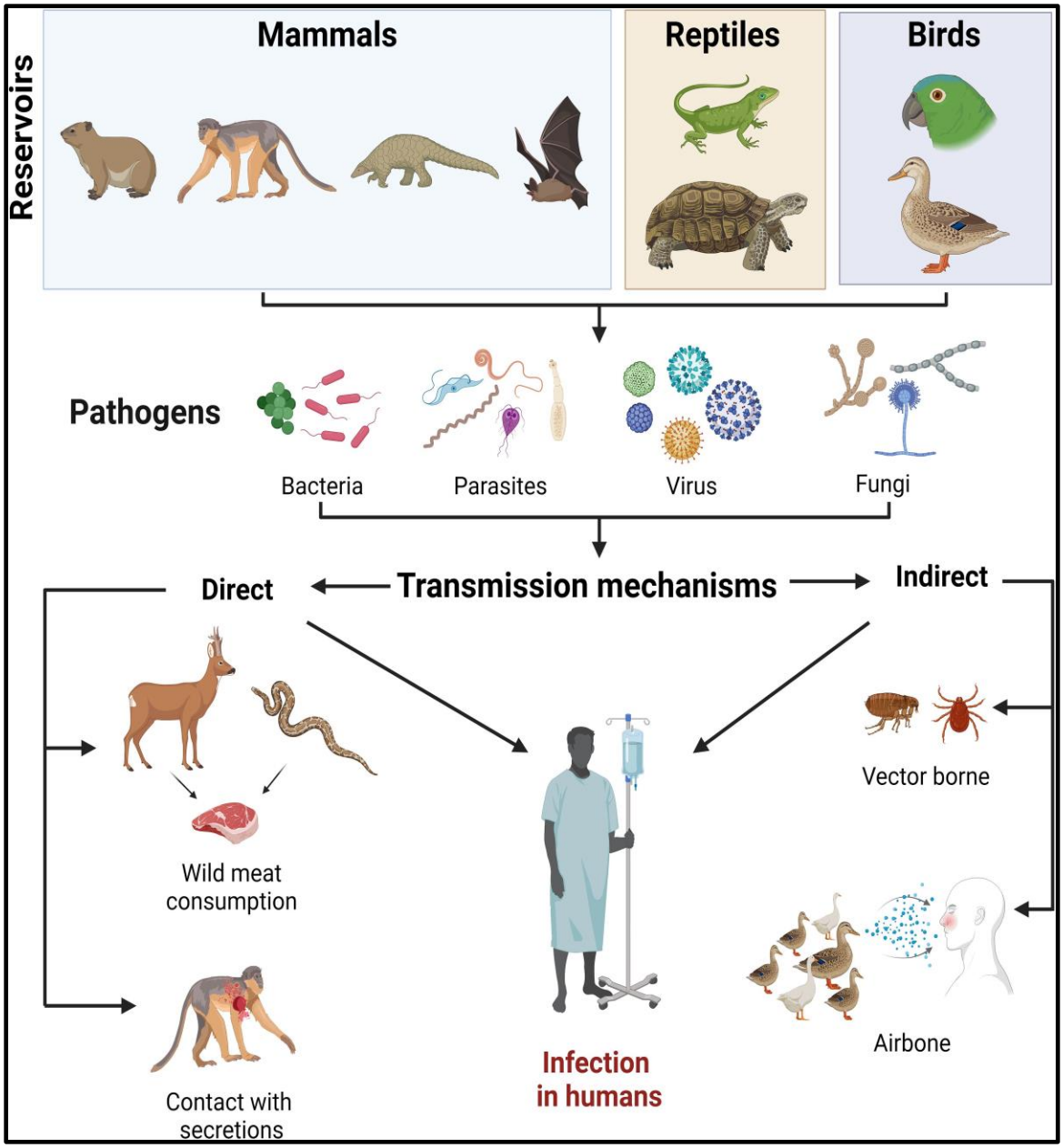

Figure 16: Integrating environmental conservation and public health strategies to combat zoonotic disease emergence.

This approach is straightforward if the exposure is to a dog or cat in the developed world (rabies, bartonellosis, and Francisella tularensis). However, the number of infectious agents potentially transmissible from a specific animal to humans may be great, and many of these infections are limited geographically and need not be

considered unless a bioterrorist event or the introduction of an infection to a new area is a possibility (Recht et al., 2020). From these studies, the percentage of patients in whom a travel-related diagnosis is made is about 70%, whereas physicians fail to consider or present the correct diagnosis in at least 65% of such patients. When there are few data about a particular animal and its role as a reservoir of zoonotic agents, it is worth considering biologically similar animals from which zoonoses have been acquired. For example, biologically similar wild animals, such as the jackrabbit, are alternative sources of zoonotic agents to the European hare. Many infectious diseases are transmitted only by a bite, exposure to face-to-face saliva, or handling of the carcass or body fluids of the infected animal. Fewer infections are the result of inhalation of infected animal products, direct contact with the animal's respiratory droplets or body surface, or ingestion of immensely undercooked animal products or water supplies contaminated by the animal. Indirect transmission by vexing arthropod vectors poke into the blood, which subsequently results in systemic infections such as babesiosis, Q fever, and marine typhus; infection from the solid waste or arthropod species of the infected animal via the conjunctivae; contaminated fomites such as the hair cut or skin removed from the infected animal.

8.2. Pandemic Preparedness

The 1918 Pandemic took everyone by surprise, and the H1N1 virus spread around the globe with great fury. In the 1970s, vaccination staved off the H1N1 resurgence, but 80 percent of the world's population remained unprotected. In 2009, a fresh dose of the H1N1 vaccine was distributed to 90 nations, which ordered a vaccine based solely on preliminary H1N1 lab isolates. The following were among the problems of the 2009-2010 Pandemic:

1. The 2009-2010 H1N1 flu pandemic has shown the world it is still unprepared to adequately counter a serious emergency. Indeed, the immediate result is that it is now viewed by the population as government "crying wolf," leading to potentially disastrous results on medical and social infrastructure.

2. Instead of the predicted situational tightening, the situation appears to be has seriously degraded to a far below even current preparedness. The 2009-2010 H1N1 problem was less severe than the Spanish flu of a hundred years ago.

3. The avian flu virus was discovered no more than 13 years on 30 November 1997 in patients with H5N1 avian flu in Hong Kong. The WHO's global H5N1 awareness in humans was only in February 2003 (Horby, 2018).

4. The U.S. has not yet developed since 1945 soda can military technology with highly chemical basic fabrication.

5. The incubation was too short to generate and deliver hundreds of millions of vaccines for an H5N1 surge.

6. There is still no robust legally required traditional combining ideological reaction and formulation.

7. There is no TB traditional and new vaccine.

8. The head of the FDA was asked about the current education system, where about two thirds of scientists received money from a drug company.

9. HMOs were proclaimed in the Great Depression, but they have not yet been handed over during the possible economic collapse.

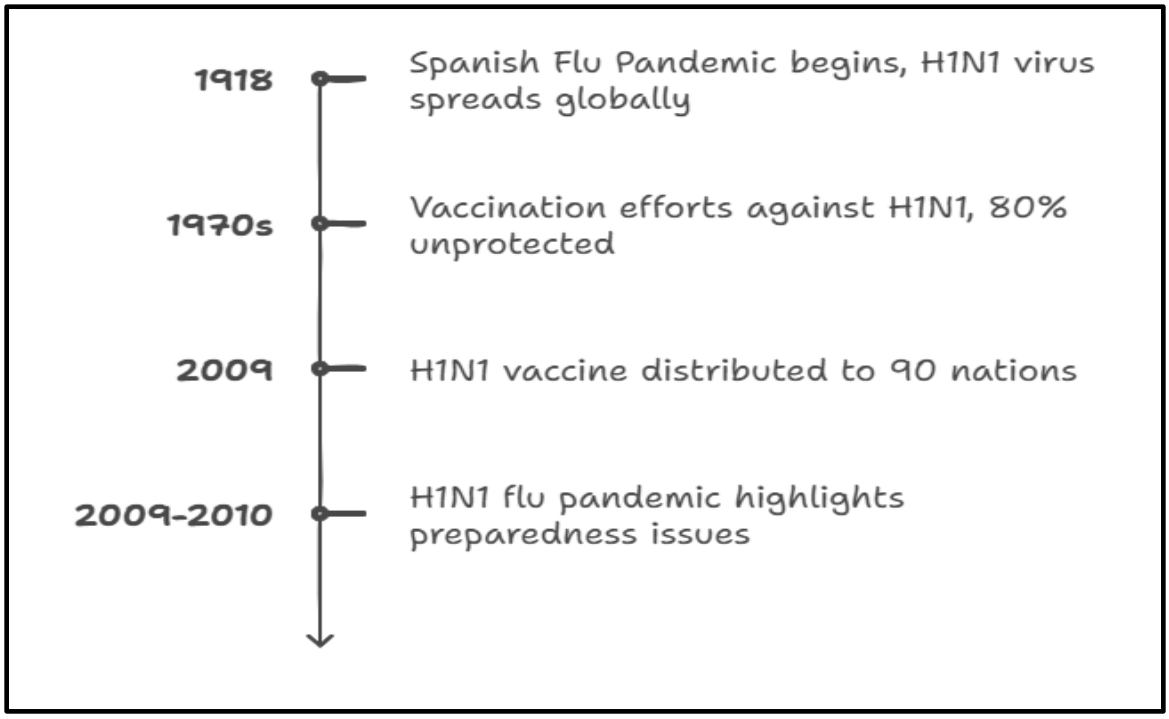

Figure 17: Global Responses to H1N1 and Pandemic Preparedness.

The epidemic can be compared to a jet aircraft with a critical vulnerability, a tiny hole in the fuselage. For the present, the danger is that it will be completely overlooked. At some point, the plane flies into an excessively cold facade with a considerable humidity. A significant proportion of the passengers on board get sick. The hole in the market is the monkey, an aggressive and viral agent, the consequences are infectious paralysis with severe complications on the respiratory and gas exchange system, hemorrhage and harmful actions of the nervous system.

At the moment, there is no possibility of long-term effective psychoprophylactic and psychoprophylactic combat both for virology and its drug agents (Hoven et al., 2022).

9. Immune System Disorders

As bugs exhibit the ability to spread rapidly in every corner of the world, the human body resists these bugs by activating the immune system. The immune system is a remarkable system in the human body. It has the ability to recognize invading bugs (antigens) almost instantaneously and then selectively kill them. Diseases are caused by disorder of the immune system, causing it to attack the body's organs instead of killing pathogens.

Parasitic flatworms that infect nearly 300 million people worldwide are the main hidden attackers targeting the human immune system. Giant roundworm is responsible for a broad spectrum of diseases that create complications in human beings. Blocking or a lack of the key molecules in the immune system results in the blocking of the machinery needed to control T and B cell expansion. The immune system gears up as soon as it encounters the pathogen, but if the result does not come out in the right sequence, the immune system remains at the disease state. The immune system is engaged in defending the body against the host of diseases (Gupta et al., 2016). As the number of bugs increases, the immune system struggles a lot. This goes hand in hand with disease spread. Whenever the human body is infected, the immune system is activated and starts the process of killing the pathogen.

9.1. Primary Immunodeficiencies

Primary Immunodeficiency Disorders (PIDs) represent a heterogeneous group of inherited disorders of the innate and adaptive immune systems. The clinical course of infection is more severe than in individuals without PID. Since treatment is usually long term, a revaccination protocol is essential for a better outcome. Our study is aimed at examining this issue in detail. A retrospective study of PID cases was made and their data was collected and evaluated. The types of PID, age, gender, clinical features, affected organs, infections, vaccinations and family history were evaluated. Revaccination in patients with PID was also discussed. In 2 years, 39 PID cases were investigated. Patient ages ranged from 1874 years and children constituted only 23% of cases. The most common types of PID were common variable immunodeficiency and specific antibody deficiency disorders. Data on family history of PID was only obtained from three PID patients. Cases of PID were observed but not examined. The age at the time of diagnosis is over 50% of the PID cases. In more than half of the cases, infections of the respiratory tract were detected. Direct information was obtained from 25 patients regarding the severity

of infection presentations after the diagnosis of PID. Routine blood tests and immunoglobulin levels were requested for 30 patients. Involvement of the affected organs was detected in the 30 patients. In 35 patients older than 16 years old, their vaccination status is interrogated. Of the 39 PID cases, no revaccination history has been recorded in 38% of cases. In 33% of patients, the number of vaccinations for each infection changing between 1 and 4. Vaccinations following the onset of PID diagnosis in 9% of cases occurred within the first 6 months. In patients with PID, vaccination is important to prevent severe infection, however the absence of PID diagnostic kits, PASADA centers, and vaccinations surveillance programs impair patient safety (Chowdary Peddi et al., 2023). Infection is the movement of an organism into an organism's surrounding tissue and its propagation. There are several diseases that a person can suffer due to an infection. That person is said to be infected with the pathogen. Although the body is built to resist many diseases and infections, some people have weak immune systems that allow the pathogen to propagate easily. Infections due to mild deterioration can sometimes lead to fatal consequences if not curbed in a timely manner. One step that can help slow the spread of the disease and thus overcome the complications is through vaccination. Patients with Primary Immunodeficiency Disorders are provided vaccines to enhance their immunity against pathogens. Vaccination of PID patients also protects people around it, known as the principle of the herd. There are 9 types of vaccines recommended to prevent diseases. Every human being should be educated about vaccinations, especially in regions with common diseases that sometimes cannot be avoided. This is a very effective and less risky way to maintain well-being. Although specialized committees insist on the importance of vaccination in PID patients and their relatives, efforts should be made to prepare them strictly. This is not only for the benefit of patients with PID, but also for protecting the population. It is also important that there are occasional comprehensive studies on vaccinations, especially in times of virus outbreaks. Public awareness of vaccinations can play a more effective role in overcoming pandemics like those that are currently occurring worldwide.

9.2. Secondary Immunodeficiencies

Secondary immunodeficiency (SID) occurs when the immune system is weakened by another treatment or illness. The signs and symptoms of SID are the same as for primary immunodeficiency; that is, frequent, prolonged or unusual infections. If the underlying cause can be removed, then in many individuals the immune system returns to normal. For others, this is not the case and the treatment is long-term, even life-long.

10. Ethical and Social Implications of Immunization

Although preventive inoculation is usually a one-time event in childhood, its medical, social, political, economic and moral repercussions extend far beyond that single occasion. The experience of smallpox shows how obligatory care can lead to the disease being declared eradicated, and it has been claimed that a decrease in vaccination foil results from an erosion of individual and collective vigilance in the face of vaccines. The particular status of vaccines in therapy often obliges states to forge a momentous partnership with the pharmaceutical industry (J. Mehlman and M. Lederman, 2020). However, the legitimacy of enforced vaccines is regularly questioned in French law courts. This article intends to account for these paradoxes by focusing on French experiences, noting, first, that care is used on a long-term basis to create resilience in children, who are at risk of various contagious diseases. Second, France illustrates the singular effectiveness of the permanent police of public vaccinations observed since the end of the 19th century. Third, concern for scientific precision has sometimes led to rendering compulsory the administration of vaccine doses that are still in the experimental phase of development.

10.1. Vaccine Hesitancy

Vaccine hesitancy is defined as the reluctance or refusal to vaccinate despite the availability of vaccines. Although vaccines are one of the most effective ways to prevent infectious diseases, a part of the population continues to refuse them. Vaccine hesitancy arises from poor confidence, lack of access and low perception, which are influenced by factors at personal, societal and health-system levels. An extensive review of scientific literature was done on vaccine hesitancy and three specific research questions were addressed: What are the characteristics of different age groups likely to refuse vaccines? What are the key messages that should be transmitted taking into account these characteristics? What are the best approaches to transmit these messages to these age groups? The results are focused on the answers to these three questions transcendentally in a personal and opinion-based way.

In the post-truth era, a considerable part of the population is also influenced by the untruthful or false information they read on the internet. The rapid evolution of the internet, especially social media, allows people to access and immediately disseminate false information that can be misleading or directly harmful. Messages are more easily propagated when they evoke emotions and when they are simple and replicable. It is true that there are many people who remain completely certain and resolute about their opinions against vaccines. For other (and often younger) people, the decision not to vaccinate comes not only from a lack of information about vaccines, but a distorted and contaminated understanding that lingers in their own skepticism ((Arede et al., 2019)).

10.2. Public Health Policies

The authoritative Morbidity and Mortality Weekly Report publishes periodic special reports of important infectious and noninfectious diseases (H. Tulchinsky and A. Varavikova, 2000). For many of these diseases, an infectious agent can be identified with certainty as the cause, and effective means of control are available. It is critical that the physician recognizes symptoms and signs of these diseases and is familiar with the control measures available. Control of the disease means a reduction in the incidence, prevalence, morbidity, or mortality. Elimination can be achieved through a sustained intervention program. Eradication is the reduction to zero incidence, the total interruption of transmission and the presence of the organism in nature. Arrangements for an eradication program must have detailed planning and organization, as well as specific periodic evaluations. All tools apply anti-disease strategies and interventional public health measures, including the wide application of vaccines.

The response to the global resurgence of major epidemic infections in recent years has fully indicated that public health applies a wide variety of tools for the prevention and control of infectious diseases as well as timely emergency contingencies. Planning measures to control and eradicate communicable diseases at the national and global level constitute a major responsibility for public health. The first and fundamental step once the disease status is ascertained is to seek to determine an etiological diagnosis and then take measures to control the source of infection, including regular examination and treatment of clinical cases. Reservoir and carrier locations should be identified, therefore people can be isolated or be treated, even with force if necessary. Barangays or isolated locations should be isolated and controlled. Sentinels should be assigned for full time observation of the disease status. Rat extermination squads must be organized to control. Vectors should be eradicated by the wide application of DDT and the possible fishing in reservoirs. Strict isolation of infected people should be enforced. The distribution of

protective prophylaxis should be ensured for vulnerable population groups. The corpses should be burned with the presence of a sanitary officer. Efforts should be made to limit exposure and harm to uninfected persons. A laboratory for the examination of samples from dubious cases should be organized. Health education should be systematically organized. The costs of drug use should be controlled. There should be provision for rats to be found and eliminated or poisoned. In these and many similar diseases the problem is to exterminate every single infected parasite. Treating infection is the key to control a communicable disease. Successful treatment of the infected person reduces potential transmission. Chemotherapy of communicable diseases with drugs has been particularly important. Chemotherapeutic drugs comprise bacteriostatic agents that inhibit growth or otherwise stop the replication of infecting organisms and bacteriocidal drugs that kill the pathogenic organisms. Similarly, the emphasis on treating communicable diseases has shifted progressively from certain traditional concerns to others and to new ones. Pharmaceutical companies devote considerable resources to the development of antibiotic drugs. Since then antibiotics have been widely used in the treatment of infectious disease and communicable diseases have been a major concern of public health. There are specific recommendations – and in some cases even laws – concerning antibiotic use and abuse, including drug resistance in pathogenic organisms. Organized public health services propose legislation, regulate, and monitor the control programs needed to curb the occurrence of communicable diseases. Public health authorities may consider immediate action to halt the spread of the disease. Inform General Conference that the present situation and development of communicable diseases constitute a public health risk of other states.

11. Future Directions in Immunology Research

Immunology has made few inroads in developing and expanding undergraduate programs compared with other fields such as microbiology and neuroscience (A. Bruns et al., 2019). The training of students in immunology is now clustered almost exclusively at the graduate level. Yet, with recent biomedical and life sciences advances and the explosion of technologies such as high-throughput sequencing, big data, and single-cell imaging, the need for individuals with a broad understanding of immunology and experience in laboratory techniques focused on probing immune mechanisms has also expanded. Relevant opportunities in the workforce include researchers en route to independent research careers, translation of groundbreaking discoveries into new therapies, and everyday application of knowledge of the immune system within the clinic. Accompanying these scientific and technological advances has been a growing appreciation that the immune system plays a direct or indirect role in nearly every facet of human health and

disease. A strong, broad-based understanding of the entire discipline and the innate and adaptive immune systems will be critically important for efforts to improve the health and wellbeing of human subjects; one example of this is understanding the complex interplay of the microbiome and the host and its implications in numerous disease states. Considering the national and global focus on the microbiome, biodefense and immunotherapies this has helped push immunology into the spotlight. Unfortunately, access to immunology courses at most undergraduate institutions is limited. Most students nationwide, regardless of the quality of their life science programs, are exposed to immunology in only one, and at most two courses. Overall, a cursory evaluation of the priority given to immunology in the life science education tract shows the discipline at a disadvantage relative to physiology, cell biology, molecular biology, genetics, and the behavior of model organisms used in laboratory settings. Consequently, as the rigor of the foundational life sciences sequence has typically increased for students entering graduate or professional schools, on average 25–30% receive exposure to immunology that may in the future serve as the catalyst to pursue advanced training in the field (or a related area). Since the majority of the roughly 6,000 post-baccalaureate matriculants each year are interested in biomedical or NIH-funded basic research, the lack of appropriate foundational coursework in immunology perpetuates and potentially exacerbates the current imbalance. Further underscores the lack of emphasis on undergraduate immunology education.

11.1. Immunotherapy Innovations

The emergence and reemergence of new diseases and the increased incidence of drug-resistant organisms has accelerated the need for the development of novel preventive and therapeutic approaches to help control a broad spectrum of infectious diseases. Infectious diseases are the second leading cause of death worldwide, with 25% of annual deaths attributable to infectious diseases. Additionally, severe diseases with longer duration impose an excessive socioeconomic burden. The drug resistance and emergence of new infectious agents has renewed interest in the immune system as an alternative target for interference. The immune system plays a crucial role in defending against infectious agents through the interplay of different immune cells and soluble factors (Ramamurthy et al., 2020). The host immune system is highly developed to target and eliminate infectious agents effectively. For example, coordinated subsets of host immune cells are recruited to the infection site upon pathogen entry to eliminate the infected cells and the pathogens. Infectious agents have evolved strategies to counteract the host immune-elimination processes and, in turn, the host immune system counter-adapts. This leads to evolution pathways for the host immune system, as well as to the pathogens leading to a favourable immune-editing extension for diseases. While

there are several highly effective preventative measures available for infectious diseases, there is a paucity of licensed therapeutic avenues currently available. Indeed, even after more than a century of intensive effort, no curative therapies are available for many chronic infectious diseases and emerging infectious agents. The emergence of drug resistant pathogens further exacerbates the situation. Taken together, there is an urgent need to explore the potential development of alternative approaches to treat infectious diseases. In this context, the emergence and re-emergence of new diseases, abnormal host immunity evolution, highly virulent strains that evade immune responses, and HIV, stimulating the immune system to correct disease-associated immune abnormalities is a recognized strategy to develop immunotherapeutics as well (Naran et al., 2018). In recent years there has been a substantial growth in understanding pathogen–host interactions to uncover mechanisms that evade immunity. Such understandings may pave the way to innovative immunotherapeutic strategies to target virulence factors used by pathogens to evade host defense.

11.2. Precision Medicine in Immune Disorders

The application of fundamental genetic and immunological insights is rapidly changing medicine. This fundamental work is leading to significantly improved understanding of the principles of immunity and the factors regulating it. Two concepts are of particular importance. Firstly, the ways in which different pathogens are sensed by the innate immune system. Secondly, mechanisms of inducing and controlling adaptive immunity by T- and B-lymphocytes underpin immune-defence and immune-tolerance (Lamborn, 2016). Powerful new approaches are being developed to modulate immune responses in ways that are much more powerful, and directed, than were previously taking seriously the philosophical basis of immunology, and entering the clinic with treatments such as organ grafting and insulitis. Very recently, the first controlled cure of HIV infection.

The ongoing dissemination of these ideas into the medical research and practice across many applications is seen in a diverse sample of approaches that are deeply rooted in immunological understanding. The genetic, molecular, physiological, and structural bases of bacteria and protozoa recognizing pathogens. An antigenic serotype polysaccharide. The genetic, molecular, and physiological bases of the SAP2 protein binding immune complexes initiating the classical complement pathway. At a figure of 1.5% of the human genome, C2 is in fact a serine protease that is a genetically unstable acute phase protein. An immunological point of can start to fight an infection, and patients with the C1q, C4 or C2 components of the classical complement pathway are particularly susceptible to infection by acidic bacteria and protozoa (Segura-Tudela et al., 2024). Strengthen the polymorphism

and copy number variation those once formulated apply to MHC class I (HLA-B) and II (HLA-DRB1).

12. Conclusion and Summary

Infections are a leading cause of morbidity and remain a life-limiting factor in a large number of children. Infections and immunity is a vast subject and one can appreciate that it is not covered comprehensively in this textbook. However, an attempt has been made to describe infections in a comprehensive manner, including those that are prevalent not only in a particular part of the world but also throughout the globe. Recent advances in treatment options have been described to some extent, along with those preventive modalities, be it prophylactic antibiotics, vaccination or other modalities. Needless to say, a lot of work has to be done to decrease the morbidity and mortality due to infections in both developing and developed countries.

A critical read of this textbook will enable readers to comprehend the basic concepts of immunization, the body's defence mechanisms, microbial flora, and infectious disease processes including clinical manifestations, management options, and prevention strategies. These strategies range from simple washroom practices to the development of novel vaccines. This text describes and explains infectious diseases that are of interest to medical students, doctors, and particularly those practising paediatrics or paediatric surgery. For those wishing to pursue further studies, epidemiological studies of infectious diseases, bacteriological isolations, antibiotic sensitivity tests, and training about drug choices could be considered. This comprehensive study of infections and immunity should aid researchers in understanding recent advances in the understanding and treatment of infectious diseases involving drug-resistant organisms.

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Chapter Nine

The Role of Microorganisms in the Treatment of Infections

1. Introduction to Microorganisms and Infections

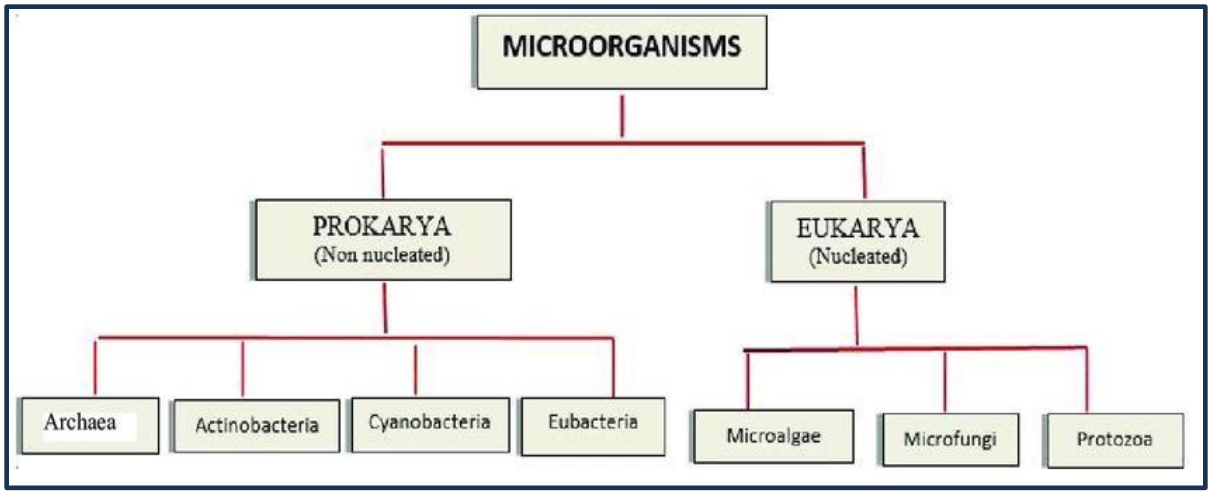
Micro-organisms have been shown to be present in virtually all habitats on our planet, regardless of the extremeness of these environments. It is now known that these micro-organisms play a critical role in human existence. These organisms have been essential for the evolution of higher life forms through processes like the production of oxygen by photosynthesis and the nitrogen-fixing ability of bacteria which enables plants to grow. Micro-organisms also degrade complex organic matter to provide nutrients for plant roots, eventually forming the "soil". For humans, some colonizing flora that inhabit the mucosal and skin surfaces synthesize essential nutrients (Fierer et al., 2016). The field of microbiology has come a long way since Antonie van Leeuwenhoek first observed the world of micro-organisms using a simple lens magnifying 200 times. In the following years, the controversial question of spontaneous generation was put to rest by Louis Pasteur. The knowledge that micro-organisms are living beings led to the experimentation with respectful handling of cultures, which, in the hands of Robert Koch, allowed the postulates that bear his name to be formulated. This milestone paved the way for a more rational understanding of the nature and pathogenicity of bacteria. In particular, the observation of clinical specimens from R. Koch's hospital in Berlin, coupled with a growing interest in staining, led Hans Christian Gram to develop in 1884 the method that bears his name. He observed that after staining with crystal violet, all bacteria were colored blue/ purple. However, after the addition of iodine, the Gram-positive bacteria retained the color, whereas the Gram-negative bacteria were decolorized (Soni and Pandey, 2024).

1.1. Definition and Classification of Microorganisms

Microorganisms (also called microbes) are microscopic organisms comprising a broad and diverse group of life forms. Microorganisms belong to the prokaryota of bacteria and archaea, the eukaryota of fungi, and micro-algae. Microorganisms may be either pathogenic or non-pathogenic. Some microorganisms are even involved in

beneficial symbioses with their higher hosts and exhibit biotechnological potential. For instance, lactic acid bacteria and Bifidobacterium species are able to ferment food and produce acid, resulting in food conservation as well as health benefits for the host (Weiland-Bräuer, 2021). The majority of bacteria do not cause diseases and are referred to as commensal bacteria. Commensal bacteria are important for human health and wellbeing. For example, commensal bacteria in the human gut facilitate the fermentation of indigestible compounds and the absorption of essential nutrients. Commensal bacteria also serve as competitors and defend the host organism against pathogenic bacteria, thereby preventing the colonization with opportunistic pathogens. Furthermore, commensal bacteria colonizing bodily surfaces prevent the colonization of the same niches with pathogenic microorganisms that may harm the host (Soni et al., 2024). In contrast, pathogenic bacteria have evolved sophisticated ways to evade the host immune system, to adhere to host tissue and to obtain beneficial compounds. Most pathogenic bacteria enter the human body through the respiratory or gastrointestinal tract and overcome the initial adjunct immune response. The ability of bacterial pathogens to counteract the host immune defense is performed either by secretion of virulence factors or by invasion of host cells. Once bacteria reach a new location within the host, they can use lytic enzymes to degrade host tissue or toxins that damage host cells to acquire beneficial nutrients. Moreover, invasive motile bacteria can access the nutrient-rich interior of host cells, thereby avoiding the attack of the immune system. Finally, some bacteria are able to interfere with the communication of immune cells, thereby interfering with the concerted action of immune defense.

Figure 1: Classification of Microganisims.



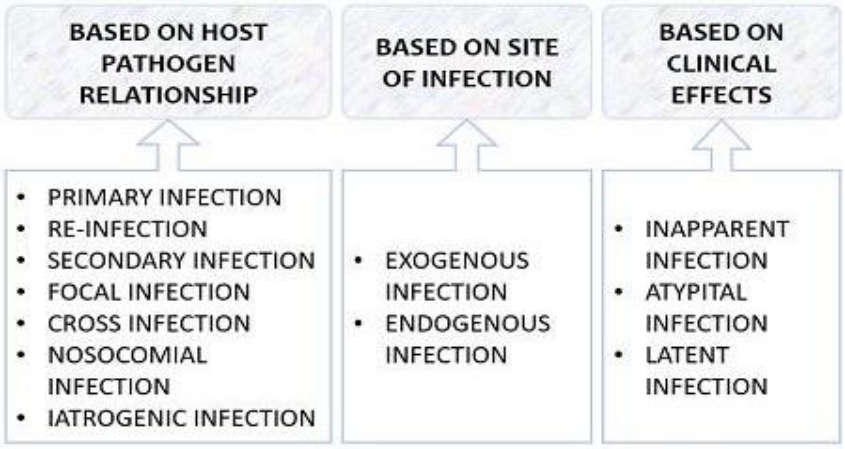
1.2. Types of Infections

Microorganisms such as bacteria, fungi, viruses, and parasites are the causative agents of a wide variety of infections. Pathogenesis is a process that damages host

tissues while benefiting the microorganism and is the basis for understanding the development of infectious diseases . Pathogens have different strategies to adapt to the host, resulting in a variety of symptoms. Some pathogens can be easily eliminated by host responses in the early stages of infection. However, some pathogens can persist for long periods in their hosts and cause persistent infections. In comparison to acute infections, persistent infections are more difficult to clear. Moreover, they have various pathologies, which can lead to the development of complex chronic diseases over time.

Infectious diseases are the second leading cause of death worldwide. Approximately one-fourth of human deaths are related to infectious diseases. Death from infection is particularly high in infants, children, and the elderly.

Table 1: Classification of infections



Babies are born without resistance to infection, and some of them are easily infected. Older people, especially after the age of 65, are more susceptible to infection due to a weakening immune function. In contrast, young adults have a substantial decrease in infectious diseases. This can be explained by the fact that young adults generally maintain their immune functions at an optimal level. In contrast, the immune system of the elderly may be impaired due to the aging process, malnutrition, underlying diseases, and drug use. In addition, people with abnormal backgrounds are prone to various infectious diseases. Many infectious diseases are opportunistic infections caused by the lack of active defense mechanisms in individuals suffering from serious chronic diseases. Successful management of infectious diseases requires accurate diagnosis and timely treatment. Physicians must understand the nature of pathogens if they are to develop appropriate treatments. Detection methods that can identify the pathogens are also essential. Additionally, vaccination plays a crucial role in the prevention of infectious diseases. If infections can be prevented, it may not be necessary to develop new drugs. Therefore, preventing infection should be the most

important area of treatment. Disinfection, sterilization, and other efforts must be made to control infection.

2. Historical Perspective on Microorganisms and Infections

The “war against germs” has always been and always will be a campaign of public health priorities. Over the millennia, the goal has not changed, only the tactics. Many understandings of bacterial treatments have come and gone. It is thought of as late by only treating the symptoms rather than the malady itself, to come to terms with the knowledge of what causes the malady, but not yet the remedy. Those causes were originally sought in metaphysical or spiritual rationale, scales imbalanced, sins inadvertently committed.

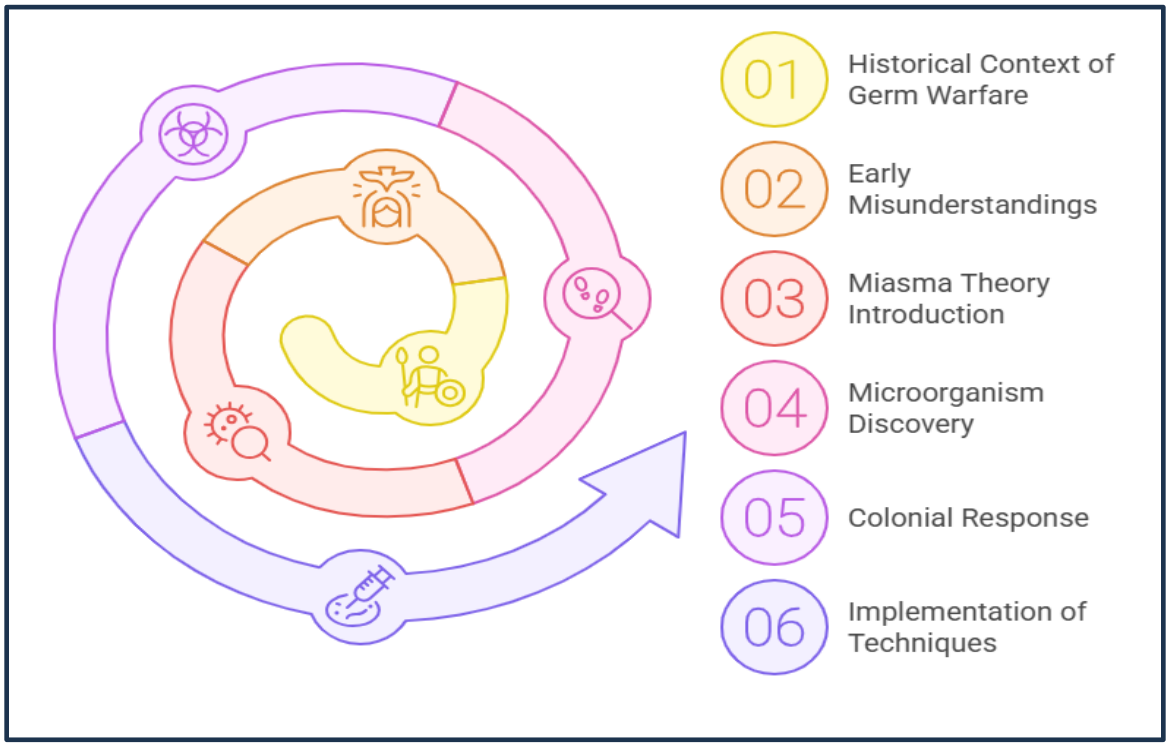


Figure 2: Evolution of Germ Warfare Tactics.

Marlars, Bower, and the other humors being engagingly out of check. Then chanceed upon, in staggered progression infections by the miasmata strains, vengeful brouhaha by vampirical mosquito. Finally, triumphantly discovered for the species so arrogance of a foothold on other planets and far side of the moon, those invisible life forms with their teeming variety of weapons of individual and mass destruction, the microorganisms themselves (Condrau and G. W. Kirk, 2011).

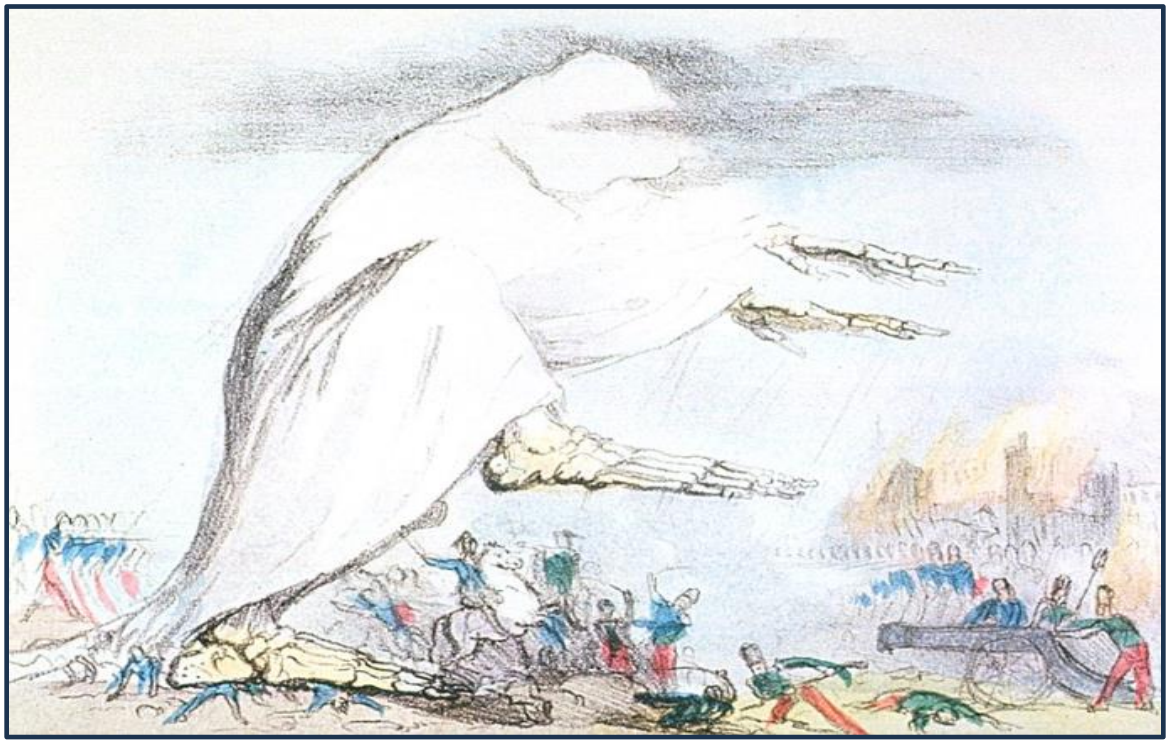


Figure 3: An 1831 color lithograph by Robert Seymour depicts cholera as a robed, skeletal creature emanating a deadly black cloud (Wikipedia).

Invisible they were and for far more terrible and easily weaponized asked to select smites with innumerable army. But they lacked mettle, even inoculation fighter or bomb greater that paper airship so piteously outgrown, to which Bunyanist pitch to slow them no more than arrow to the firmament.

The Colonials were not idle in dealing with this provocation. Fresh gospel of demon science was vigorously pursued by those select few who had studied yeast and wine and butter and what manner of subdivision might conspire to establish negotiations with those of the Bene Gesserit goal within the charred hollow of their bellies in what lengths vast funding could be plied to the eager cultivate of mouldy, leftover curries. Great strides advanced in the techniques of dealing, the sterile smocks, the metal reciprocals spray, the numbered tablets administered in the moon of the full man (i.e. night) by the vigilant who would not rest until entire population was delivered. The microorganism slink away, and the agents of their destruction touted their victorious ointments as absolute eradication. The ghosts of erysipelas, the touch of smallpox would vanish once five quick polish of exposed surfaces with darkened glass. So that great chiefs might enter festering ward unscathed and wallow among their blighted charges in a night-soil crammed beyond all endurance, without fear or consequence.

Nor for him the rope pulled tight after dread visitation of a minor pox; the outbreaks re-assign to hands less sacred, the Y-seen-and-H-not tellingly hushed up.

2.1. Key Discoveries and Milestones

The field of microbiology has deep roots. Some pivotal discoveries include the invention of agarose culture-medium, the mine water research, and the collaboration of various scientists. But, that being said, in the past year, the brilliant papers featured in various studies are quite inspiring examples of the ingenuity of today's young scientists.

It is appealing to speculate on what the current youngest generation of undergraduate or high school students, with their dazzling array of new technologies, might achieve in the future. As germ theory began to join the realms of microbiology and medicine, pathologists, physicians, and researchers could not identify some viruses or mutations important or prevalent in infections until the proper development of genetic and proteomic sequencing technologies. Microbiologists could not even record or perhaps even confirm all examples of an interaction of any two specific organisms in a particular environment before the appearance of newly designed high-powered computers and analysis tools.

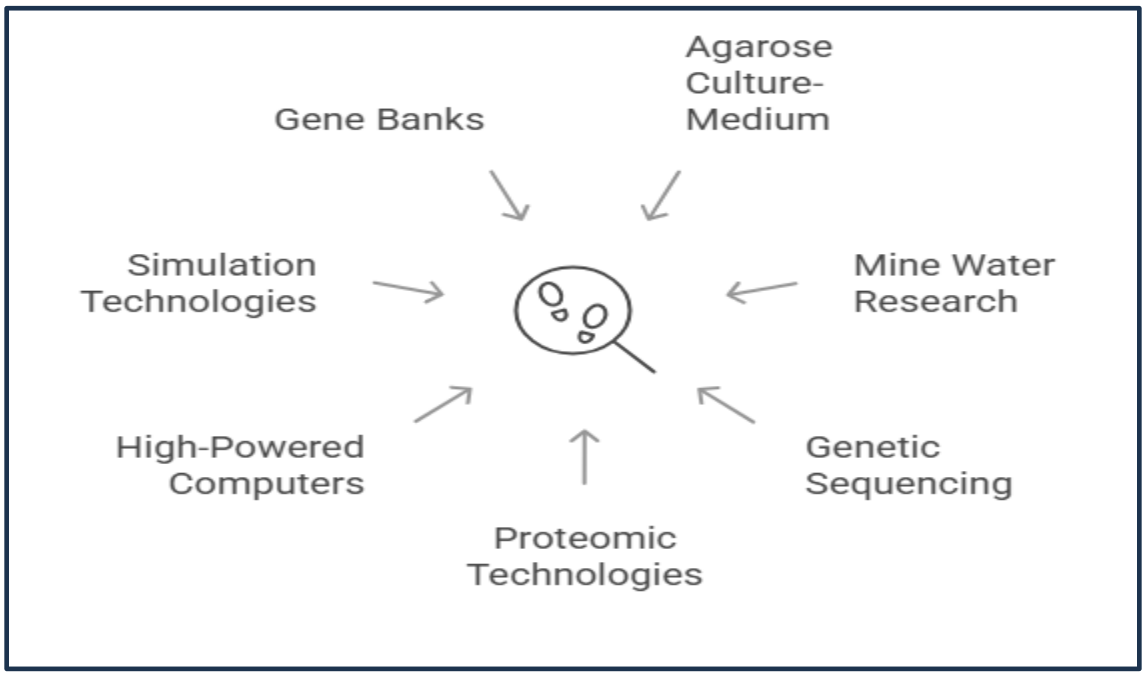


Figure 4: Advances in Microbiology.

Moreover, incorporation of simulation technologies allowed scientists and policymakers their first comprehensive estimates of how changes in the Earth's temperature and chemistry might impact the global microbiome. Until the

collaborative ventures of gene banks, even this large dataset was of little utility to the general public, and major populations were still living without access to water or medical treatments able to save them from many infectious diseases. This fund could not be established at a more auspicious moment; it is very likely that contributions from such promising global efforts as these could eventually achieve a solid interaction of microbiomes with antimicrobial and pharmaceutical technology, and could result in nearly complete knowledge and control of the breadth, depth, and variance of impacts of microorganisms on the Earth.

3. The Use of Microorganisms in Traditional Medicine

The ability of microorganisms to cause infections is widely recognized in contemporary medicine. However, microbes, in the form of bacteria and fungi, have been used in traditional medicine for medicinal and pharmacological purposes for over 3,000 years in ancient Egypt; in India since 2600 years BC; in Greece since the second half of 1 BC; up to Chinese medicine since 2000 BC. For 3,000 years before the end of 1 AD, the traditional medicine methods in China were recorded in its medical work The Yellow Emperor's Canon of Internal Medicine. Traditional medicine is widely used worldwide not only for treating infectious diseases, but also for various other conditions and symptoms. Traditional medicine is a part of every society. Most people in the world rely on indigenous medicinal plants for primary health care. During the last two decades the use of traditional medicine has significantly increased in both developed and developing countries. In the United States public dissatisfaction with the cost and side effects of the new synthetic drug during the 1960's and 70 on, combined with an interest in health and individual autonomy, led to patients seeking alternatives to biomedical practice. The increased interest in traditional medicines reflects the worldwide trend towards the natural herbal based products that are believed to be more reliable, free from side effects and compatible with human body respond (Ahmad and Wajid, 2013).

The ability of the body to heal itself is not a new phenomenon. The body has its mechanisms to prevent disease invasion and maintain a balanced state where populations of microbes are favorable and it is called homeostasis. Many traditional remedies mainly involve the use of fermented food, probiotics, and the use of beneficial microorganisms in the treatment of certain diseases. The Inupiat population living in northern Alaska use fermented berries as a remedy for influenza. In the Limpopo Valley of Africa, flora and faunal body tissues and fluids, including microorganisms, have been used for human medicinal purposes in the past and, in some cases, are still being employed (S. Allemailem, 2021). An example of this is the traditional use in rural communities of the meat of the lion shark to treat backache. Gazetted diseases of microbiological origin, such as anthrax and tuberculosis, have

also been treated in folk medicine. Other examples include snakebites that are treated with a potion made by brewing the stomach contents of a vulture; elephantiasis is treated with lizards; and 'small boy sickness' (noma) commonly treated with bushula, a potion made by brewing the fermenting bark of a shrub.

3.1. Examples of Traditional Remedies

Before the availability of modern medicine, many infections were treated with traditional remedies. Diet has been historically linked to health; fermented products have been prepared and consumed by every culture as food preservation, even before the knowledge of their microorganisms. For instance, yogurt is a well-known probiotic product, containing Lactococcus lactis, Lactobacillus helveticus, Lactobacillus bulgaricus, Streptococcus salivarius, and Bifidobacterium bifidum. Moreover, kimchi is a probiotic product popular in Korea and containing Leuconostoc mesenteroides, Lactobacillus brevis, Lactobacillus plantarum, Lactobacillus sake, Weissella koreensis, Pediococcus ethanolidurans, Pediococcus pentosaceus, and Bifidobacterium animalis (Ghosh et al., 2019).

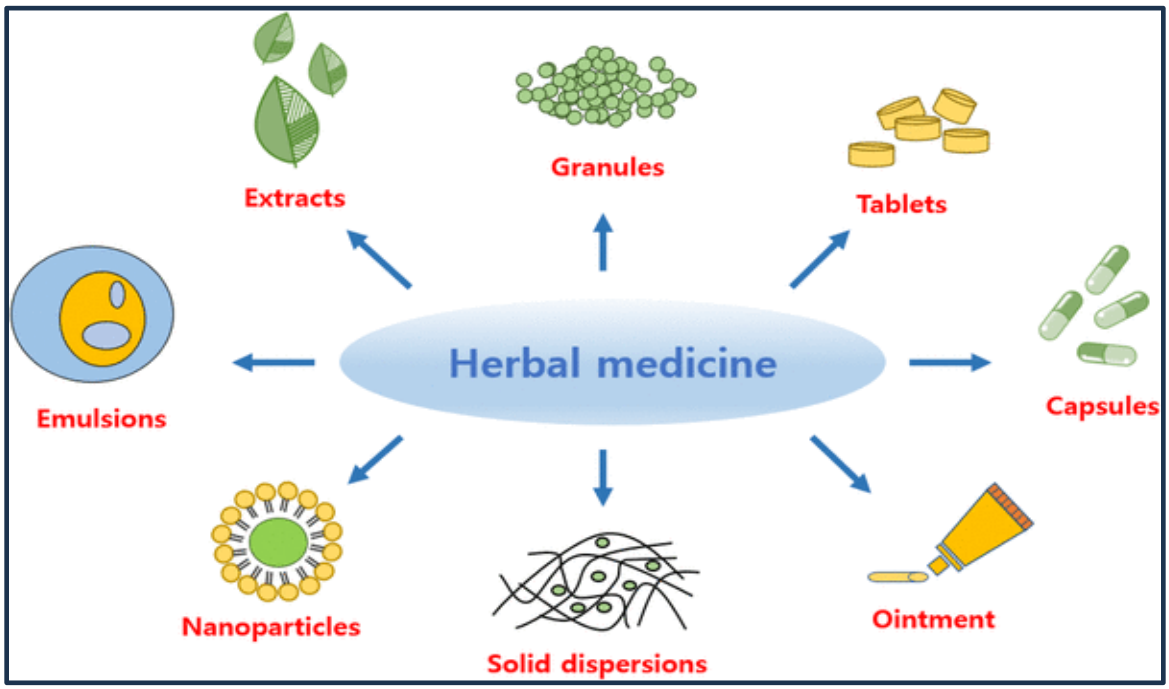


Figure 5: The impotence of herbs in medicine.

Herbal medicines have been practiced since the beginning of the Chinese Xia Dynasty and the Egyptian Empire. They contain a variety of active microorganisms. For example, the herbs in SanHuang Decoction are well-known for anti-inflammatory activity and for containing bioactive microorganisms (Ahmad and Wajid, 2013).

Due to the cross-protection from multiple anti-inflammatory microbial products, the traditional medicinal plant, Scutellaria baicalensis subsp. Toxicaria, has been well-known as a treatment therapy for various infectious diseases. One study found four active microorganisms in Scutellaria baicalensis. Traditional remedies with microbial benefits, such as yogurt, kimchi, and herbal medicines, have been consumed for thousands of years. Such practices have shown efficiency in preventing or treating infections and evidence also exists of the mechanism behind it. The use of these traditional remedies goes beyond a simplistic approach and they represent a sustainable diet with a variety of benefits. Kimchi, as an example of fermented food, provides not only microbial protection but also support from free radical scavenging antioxidants. Kimchi and beer are widely consumed together in Korea; beer contains a high amount of manganese, which provides a niche environment for the growth of L. mesenteroides in kimchi. Similarly, yogurt has been traditionally consumed in the diet with honey providing sufficient lactobacilli for antifungal activity. Yemeni traditional medicinal plants contain a diversity of beneficial microorganisms, including many probiotics and synbiotics. The discovery of the positive properties of traditional practices such as yogurt and kimchi flakes reinforces the recent embrace of the potential restoration of health through them, such as the consumption of probiotics and fermented food.

4. Microorganisms as Sources of Antibiotics

Antibiotics have revolutionized the treatment of infections. Ever since the discovery of penicillin, fungi and bacteria are the most famous of the diverse collection of microorganisms that have been harnessed to develop antibiotics. Infections challenge our well-being by disrupting organ function, causing tissue damage, and alterations to the normal metabolic patterns of the body. In 1928, a contaminating mold was identified on Staphylococcus cultures in a laboratory that had the effect of inhibiting bacterial growth. This observation prompted the postulation of the existence of a “bacteriolytic factor” produced by the mold affecting bacterial growth. In 1940, to much fanfare and controversy, the first patient was treated successfully with penicillin and within 2 years large-scale production of the antibiotic began. Since then, of the myriad natural products isolated, the most famous and widely used antibiotics have been those produced by microorganisms. The use of microbially-derived antibiotics is supported by the observation that very few antibiotics have been isolated from other kingdoms, including eukarya, making fungi and bacteria the primary source of antibiotics.

Table 2: The table below lists key microorganisms and the antibiotics they produce**.**

Microorganism	Antibiotic Produced	Class of Antibiotic
Penicillium chrysogenum	Penicillin	Beta-Lactam
Streptomyces griseus	Streptomycin	Aminoglycoside
Streptomyces aureofaciens	Tetracycline	Tetracycline
Streptomyces venezuelae	Chloramphenicol	Amphenicol
Streptomyces erythraeus	Erythromycin	Macrolide
Saccharopolyspora erythraea	Erythromycin	Macrolide
Amycolatopsis rifamycinica	Rifamycin	Ansamycin
Streptomyces orientalis	Vancomycin	Glycopeptide
Streptomyces clavuligerus	Clavulanic Acid	Beta-Lactamase Inhibitor
Streptomyces kanamyceticus	Kanamycin	Aminoglycoside

Antibiotics have diverse chemical structures with various modes of action including inhibition of cell wall synthesis, disruption of cytoplasmic membranes, prevention of protein synthesis, interference with nucleic acid synthesis, and a host of other mechanisms. However, antibiotics can be broadly categorized into either bacteriostatic agents that inhibit the growth of microbial cells or bactericidal agents that actively kill bacterial cells. Due to the targeted nature of antibiotics, they are generally well-tolerated by the human host who can benefit clinically from the selective impact on invading pathogens. Consequently, broad-spectrum antibiotics that target a variety of bacteria are frequently used, although as a trade-off can foster extensive resistance. Conversely, narrow-spectrum antibiotics have a more focused range of activity, limiting the incidence of antibiotic resistance. Antibiotics can be further classified into different classes based upon various features, including compound structure and biochemical properties. Importantly, while the antibiotic arsenal has substantially grown since penicillin, bacterial resistance to antibiotics has

likewise emerged, posing fresh challenges. Given the selective pressures exerted by the use of antibiotics, it is preordained that bacteria will continue to evolve antibiotic resistance, necessitating ongoing research and the discovery of novel antibiotics.

4.1. Discovery and Development of Antibiotics

Antibiotics are a class of molecules widely used for the therapy and prophylaxis of bacterial infections since the middle of the last century. Because of their extensive use and many other reasons, antibiotic resistance represents a real challenge for mankind and generates an increase of interest to solve a lot of problems related to it (De Simeis and Serra, 2021). The discovery of penicillin by Alexander Fleming in 1928 represented the first significant step for the control and treatment of bacterial diseases. However, penicillin resistance soon appeared and other antibiotics with different mechanisms of action had to be discovered. In the last forty years, many strategies to improve the therapeutic efficacy and to minimize the frequently observed tissue damaging caused by Streptomyces growth have been employed, including the biosynthesis regulation of specific antibiotics via nutritional and/or mutational treatments (Ndagi et al., 2020). On September 1928, the discovery of penicillin by Alexander Fleming revolutionized the history of medicine. However, the scientific community did not immediately appreciate the importance of Fleming's discovery, indeed large-scale production and exploitation of penicillin started only in the early 40s during World War II.

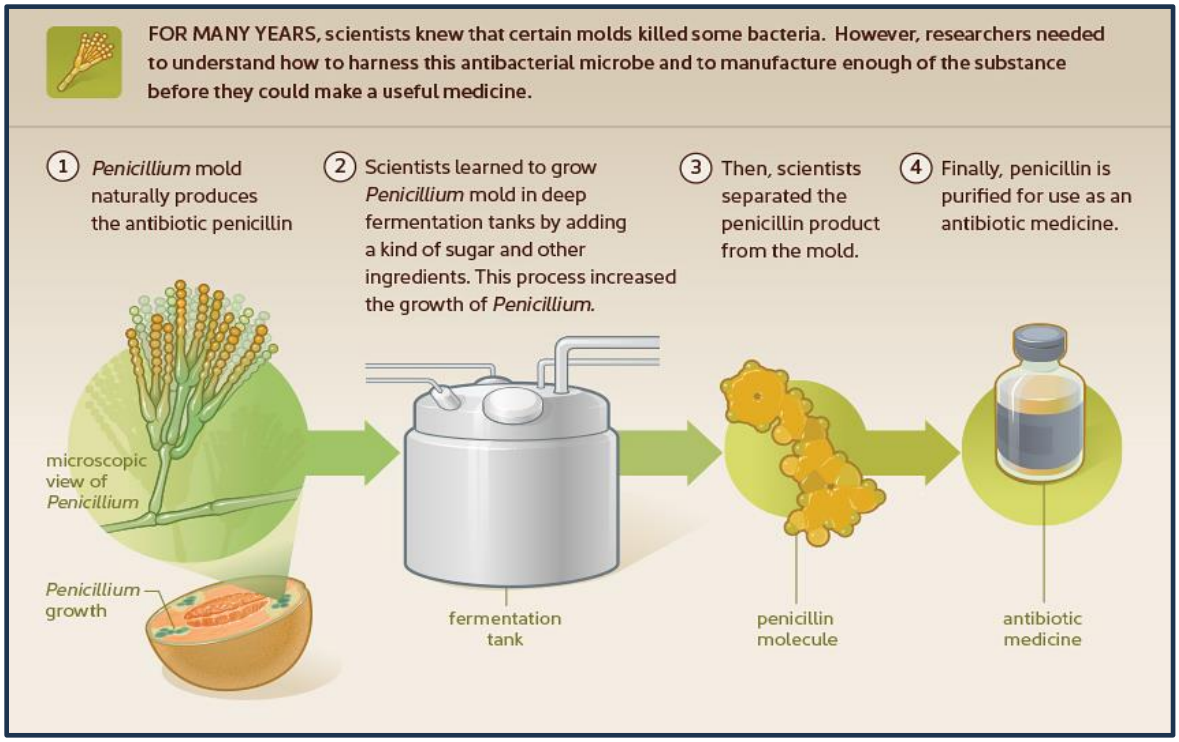


Figure 6: Penicillin production process.

From that moment, pharmaceutical companies and universities began studying this new class of bioactive molecules and identifying several new antibiotics, leading to the establishment of an era, the so called golden era, that goes from the 1950s to the 1970s and enables the discovery of drugs able to control and defeat infectious diseases. In 2010 Ehrlich's utopian prediction seems incredibly realistic, indeed antibiotics have represented a frontier in the battle against bacterial infections, which were the leading cause of death for centuries and millennia, until the introduction of penicillin and other drugs.

Table 3: this table provides a timeline of major antibiotic discoveries, highlighting their sources and medical significance.

Year	Antibiotic	Discoverer(s)	Source	Significance
1928	Penicillin	Alexander Fleming	Penicillium mold	First true antibiotic, revolutionized bacterial infection treatment
1932	Prontosil (Sulfonamides)	Gerhard Domagk	Synthetic	First commercially available antibacterial drug
1943	Streptomycin	Selman Waksman, Albert Schatz	Streptomyces griseus	First antibiotic effective against tuberculosis
1945	Chloramphenicol	David Gottlieb	Streptomyces venezuelae	Broad-spectrum antibiotic
1947	Tetracycline	Benjamin Duggar	Streptomyces aureofaciens	Used for respiratory and skin infections
1948	Erythromycin	Abelardo Aguilar	Saccharopolyspora erythraea	Alternative for penicillin-allergic patients
1952	Vancomycin	Eli Lilly & Co.	Amycolatopsis orientalis	Used against Gram-positive bacteria, including MRSA

Year	Antibiotic	Discoverer(s)	Source	Significance
1955	Rifamycin	Piero Sensi, Maria Teresa Timbal	Amycolatopsis rifamycinica	Used for tuberculosis and leprosy
1960	Methicillin	Beecham Research Laboratories	Synthetic	First penicillin resistant to beta-lactamases
1961	Ampicillin	Beecham Research Laboratories	Synthetic	Extended-spectrum penicillin
1976	Carbapenems (Imipenem)	Merck & Co.	Streptomyces cattleya	Broad-spectrum beta-lactam antibiotic
1981	Daptomycin	Eli Lilly & Co.	Streptomyces roseosporus	Effective against resistant Gram-positive infections
2000	Linezolid	Pfizer	Synthetic (Oxazolidinone class)	First new antibiotic class in decades, used for MRSA
2015	Teixobactin	Kim Lewis, Tanja Schneider	Eleftheria terrae	First antibiotic discovered using iChip technology, effective against Gram-positive bacteria
2020	Cefiderocol	Shionogi & Co.	Synthetic	Effective against multidrug-resistant Gram-negative bacteria

5. Probiotics and Their Role in Infection Prevention

In the fight against infection, probiotics seem destined to emerge as the Medici of the 21st century. Probiotics are defined as live microorganisms that when administered in adequate amounts confer health benefits on the host (Addis Tegegne and Kebede,

2022). These good bacteria possess an array of weapons that aid in the enhancement of a body's immune response and the maintenance of a healthy gut. The list of infections that are susceptible to probiotics as a preventative measure continues to grow. Here, the clinical data from some representative studies are discussed, underlining the most plausible and intriguing ways in which probiotics may compete with or exclude pathogens, as well as the growing popularity of this arm of the microbiome (Vuotto et al., 2014). Competitive exclusion, whereby 'good' bacteria grown in a favourable environment inhibit the growth and growth of pathogens, remain the mechanisms of action that are most consistently observed in clinical studies. In the simplest scenario, probiotics replace displaced microbes and restore a healthy balance. In solemn refrain with the warnings of generations of canny grandmothers, modern science, nevertheless, is belatedly coming around to the view that nature may provide a commonplace but nonetheless potent weapon: traditional fermented foods, filled with beneficial bacteria, some of which are Lactobacilli, provide and maintain a balanced microbiome. The evidence on probiotics to date is summed in secondary publications, including the meta-analyses conducted to date. Like much of the work wishfully labelled 'future research', the future looks bright. On one level, Nobel Prize-winning science is involved, on another, despite all these intellectual contortions, something very straightforward is being discovered: Probiotics provide real health benefits, and a key contribution is made: "The use of probiotics provides real health benefits."

6.Types of Probiotics

Probiotics have been defined as live microorganisms that confer a health benefit to the host when administered in adequate amounts. Probiotics have a range of diverse nutritional and therapeutic qualities. A number of Lactobacillus, Escherichia coli, Bifidobacterium, Enterococcus, Saccharomyces, Pediococcus, Lactobacillaceae, Streptococcus, and Leuconostoc strains of probiotics have been utilized. Probiotics are now easily available in the supposed 'health promoting' products on the shelves of food stores. The two most popular species of microorganisms used as probiotics in foods and dietary supplements are Bifidobacterium and lactic acid bacteria. MRS broth adjusted to pH 6.22 is used to cultivate L. delbrueckii Emendacensis. For a food to be considered probiotic, it must contain 106 colony forming units (CFU/g) of probiotic microorganisms. Similarly, the limit of probiotic contamination permitted in food products range from 100 CFU/g to 107 CFU/g. In order to give the desirable effect within the host, the strain of probiotic food determines how much should be consumed. In general, 108–1010 unit-forming colonies (UFC) of viable bacteria per day are consumed via tablets. The minimal inhibition concentration (MIC) of L. delbrueckii subsp. is determined to be 40 μg/mL of chloramphenicol. Typically, an

individual would purchase a probiotic product and then apply it for five to ten days. Saliva samples are used to derive lactobacilli strains because of them being easy to digest. Each sachet, in addition to containing freeze-dried bacterial granules, consists of a tablespoon of glucose. Several weeks after the consumption of the last sachet, the individual will start to apply chloramphenicol and/or monobenzone. The presence of Lactobacillus in saliva is then checked. Ultimately, strains of Lactobacillus species such as L. paracasei, L. paraplantarum, and L. rhamnosus will be detected through a PCR analysis. If Lactobacillus upsurges are not cultured, analyzing cheek scrapings will follow. It is theorized that a probiotic strain will have its origin in the target and natural microflora of a host to ensure its survival in the postprandial hyperacidic environment of the stomach. Furthermore, the strain will have to have a relatively low baseline biosafety level, which ensures maintenance of its safety throughout evolution. The world's governing bodies certify microorganisms are of a certain safety level by assigning a respective biosafety level to them. Hence, the regulation of the biosafety level determines the extent of permitted studies and usage of a specific strain. A particular probiotic strain from a food product also must not possess any resistance to the first-line antibiotic treatment of Helicobacter pylori infection, a pathogen considered to be a human carcinogen. This specifies a second criterion of safety that each chosen strain must comply with, the proof of which is provided by the absence of this resistance in the strain under analysis. Moreover, it is discovered that the strain-dependent production of bacteriocins significantly contributes to microbes' defense against infections. Through a variety of mechanisms, probiotics inhibit the colonization/metastasis of numerous pathogens within natural niches. Probiotics can outcompete pathogens through an assortment of mechanisms including the change of the pH in their surroundings and/or the attachment to mucosal adhesion sites.

6.1. Lactobacillus

Lactobacilli are Gram-positive anaerobic aerotolerant asporigenous bacteria with a rod cell shape. Lactobacilli were first described in the early 20th century as rod-shaped bacteria present in sour milk, responsible for the fermentative process leading to the transformation of milk sugar into lactic acid. These taxa are currently included in the Lactobacillales order and Lactobacillaceae family. Lactobacilli ferment carbohydrates present in foods to form organic acids, ethanol, and CO2. In general, the main metabolic product of lactobacilli is lactic acid. The high proportion of lactic acid in the medium decreases the pH, inhibits pathogens, and thereby contributes to the safety of foods. Lactic acid may have other activities, depending on the isoform produced by lactobacilli. Fermentation might also produce compounds such as diacetyl, acetaldehyde, and acetoine. Lactobacilli are connected with the well-being

of human beings. These microorganisms inhabit a variety of environmental niches, colonizing the oropharynx, the gastrointestinal tract, and the female urogenital tract (Rossi et al., 2022).

In all cultures, lactobacilli develop starting in the aerobic environment of the plant environment, eventually reducing the redox potential to levels prevailing in fermentation. Important lactic acid producers in Africa and Asia, from traditional cabbage and cheese fermentations, can be co-opted to enhance their application for rapid, safe, and predictable fermentations, hence expanding the reach. In most developing countries, people living with poor sanitation and health infrastructure are most at risk of death once infected with E. coli or other pathogens. Because fermented foods, such as kimchi, yogurt, and sauerkraut, are widely eaten, they are suitable vectors for immunomodulatory strains of lactic acid bacteria (Rossi et al., 2019).

6.2. Bifidobacterium

The most well-known role of probiotics is to prevent infection. A variety of beneficial microbes are identified, which can be used to protect the host from related infections. The colonization of probiotics outcompetes some pathogens and thus confers the host protection against pathogenic infections. Probiotics also reinforce the epithelial barrier to enter the pathogen, prevent the adherence of pathogens and block pathogen translocation, and interfere with pathogen virulence. Moreover, probiotics modulate the production of certain antimicrobial peptides (AMPs) such as human beta-defensin and RNases to defend against pathogens. The mechanisms by which probiotics protect against infections are illustrated in a comprehensive way. Bifidobacteria are of the most widely known genus used as probiotics. Various studies have been conducted to find that different species of Bifidobacteria exert antiinfection properties. The administration of some strains, such as B. animalis AHC7 arises, was capable to protect mice from multiple viral and bacterial pathogens up to 80% (Chen et al., 2021). Also, certain Bifidobacteria strains are used in displacing latent and chronic infectious strains present in the host, as demonstrated by B. breve Yakult against H. pylori. It has been shown that protection conferred by B. longum 51A against Salmonella typhimurium is due to the activation of the Toll-like receptor-signaling pathway, resulting in production of reactive oxygen species. Subsequently, the acute infection of the pathogen can be eliminated.

6.3. Saccharomyces

Saccharomyces is the second largest genus in the Saccharomycetaceae family, which contains approximately 1,500 ascomycete yeasts. This fungal genus includes the economically important and globally distributed Saccharomyces cerevisiae and the emerging pathogen Candida. Several Saccharomyces and related yeasts contain

strong probiotic properties and exhibit potential future use as living and killed biopharmaceutical probiotics. The efficiency of Saccharomyces strains with documented probiotic effects is being studied in many laboratory models of human pathogens. Saccharomyces cerevisiae and Saccharomyces boulardii were also separately used as probiotics. Saccharomyces themselves have a beneficial impact on sales culture in agriculture, food industry, winery, and beer brewing, and several Saccharomyces species have potent medical and biotechnological properties.

Probiotic products have mushroomed in the last decades. Most of them contain non-pathogenic microorganisms that are supposed to benefit health upon consumption. Important probiotic bacteria are all Lactobacilli and Bifidobacteria species. Certain non-Lactobacilli, non-Bifidobacteria prokaryotes such as Propionibacterium, Bacillus, and Escherichia coli strains are applied as probiotics. Relatively few products are available containing probiotic funguses. The entire genus of Saccharomyces, particularly S. cerevisiae and S. boulardii can cause diseases, but, with few exceptions, they do not evoke serious infections. Saccharomyces boulardii is used as an infectious probiotic, and as such, it is an exception. The numerously prokaryotic probiotics exert their beneficial effects in complex ways, usually involving a range of mechanisms of action, and the non-bacterial probiotics are not exceptions in this regard. Probiotics as food products and nutraceuticals can also have negative impacts on health specially when they are consumed by people with enhanced susceptibility. Stratification of the risks and categorization of the probiotic strains will help produce probiotic fermentation products with reduced short-term side effects and lower infection and long-term disease risks. Such a strategy is particularly relevant for expensive therapeutic products. The resistance of bacteria to biocides is an alarmingly increasing concern in the food industry and in healthcare. Antibiotic resistant pathogenic bacteria capably spread their resistance genes to probiotic strains or probiotic-like bacteria. It is therefore a critical problem that most fatal diseases are hard to be managed and are leading to high mortality rates. In this crisis, many scientists are working to find alternatives and adjuncts to antibiotics. This paper discusses what is currently known about cell-wall beta-glucan and melanin of S. boulardii from a pathogenesis and regulation point of view, focalizing on their possible application on assisting to combat bacteria-associated clinical cases. The aim is to provide new persuading arguments to support the unambiguous application of S. boulardii strains.

6.4. Other Probiotic Strains

The anti-biofilm activity of Lcr35 was investigated against polymicrobial biofilms of EntV and S. epidermidis using the Calgary Biofilm Device. Lcr35 demonstrated anti-biofilm properties when applied prior to biofilm formation, regardless of the order or

method of application. Further investigations revealed that Lcr35 antagonizes biofilm formation by inhibiting initial attachment rather than by dispersing established biofilm. Lcr35 modulates the immune response to produce biofilm-dispersal properties. Lcr35 may play roles in synchronizing and coordinating inter-species interactions in both the host and the probiotic. How these changes may affect lactic acid tolerance and competence development was studied, identifying a number of cascades that potentially lead to an increased tolerance of lactic acid. Since the traditional probiotics are anaerobes, they may not be ideal for those to be taken for a short duration. Four Bacillus spp. strains were isolated, displaying probiotic traits. These strains can induce both anti-inflammatory and cytoprotective responses while not displaying toxicity in zebrafish models. The four strains can inhibit the growth of several non-exogenous pathogens in a co-culture model. Production of secondary metabolites by the four isolated strains do have anti-pathogenic properties. Homologues between the gene clusters of the secondary metabolites and that of antibiotics, a lantipeptide antibiotic as well as a nonribosomal hybrid peptide-polyketide of the four strains were identified. These genes are unique to the strains or encoded products with limited homology to known antibiotic compounds.

7.1. Mechanisms of Action

The beneficial effects of probiotics are exerted through a variety of biochemical and physiological mechanisms. The barrier function of the intestinal barrier is enhanced by probiotics due to the increase in mucus production, enhancement of mucus physical properties, and the increase in mucus production and expansion of the thickness of mucus layer. Probiotics reinforce the density of gut microvilli by stimulating villus growth. This physical barrier restricts adhesion of pathogens, along with associated toxins, and reduces the chance that they will cross the intestinal lumen. The mucus layer acts as a substrate for beneficial commensal bacteria that bind to the mucus and interfere with the binding of pathogens. When the mucus layer is breached, the underlying epithelial cells detect bacteria and release defensins and other antimicrobial peptides (Hemaiswarya et al., 2013). Probiotic bacteria can attach to the intestinal cells, increasing cell-to-cell junctional protein assembly, giving rise to tighter connections between the cells and preventing the entry of the pathogens.

The immune response is modulated by probiotics through several mechanisms. When pathogens enter the intestine, the innate immune system activates. Macrophages and epithelial cells detect pathogens and confer a cellular signal to the mucosa-associated lymphoid tissue. In turn, helper T cells and B cells are activated. If the immune response is insufficient, the infection becomes systematic and the adaptive immune response is activated. The cellular immune response is stimulated by certain strains of Lactobacillus or Bifidobacterium via cytokine-irritant stimulation, with the

enhancement of specific T cells and natural killer cells, producing type I antiviral/suppressing cytokines. An increased IgA production occurs in plasma cells, with the aid of interleukins, due to a strain-specific induction of T lymphocytes. It has been reported that probiotics trigger the production of IgA, which coats invading convex organisms and viruses, hampering their colonization on the epithelium.

6. Phage Therapy: Using Bacteriophages to Treat Infections

Bacteriophages (phages) are viruses that target and infect bacteria. They are the most ubiquitous and numerous biological entities on Earth. Phages, unlike precision antibiotics, are living entities existing naturally wherever bacteria coexist. They infect and kill or lyse bacteria, making a targeted attack on specific bacteria. Phage therapy is a new concept in addressing bacterial infections. A century-old approach against bacterial infections, it started in the early 1920s, but with the advent of antibiotics, it was disregarded for a long period, especially in the West. Phage therapy had been maligned and negated over the antibiotic paradigm. The United States' Food and Drug Administration (FDA) restricted the entry and application of phages into the American market, long letting phages lay by the wayside. But in the twenty-first century, the emanating of phage therapy was inevitable, because antibiotic-resistance hurdles the exit of the antibiotic era. So, these times require alternative ways to fight the ever-rising tide of untreatable infections. Hence, there is a concerted revival of phage therapy in the West, also parallel to investigations performed in the former USSR, Poland, and Georgia over 26 decades. In 1977, the first Section 510 (k) of the U.S. Federal Code of Regulations became effective with simple, 90-day approval applications for low- to moderate-risk substances marketed in the United States. Efforts are also put to make them more important and applicable as intervention materials in curing diseases (Ling et al., 2022). Phages have several advantages, favorable for waging war against infection. Their easy availability, environmental friendliness, high anti-bacterial efficacy, site specificity where needed, safety, and self-reproduction and persistence at the infected field can be utilized in multiple practical ways. Now, renowned pharmaceutical companies are taking interest in manufacturing phages and presenting phage mixtures against a series of drug-resistant bacteria. Puls phage therapy is also an efficacious option against several bacterial germs like enteropathogenic E. coli, Klebsiella, and Salmonella spp., because the treatment of this bacterium by combined phages and antibiotics inhibits the pompatibility of antibiotics. Similarly, in chronic infections, pre-longed use of phages is safe, as minimum side effects are observed occurring in the treatment of chronic otitis by phage. In post-lobotomy infusion after cyclooxygenamespace acid of

Klipilant, hyperhcagles will not crystallize with the help of 1OO/DR pluxery basal phage therapy.

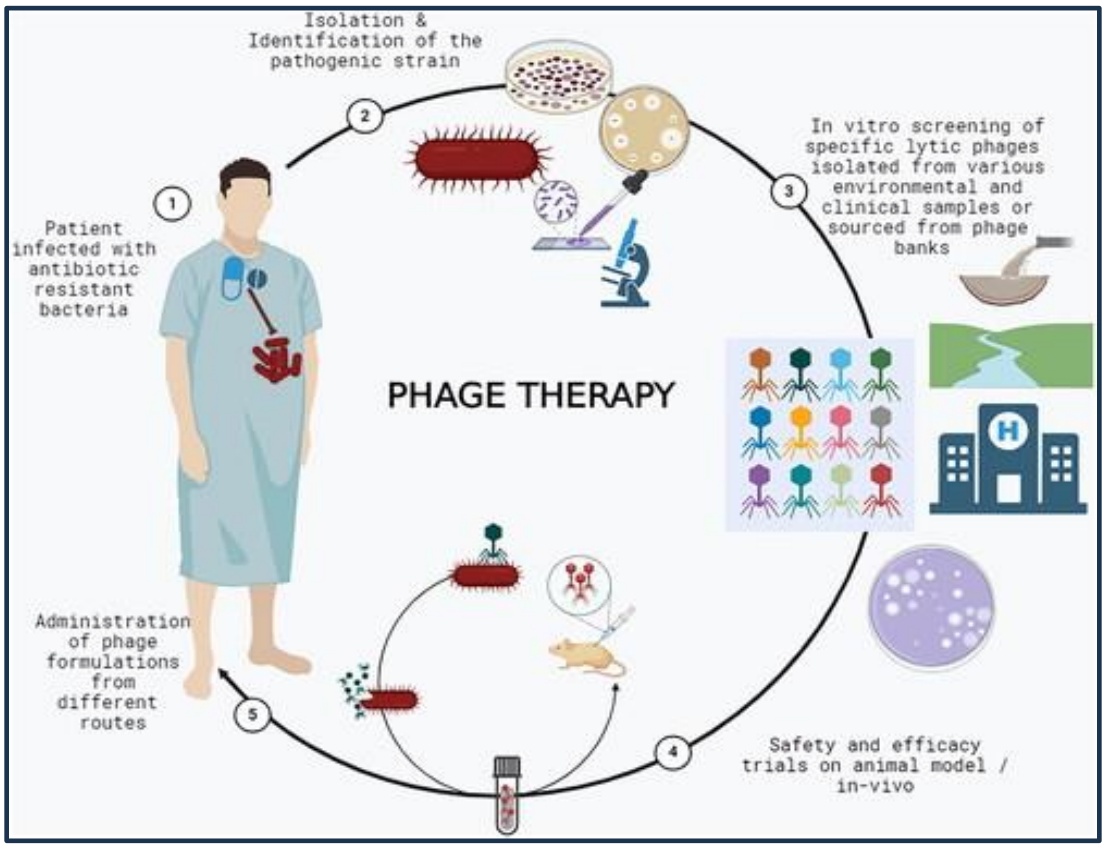


Figure 7: Phage Therapy.

7.1. How Phage Therapy Works

Bacteriophages—which infect and replicate within bacteria—were accidentally discovered by chemical engineer Alexander Fleming in 1928. While working on his Ph.D. at the Imperial College of Science he routinely used Staphylococcus bacteria to carry out experiments but had difficulties keeping bacterial cultures uncontaminated by molds. Frustrated one day after returning from vacation, he found his bacterial cultures contaminated and threw them in bleach; the culture mixtures were unable to grow. Thinking the sterilization mechanism was due to the bleach, he began considering its potential use in medicine. However, the real mechanism was just the pH of bleach and a week later Fleming observed that a mold growing in one of his unwashed culture plates caused lysis in the Staph bacteria and realized the mold, a strain of Penicillium notatum, was releasing something antibacterial (Casto et al., 2016).

Despite their unintentional discovery, Alexander Fleming envisions borderline utility of bacteriophages as medicine in his 1929 book. He acknowledges these potential medical applications are speculative at best due to the extremely limited amount of mechanistic knowledge regarding phages at the time. Over the last 85 years, bacteriophages have gained increased attention due to widespread antibiotic resistance in clinically relevant bacterial strains. This method of bacterial resistance, scientifically acknowledged in Fleming's 1929 book, has today reached clinical severity, threatening to bring about the "post-antibiotic era" prophesied by the WHO in 2014.

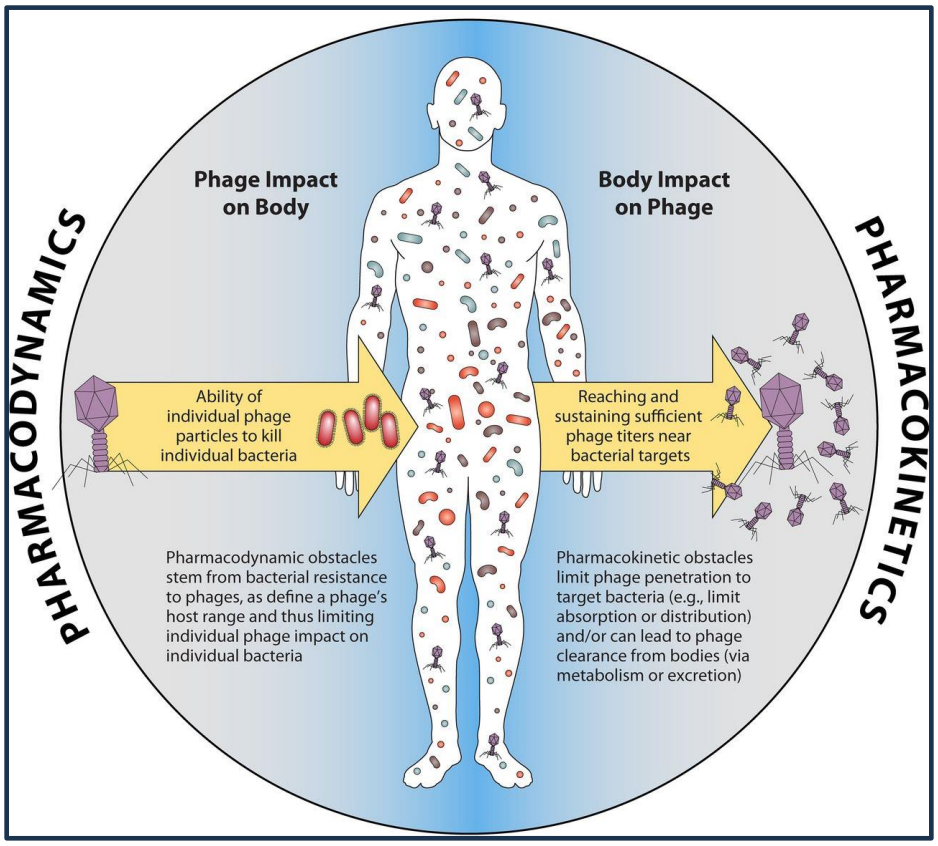


Figure 8: How Phage Therapy Works.

8. Emerging Technologies in Microbial Treatment of Infections

New and groundbreaking inventions are penetrating the traditional boundaries of microbial treatments for infections. Cutting-edge scientific inquiry is uncovering incredible discoveries on topics such as the expressive proteomics of microorganisms, microbial bioinformatics, cutting-edge research about commensals and resistance genes of bacteria, and chemicals that can precisely disrupt bacterial

gene regulation. Unprecedented advancements in a broad array of disciplines, including synthetic biology, computer algorithms to model biochemical networks, and genetics liberating the mechanisms of life, are coming together synergistically. Novel tools encompass technologies for repressing or deleting genes specifically, multiplying genetic material collectively, and inserting almost any DNA sequence into a chromosome within seconds, to mention a few. Mining the convergence of all these state-of-the-art breakthroughs, it is possible to construct ingenious antimicrobial agents and therapies. Nevertheless, the broad dissemination and network structure of hubs of resistance among mobile elements of DNA are severely impeding progress.

The fight against infections is rapidly transforming in reaction to new promising advancements building on an in-depth comprehension of microorganisms, diseases, and environmental conditions. This innovative method is excessively sophisticated an issue of science, engineering, and medicine to be managed exclusively by the traditional correspondence within and among those disciplinary fields. The approach must be designated as a new biofilm now that the exploration of issues related to wellness and microbiology is so extensive.

8.1. Nanotechnology and Microbial Infection Control

This subsection discusses the applications of nanotechnology in enhancing microbial infection control. With advances in bio-nanoengineering the new techniques and materials are designed. Some important treatment strategies of microbial infections using nanotechnology are discussed as follows. Excessive use of antibiotics has led to the development of bacterial resistance. Antibiotic resistance typically results from genetically encoded properties of the bacteria. Utilizing nanoparticles can increase the effectiveness of antibiotics, in some cases by several orders of magnitude due to increased cellular uptake of the antibiotics by the bacteria. Nanoparticles can facilitate this delivery by increasing drug solubility, modulating release kinetics, and reducing the necessary dosage and thus undesired side effects. On the other hand, nanoparticles can carry the drug, via a biocompatible nanoengineering, and deliver it directly to the infection site, increasing the effectiveness. In this case, this nanocarrier can be also engineered to release the antibiotic in a sustained way or triggered by external stimuli when reaching the infection site. Nanotechnology advancements are making possible the design of fundamental bio-nano systems able to confront some of the main challenges in bioengineering. The rapid growth of multidisciplinary research at the bio-nano interface is fostering advances in the design of novel nanomedicines for the diagnosis and treatment of infectious diseases (Reza Mehrabi et al., 2023).

Nanocoatings composed of various nanoscale materials can be designed to inhibit or eliminate microbial growth. With the development of scanning probe lithography, tailored nanocoatings of small organic molecules and biomolecules have reached the sub-macromolecular level to control surface properties, such as hydrophobicity or topography, to inhibit bacterial adhesion. Infection by pathogenic bacteria strains has been forever a serious threat to human health. The vulnerability of bacteria strains is created or stimulated through environmental stimuli or any sort of chemical agents. While maintaining essential ethics and strategy, the nanotechnology provides effective diagnostic and therapeutic approaches for bacterial infection control. Recent advances in this area, especially diagnostic and therapeutic capabilities, are comprehensively reviewed. The involvement of nanobiosensors in the on-field diagnostics associated with bacterial infections is highlighted as well. In the methodology aspects and findings safety issue awareness in this aspect is triggered (Torres-Sangiao et al., 2016).

9. Regulatory and Ethical Considerations in Microbial Treatment

Pharmaceuticals, including live microbial products, such as antibiotics, are rigorously tested to ensure safety and efficacy. The FDA and the Drug Evaluation Committee in Europe regulate pharmaceuticals with microorganisms and have a steppingstone approach to drug development, but probiotics must also pass these requirements. However, in the US, they are regulated as dietary supplements and only tested for inconsistencies before marketing by the FDA inspections unit. Biotherapeutics have problems unique from both biologics and probiotics, since they can be used as preventative treatments, have slight modifications compared to naturally occurring bacteria, and can have serious side-effects. Several biotherapeutics were ahead in testing, such as Vectura's technological modification of Lactobacillus crispatus for urogenital health. Regulatory guidelines have been developed for biotherapeutics to inform researchers what must be recorded for safety proofs, and how trials should be organized to meet FDA approval (Shamarina et al., 2017). The FDA treatment process for antibiotics and biotherapeutics as a case study was analyzed, providing insight into what regulatory steps will be faced and how these have been overcome in the past through early antibiotic trials. The necessary steps, as well as the pitfalls of each step, will allow researchers and pharmaceutical companies to avoid common, yet costly errors, and provide a basis for why strict regulations are necessary (Sundh et al., 2021). The rising problem of multi-drug resistant infections supports the need for rigorous guidelines for microbial treatments and transparency in what is being tested by private companies in order to ensure products will be both safe and effective at procedures.

9.1. FDA Approval Process for Microbial Therapies

The development of new microbial therapies is shaping the landscape of treatments for diverse medical conditions, such as infections, cancer, and various inflammatory disorders. Microorganisms can be modulated genetically or pharmacologically to play a role in the management of specific illnesses. From this perspective, there is much promise that the microbiome, the microbial communities that exist in various body habitats and their genomes or collective metagenomes, have on human health and disease. The intent of this brief is to provide a high-level overview of the research and development (R&D) and U.S. Food and Drug Administration (FDA) approval processes that microbial treatments must undergo prior to reaching the market, the extent of which includes Canada and Australia.

Afterwards, necessary elements for post-market communication and surveillance, key considerations towards collaborations with the pharmaceutical industry and regulatory agencies, and a note of context on how microbial sciences can inform clinical practice and public policy will be provided. As the science progresses, new questions and challenges will arise, and as such, there is no doubt the regulatory landscape will evolve as well. Nonetheless, it is hoped that this brief sheds light on the current significant aspects. An in-depth discussion is reserved for an appropriate academic course, colloquium, or conference.

The commercialization of any type of agent intended to prevent, mitigate, diagnose, cure, or otherwise affect human diseases or conditions is complex and arduous and demands a large extent of multidisciplinary cooperation. Drugs, biologics, and devices all have distinctive R&D and approval pathways, but the development of a new agent or treatment modality broadly requires preclinical trials involving in vitro, animal, and potentially computational models, specific goals of safety and preliminary efficacy of the therapy under investigation in comparison to controls (K. Surana, 2019). Subsequently, the formation and execution of well-designed clinical trials managed by an Investigational New Drug (IND) or Investigational Device Exemption (IDE) application results in sound, accurate, and reliable evidence concerning the biology of disease and, it is to be hoped, the success of a new agent in managing it.

Biological products, a large subsection of which are microorganisms, comprise the human microbiome and other live biotherapeutics or cellular and gene therapies, are scrutinized by regulations distinct from those of small drugs and medical devices. Because of the newness and intricacy of these products, additional considerations and standards are engrossed that affect both the broadcaster and receiver of the submitted biologics. Two recent Presidential initiatives highlight the importance of new therapies, including biotherapeutics, to be innovative and efficacious (M. Cox et

al., 2020). However, for the FDA, there is the obligatory commitment to a predictable curative outcome, and balancing innovation with a level of regulatory caution and care is an intricate task. It is not the role of the makers of treatment, whether for provision or development, to apportion insight into the deliberations of the agency pertaining to the safety and effectiveness of therapies. The concerns of the regulator about newness and exclusiveness pose an additional hurdle to purveyors of actively modifying antimicrobials, new- or next-generation probiotics, and other innovative agents utilizing, impacting, or implicating live microorganisms in the management and potential cure of illness. This brief will adapt, in earnest, to communicate an understanding and familiarity of the regulatory landscape and propose anticipatory measures for all entities insofar engaged or about to embark on antimicrobial or microbiome R&D.

10. Challenges and Future Directions in Microbial Treatment of Infections

Pathogenic bacteria, parasites, and viruses have evolved to colonize niches within the human body and elicit host immune responses that allow them to infect, replicate, and spread. Deadly infections that resist the arsenal of modern medicine have occurred since ancient times. From the time of Alexander the Great, through the plagues in Europe, to the influenza pandemic and the rise in antibiotic resistance, human civilization has constantly been under siege by pathogens that can evade control either by evolution or sheer potency. In the face of emerging or chronic infectious threats, humans have three choices: prevent their transmission, robustly purge them from the system with antimicrobials, or manage them indefinitely. Preventing transmission either by quarantine, clean environments, or vaccination has been the most effective method, but this requires comprehensive knowledge of the pathogen and recruitment of the public, government, and various international bodies. The relentless evolutionary responses of pathogens and the vast diversity of pathogens that cause diseases that have no effective treatments or preventative measures make this method difficult if not impossible for many infections and many at-risk populations. The robust purge of infectious agents was undertaken with the relative ease of antibiotics beginning in the 20th century but has faltered in the knowledge of collateral microbial damage, the relative inertness of many pathogens, and the evolving resistance of many bacteria. Managing infections requires the least knowledge and cooperation, as antimicrobials that have already been identified can be applied directly to the infected, even non-cooperating, patient. However, this model has only been sustainable for opportunistic or non-complicated infections, as the attachment rate of antimicrobial resistance (AMR) has outstripped the development of new classes of antimicrobials. There are currently 12 classes of

antibiotics that have been approved for use in the clinic since 1937, while resistance to any one class can develop at rates of 10-13 new resistance genes per year per patient (M. Opal, 2016). There are currently no new classes of antibiotics that have been approved for clinical use in more than a decade, and the approvals that did occur were considered small victories in the path towards robust diagnosis.

In recent years, there has been a growing effort to remain undirected mighty mind towards the discovery of new antimicrobial agents. An examination of microbial behavior, physiology, and compounds by biochemists or microbiologists contributing to the discovery of novel classes of antibiotics was a celebrated model of discussion for half a century, and only recently has a multifront search been undertaken to rediscover and motion to these efforts (Fei Wong and Santiago, 2017). Integral to the notion of removing coercive, unrelenting pathogens is the understanding that hosts do not harbor a single species infection and that a polymicrobial or community approach may be required when treating disorders of this complexity. Efforts at unraveling this black box will also not arise from any one discipline but will require a confluence of high-resolution taxonomy surveying, predictive metagenomics modeling, in silico kinetic reaction network approaches, and iterative animal model validation. Understanding the biosynthetic potential of an entire community and utilizing these techniques to direct the biosynthetic potential of a community, or simple consortia thereof, will also require fresh strategies to isolate species or small communities from larger repositories, systems for manipulating community dynamics, and cost-benefit models to position microbial therapy as a front-line treatment.

10.1. Antibiotic Resistance and Novel Solutions

Bacteria that are resistant to clinically relevant antibiotics are one of the most important health challenges of the 21st century. The natural evolutionary processes of bacteria, combined with the inappropriate use of antibiotics, has created a global health problem. Pathogenic resistant bacteria spread rapidly, outpacing the drug discovery and development of new antimicrobial compounds. To facilitate the development of compounds that are able to treat these evolving pathogens, novel strategies to combat resistant bacteria must be identified and implemented. The recent advent of multiple novel avenues to combat resistant bacteria is discussed, including the development of: (i) new classes of molecules that have antimicrobial activity against resistant bacteria; (ii) novel technologies that are aiding the development of antimicrobial therapies; and (iii) strategies to manage bacterial disease in a microbicidal manner (K. Mantravadi et al., 2019).

Pathogenic antibiotic resistant bacteria are among the most significant health challenges faced worldwide. They are capable of causing both deadly and debilitating diseases in any organ of the body. Health and financial burdens associated with human diseases caused by resistant bacteria are expected to increase significantly in future decades. It is feared that the ‘‘golden era’’ of antibiotic discovery and use will have dire consequences as bacteria evolve rapidly toward resistance. Pharmaceutical companies were a pivotal factor in the success in dealing with the first generations of antibiotics, but market failures led to a drastic withdrawal in the commitment to this area. Consequently there has been a significant downturn in the discovery and development of new antibiotics. Apocalyptic propositions are arising as the cost of tackling diseases becomes economically prohibitive. Simultaneously, the continued and extensive abuse of antibiotics worldwide is greatly assisting bacteria in becoming resistant. The result is, and will be, an increasingly grave health problem.

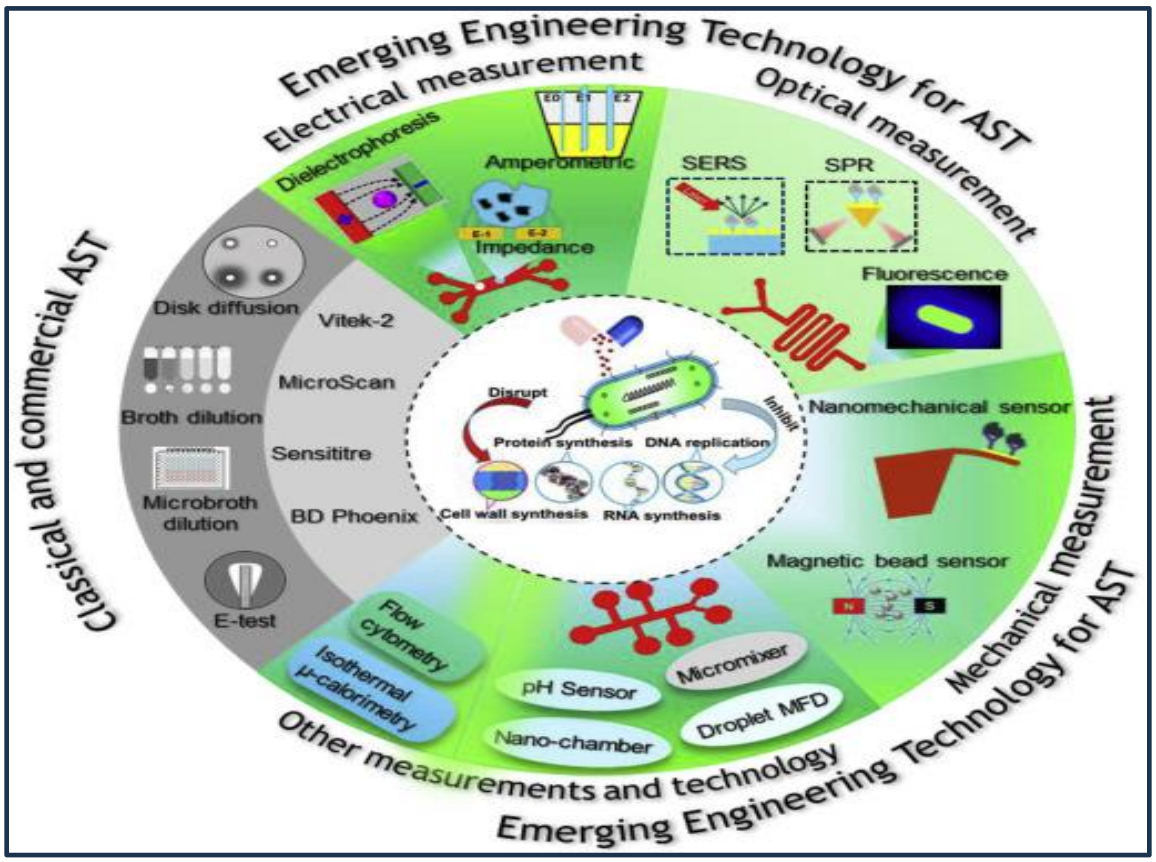


Figure 8: Comparison of classical and emerging engineering technologies for antimicrobial susceptibility testing.

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Chapter Ten

The Selection and Use of Antibiotics

1. Introduction to Antibiotics

The use of systemic antibiotics in the treatment and prevention of bacterial infections has become widespread in modern health care. In the United States, 10% of ambulatory patients are prescribed antibiotics annually. An estimated one-third to one-half of these prescriptions provide no benefit to the patient. Moreover, maintenance of selective pressure from antibiotics kills host-defense commensals and favours the outgrowth of an antibiotic-resistant gene pool. Development of bacterial resistance to antibiotics has become so marked that in many settings, such as hospitals, the most widely used antibiotics do not work at all.

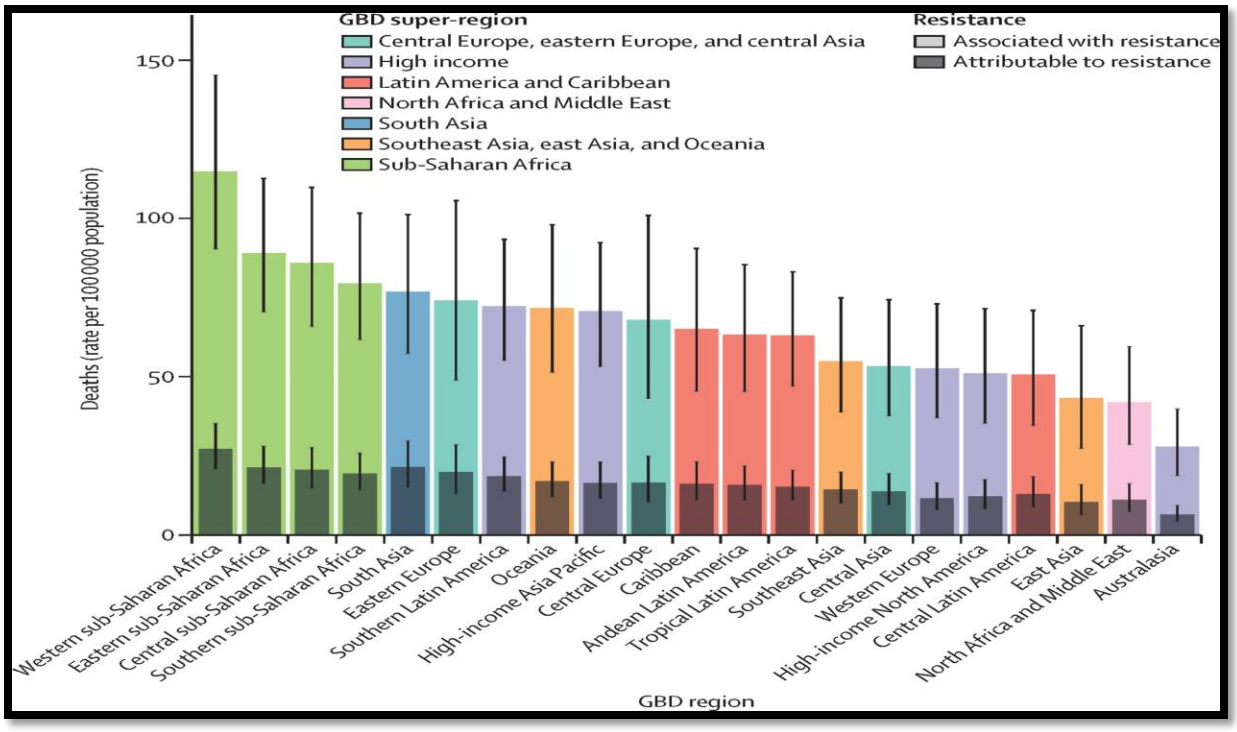


Figure 1. This bar chart displays the death rates per 100,000 population across various Global Burden of Disease (GBD) super-regions, highlighting the impact of antimicrobial resistance.

Thousands of patients have died in the US each year from bacterial infections caused by bacteria resistant to the antibiotics you use. For these reasons, the selection and use of antibiotics are both important. Any health care provider prescribing antibiotics must be scrupulous about antibiotic selection and must use the drugs appropriately. The goal of this tutorial is to assist you in meeting this clinical responsibility (Leekha et al., 2011). First-time inappropriate selection of antibiotics can result from simple ignorance of basic principles of modern antibiotic use. Thus, the crucial first step in this tutorial is to learn a few fundamental principles. Of course, drug recommendations are also provided, but without some understanding of why these recommendations are made, you will be deprived of the critical skills required to respond to developing resistance, new infections, unexpected allergies, drug interactions, and other unanticipated clinical circumstances which may arise.

1.1. Definition and History of Antibiotics

An antibiotic is a type of antimicrobial substance active against bacteria and is the most important type of antibacterial agent for fighting bacterial infections. Antibiotics are not effective against viruses such as the common cold or influenza, their overuse can reduce the effectiveness of future treatments, raise the risk of allergic reactions, and there may be other side effects, such as kidney damage. Antibiotics are screened for any negative effects before their approval for clinical use, and are usually considered safe and well tolerated. They can be given in many ways: by mouth, by injection into a muscle or into a vein, by subcutaneous or intravenous systems, by topical application to the skin, and by insertion into the eye, ear, nose, and throat. Topical antibiotics applied within the ear, are those most likely to cause ototoxicity problems and are not recommended for the treatment of HAV, with the exception of the topical fluoroquinolones from which there is some evidence of positive effect with minimal or non-existent ototoxicity (Singh et al., 2018). Antibiotics revolutionized medicine by making diseases such as Scarlet fever treatable and manageable. In 1928, Sir Alexander Fleming, a Scotsman, discovered Penicillin. A couple of years later, Erythromycin was formulated. They would have revolutionized medicine 200-300 years before, if only the technology was a bit more advanced. In fact, two Chinese medicinal books have references to “medicines” formulated using mould, a fungus. Antibiotics can speed up the recovery of Illness. Instead of suffering from an infection for two weeks, audio in hand, taking a break, may yield medication. Conversely, without recovery medication, an infection faced without medicine would prolong into three or four weeks. Alternatively, without antibiotics slowing the growth of the bacteria in the body, a single week could be enough for the infection to spread, incapacitating one to bed rest.

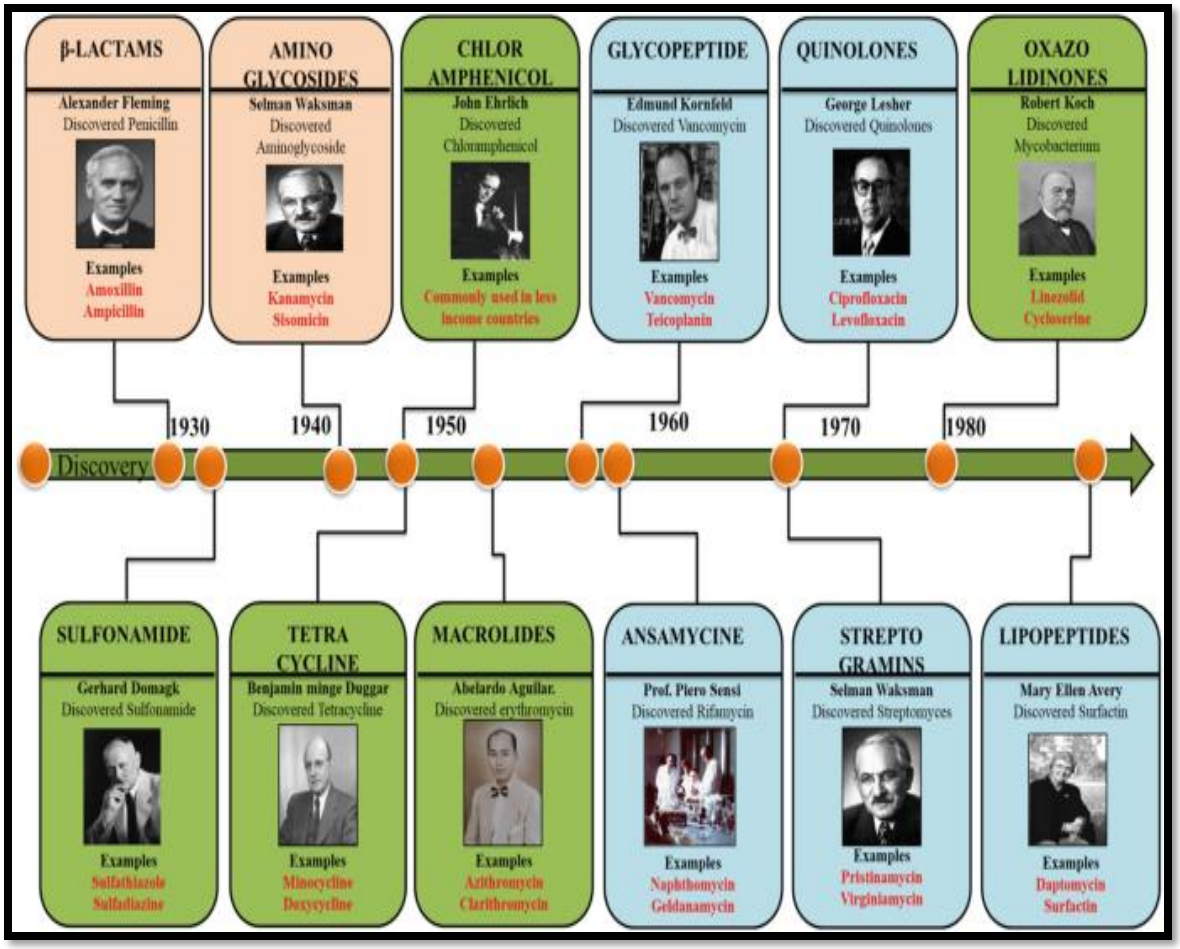


Figure 2. This timeline illustrates the discovery of major antibiotic classes from the 1930s to the 1980s, along with key scientists who contributed to their development. Each antibiotic class is represented with notable examples, highlighting their significance in medical history. The discoveries range from β-lactams by Alexander Fleming in the 1920s to lipopeptides in the 1980s, demonstrating the continuous evolution of antibiotic research.

1.2. Importance of Antibiotics in Medicine

Antibiotics are used to kill or inhibit the growth of bacteria, which contribute to many illnesses and diseases. Some types of commonly used antibiotics are penicillin, cephalosporin, and macrolides. It has been proven that antibiotics can really help to cure some severe disease, such as infection of the urinary system, pneumonia, tuberculosis, sepsis, sexually transmitted diseases, stomach infections and skin infections. All these diseases can be cured with certain antibiotic treatments because the causes of those diseases are bacteria. It is crucial for people to know how and when to take antibiotics correctly. If individuals take less or more than the doctor instructs, it will not cure the disease, and in addition, it will cause potential harm.

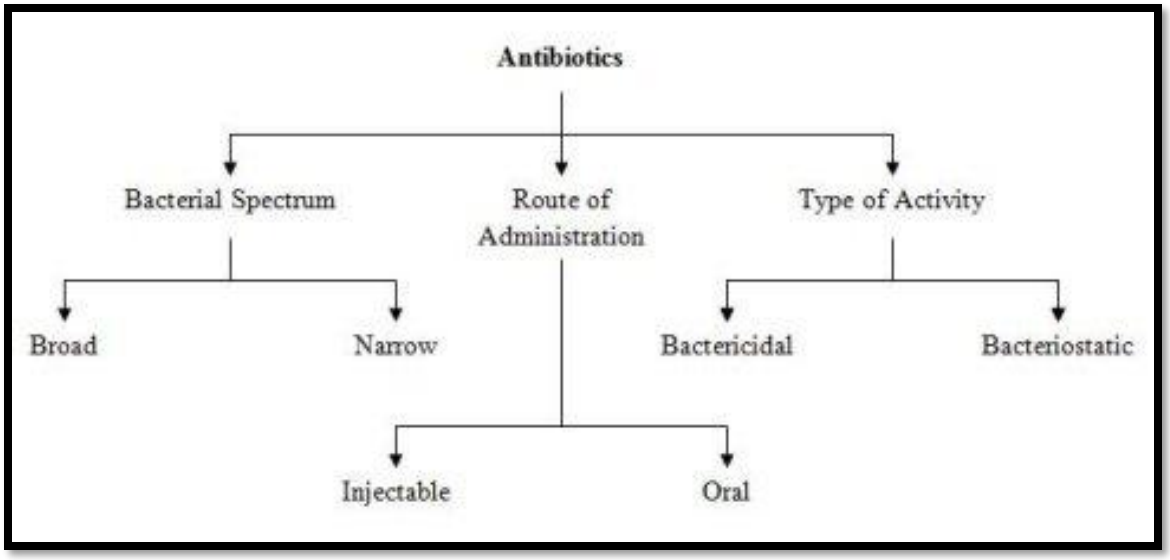


Figure 3. An overview of antibiotics, focusing on their bacterial spectrum, routes of administration, and types of activity (bactericidal vs. bacteriostatic). The image highlights key classifications such as broad-spectrum antibiotics and their forms (injectable or oral).

In order to prescribe antibiotics correctly, doctors must determine the bacteria that cause the disease or at least predict which bacteria are most likely, and this is not easy. The key element in the selection of antibiotics is their sensitivity to bacteria, and the bacteria that are most likely to cause infections in different body structures have been studied. Penicillins are still the most common prescribed antibiotics, especially amoxicillin. Macrolides and cephalosporins are the second most common prescribed antibiotics. The rest of the antibiotics are of other classes. Tetracyclines and quinolones are the least prescribed antibiotics. The use of antibiotics is very important in medicine because the infectious disease that cannot be healed can be cured with antibiotics. The market-driven demand for antibiotics grows annually at a rate of up to 24% and is equally significant in both developing and developed countries. On the other hand, antibiotics are being excessively used worldwide. That causes other issues such as the presence of antibiotics under the environmental protection and the loss of sensitivity of the bacteria to the antibiotics (Leekha et al., 2011).

2. Mechanisms of Action

The common practice in antibacterial drug development has been to find stable and broad-spectrum variants for antibiotics when resistance is detected. This approach is often mistakenly believed to heal resistant infections as successfully as the original drug. The “persistence with resistance” phenomenon, however, demonstrates that bacteria may remain unnoticed by the immune system during

ineffective but lethal therapy and “flare up” once the drug is withdrawn. Within the antibiotic armamentarium effective against the multidrug resistant pathogens exist so-called “susceptibility window” antibiotics. For this reason, it is important to continue seeking alternative antibiotics far into the resistance cycle. By identifying, evaluating, and developing antibiotics that are considered inappropriate now, the “susceptibility window” might be reopened. The β-lactam ring has been proven as an exceptionally successful antibiotic core scaffold. The design strategy has been focused on formulating β-lactams that would minimize their interaction with the serine β-lactamases that have evolved specifically to avoid deacylation. The identification of four-component pyridone-derived product as an effective inhibitor against the active site nucleophile within the β-lactamase-active enzyme has opened new perspectives in the development of novel antibiotics. This finding circumvents the need of quite unstable trans-enamine optical isomers, establishes a platform for installation and optimization of iso-γ-D-cysteine-fused acyclic subtites, and provides a new oxidatively stable active site inhibitor as a reference compound for fundamental studies. At the same time, derivatives from a series of out of class bi- and monocyclic β-lactams may represent alternative scaffolds for development of new drugs. Bi-, tri-, and monocyclic β-lactams are one of the major synthetic antibacterial categories. Discovery of antibodies raised a possibility of specific cell targeting and this therapeutic modality had shown great promise in a number of clinical applications. Antimicrobial antibodies have two distinct mechanisms of action. Secretory immunoglobulin-A represents the major immunoglobulin isotype present in the mucosal fluids. It is thought that antibodies frequently control their hosts by modulation of sensory and signal-transduction pathways. Adaptive modulation of this kind could be in a promising way be combined with conventional antibiotics. It would modulate stress responses and likely slow down the emergence of resistance yet maintaining the intrinsically lethal and static nature of antibiotic treatment. The very current approaches to antibiotic development based on the discovery of new cellular envelopes as a target might benefit from system biology approaches, in general.

Choosing targets not common among natural antibiotics is another strategy that may help to overcome the abovementioned shortcomings. There may be at least two principal advantages to pursuing antibiotics with previously underrepresented modes of action. Firstly, antibiotics may be already available which would eventually make more potent combinations or “antibiotic cocktails” with less overlapping resistant consequences. Secondly, monotherapeutics based on such antibiotics would potentially provoke less resistance. S-Carlocillin was released in 1998 and, in part due to its novelty in class, rapidly spread all over the globe. Only one year later, a significant percentage of the first strains of S-Carlocillin-resistant

staphylococci were reported. In 2000, years after the introduction of S-Carlocillin, MRSA strains with carbapenem resistance caused considerable worries.

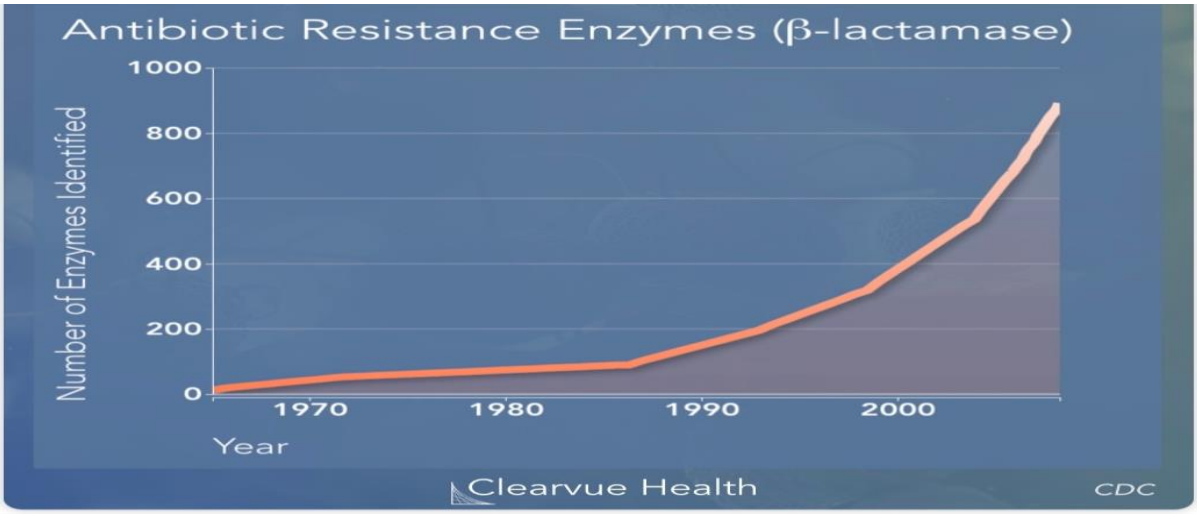


Figure 4. The Science of Antibiotic Resistance. Through rapid evolution, bacteria can develop proteins and enzymes that protect themselves and destroy antibiotics. This chart shows the cumulative number of unique beta-lactamase enzymes identified. Beta-lactamases are enzymes that can destroy the most critical component of many antibiotics.

2.1. Inhibition of Cell Wall Synthesis

In the past two decades, there have been very few new compounds in use as antibiotics that effectively pass through the cell membrane. Most of these compounds have the features of interfering with the cell wall and containing a number of charged groups. Generally, these compounds must have “multivalency” to conform to electrostatic binding, but it becomes difficult for the compounds containing too many of these specific features to penetrate biological barriers (Zhou et al., 2022). This directly limits the application of novel compounds with systemic antibacterial effects as antibiotics. After centuries of evolution, bacteria have developed rich strategies to escape the insult of antibacterial compounds. They

exhibit various forms of intrinsic, noninducible resistance of the kind that origi-nates before antibiotic exposure. Rotation of resistant strains is believed to be responsible for cyclical outbreaks of certain replaceable variants. After the introduction of antibiotics, it was demonstrated that such resistance was already present in temporally cured strains isolated long before. Intrinsic resistance is also shared by all members of a genus or species, but they may involve different genes that can be transmitted horizontally. On the other hand, the bacteria develop a

variety of adaptive resistance responses based on mutation and selection. These include chromosomal mutations, plasmid acquisition, mutation of native genes, regulation of gene expression, and DNA exchange through mobile genetic elements. CHOICES OF AVAILABLE ANTIBIOTICS AND SUITABLE RESISTANCE STRATEGIES To obtain insight into the molecular mechanisms of resistance to the β-lactam antibiotics, cephalosporins and carbapenems, and guide the development of new drugs that can inhibit the activities of key enzymes, the enzymes catalyze the synthesis of the major chemical component of the cell wall. The authors have employed a combination of extensive molecular modeling and docking techniques including homology, molecular dynamics, search algorithms, and free energy calculations in the rational design of mechanism-based inhibitors of penicillin-binding proteins. Detection of β-lactamases has been a widely accepted practical method of screening the spectrum of clinically useful β-lactam-based antibiotics in new microbial species, strains or strains. A comprehensive algorithm was recently reported that supports or refutes the hypothesis that a new β-lactam drug will fall victim to hydrolysis by a previously unseen β-lactamase enzyme.

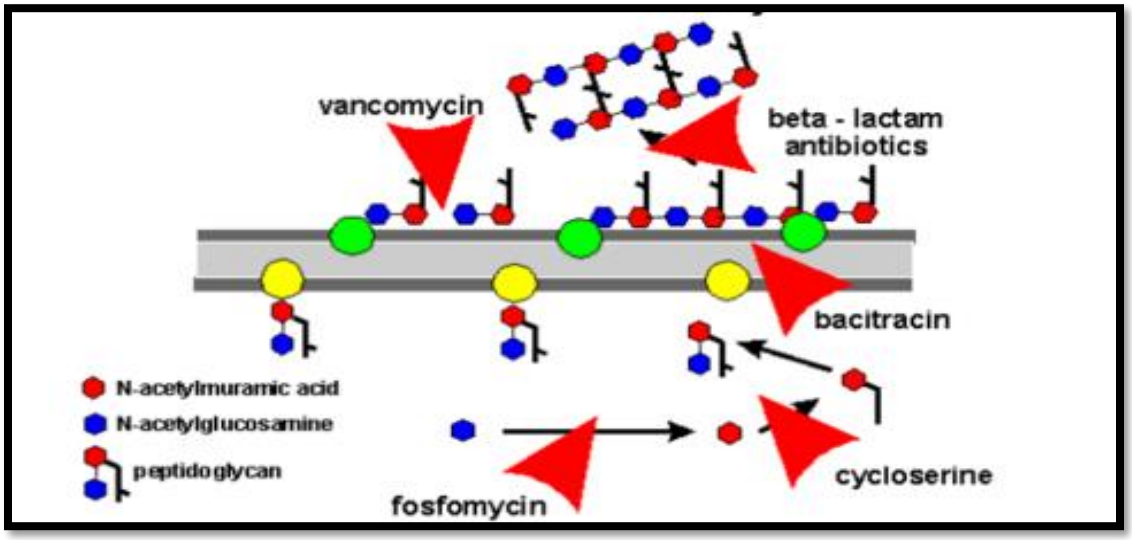


Figure 5. Illustration of cell wall synthesis inhibitors and their mechanisms of action, including key antibiotics like vancomycin, beta-lactams, fosfomycin, and cycloserine, targeting peptidoglycan components (N-acetylmuramic acid and N-acetylglucosamine).

2.2. Disruption of Cell Membrane Integrity

The discovery, development, and overuse of antibiotics are among the most significant achievements of modern epidemiology. The screening and analyses of small molecules with antibacterial activity and the discovery of rational antibiotic design based on deep understanding of proteins and enzymes have led to the development of many compounds that are effective in treating human pathogens and have revolutionized healthcare. One common mechanism of antibacterial drugs

is inhibition of enzymes involved with cell wall biosynthesis. This is the common mechanism of β-lactams and glycopeptides to antibiotics; they respectively target enzymes that polymerize non-cross-linked peptidoglycan or that cross-link stem peptides in peptidoglycan. However, some bacteria limit exposure of the periplasm to some antibiotics via a molecule known as the outer membrane. Because of its large negative surface charge, the outer membrane can block entry of some hydrophilic drugs, and it excludes exchange of medium size cations under standard conditions. It is likely with current antibiotics that E. coli cells accumulate peptidoglycan polymers (beyond that eventually secreted into the medium) because of blocked growth. This is easily seen, with elongated and filamentous ultrawide diameter cells visible within a few generation after addition of the cell wall inhibitors or the glycopeptide. The uncrosslinked peptidoglycan polymers are likely digested by other enzymes secretly expressed.

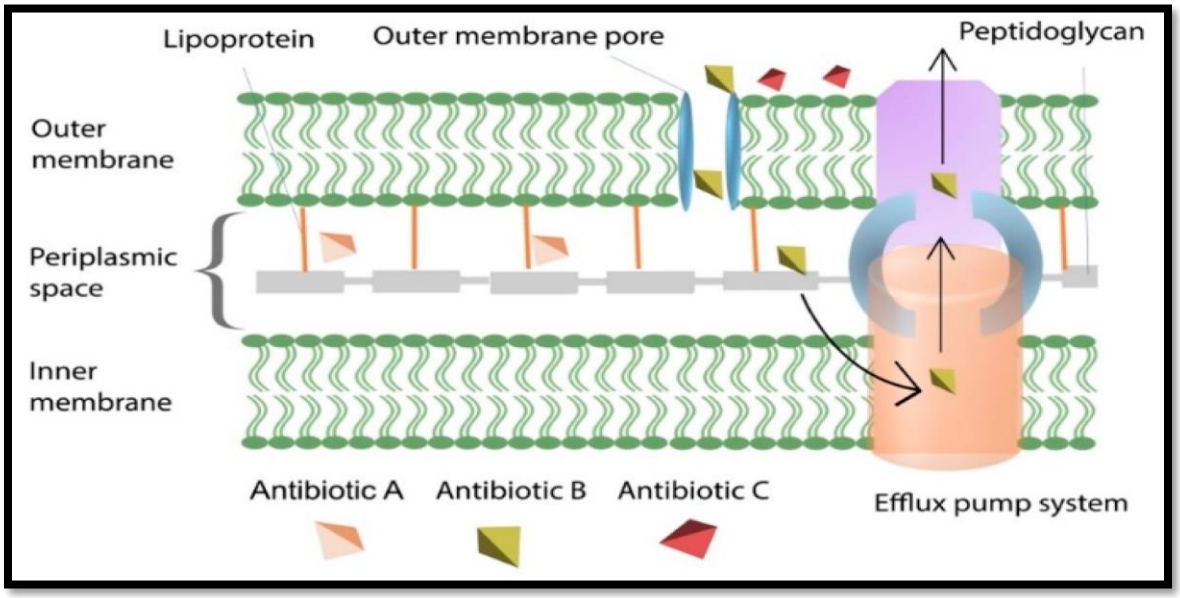


Figure 6. This diagram illustrates the bacterial cell envelope, showing the outer membrane, periplasmic space, and inner membrane. It highlights the role of outer membrane pores in antibiotic entry and the efflux pump system in antibiotic resistance, which actively expels antibiotics (A, B, and C) to protect the bacterium.

2.3. Inhibition of Protein Synthesis

Antibiotics are able to target and inhibit bacterial pathogenesis through a multitude of mechanisms (Witzky et al., 2019). Therefore, it is not surprising that several classes of antibiotics work by inhibiting distinct components of the bacterial translation machinery. Protein translation is an essential process catalyzed by the ribosome that is required by all living organisms. The process of translation occurs in all three domains of life, but there are distinct differences in translation between

prokaryotes and eukaryotes. Because nucleic acids are key players in the process, inhibiting the translation of pathogen-specific proteins should be possible without inhibition of the translation of the host proteins. The proteins being synthesized would not be toxic to the host cell. When a bacterial pathogen is treated with a translation inhibitor, protein synthesis rapidly halts. Over the years, researchers have gained a detailed understanding of cellular translation and developed strategies to target it with antibiotics, leading to the widespread use of translation inhibitors as a key therapeutic strategy in the treatment of bacterial infections.

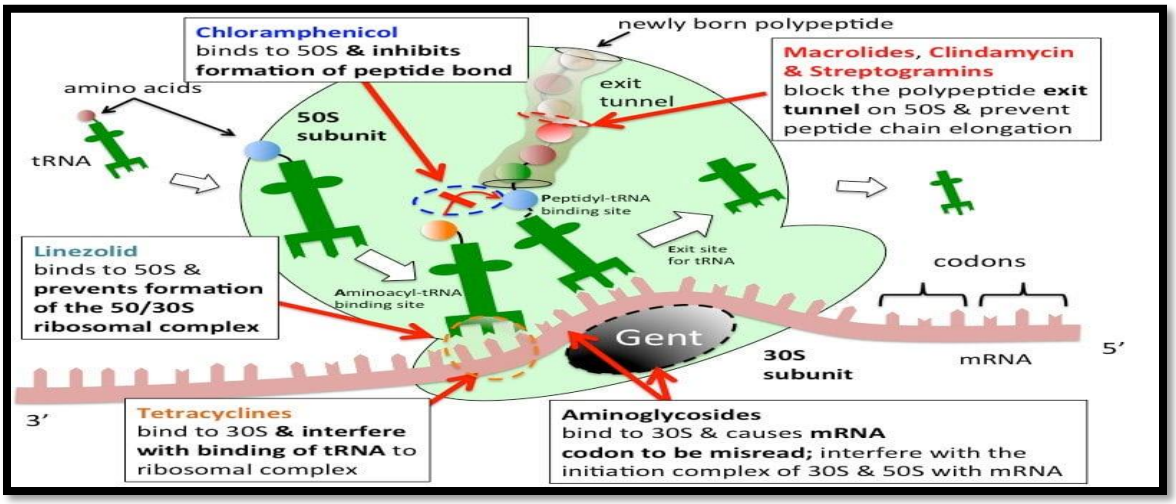


Figure7. Illustration of antibiotic mechanisms targeting bacterial protein synthesis, including chloramphenicol, linezolid, macrolides, tetracyclines, and aminoglycosides, with their respective binding sites on the ribosome (50S and 30S subunits) and effects on tRNA, mRNA, and polypeptide chain elongation.

3. Types of Antibiotics

Antibiotics play an important role since the World War II in the fight against the infectious diseases. The selection and rational use of antibiotics are unavoidable tasks of health care providers in all field of medical practices. Antibiotics acquisition from the pharmacy should also go through the receipt of proper prescription written by licensed medical practitioners. Effective treatment of the patients with antibiotics will result in a good prognosis. Antibiotics differentiate from other chemical compounds since they attack bacteria. Additionally, antibiotics should be taken as prescribed in adequate doses and duration, hence antibiotic courses intended for the patients under treatment of infectious diseases should be taken by the patients. There are large numbers of antibiotics often major consideration contented in the Essential Drug Lists (EDL) publicatively proclaimed by the Ministry of Health. Antibiotics can be utilized and utilized, like penicillins, cephalosporins, aminoglycosides, fluoroquinolones and carbapenems. Penicillin derivatives oxacillin

and amoxycillin represented the highest utilizations of antibiotics were reported in a study conducted by (Leekha et al., 2011). Unlike the findings of, Nakayama & Sasak in Japan reported glycopeptide derivative vancomycin was the most frequently utilized among the prescribed antibiotics. The fact is due to the appreciation of traditionally employed antibiotics in the hospital setting where the problem of antibiotics restrains are abundant. An examination of antibiotics resting was disregarded due to the fact that only parenteral antibiotics were reported better investigated by. According to, the problem of the technological inferiority such as improper syringe for injections was common in the developing countries. After preparation, the intravenous set should be from the Anti-Luer-Look device and must have in-line filter as well.

Table (12-1).This table presents the major classes of antibiotics, along with examples, mechanisms of action, and common uses in treating various infections.

Antibiotic Class	Examples	Mechanism of Action	Common Uses
Beta-Lactams	Penicillin, Amoxicillin, Cephalosporins	Inhibit bacterial cell wall synthesis	Respiratory infections, skin infections
Macrolides	Erythromycin, Azithromycin	Inhibit protein synthesis	Respiratory tract infections, STIs
Tetracyclines	Doxycycline, Tetracycline	Inhibit protein synthesis	Acne, Lyme disease, respiratory infections
Aminoglycosides	Gentamicin, Streptomycin	Inhibit protein synthesis	Severe bacterial infections
Fluoroquinolones	Ciprofloxacin, Levofloxacin	Inhibit DNA replication	UTIs, respiratory infections
Sulfonamides	Sulfamethoxazole, Trimethoprim	Inhibit folic acid synthesis	UTIs, pneumonia
Glycopeptides	Vancomycin	Inhibit bacterial cell wall synthesis	MRSA, severe infections
Oxazolidinones	Linezolid	Inhibit protein synthesis	Drug-resistant bacterial infections

However, reported that there was a viable loss of antibiotics suspension by 7% after reconstitution because of the syringes utilized. About 31% of antibiotics were prescribed by their internationally recognized international non-proprietary names (INN). The remaining were made according to their trade names, local names and chemical names. Almost all patients treated received at least 2 antibiotics at any given time. A study carried out by in the medical, surgical, pediatrics and gynecology wards in Namibia endorsed the findings; whereby 68.8% patients received 2 and more antibiotics. Antibiotics which were frequently prescribed in combination were also disclosed in's study, discovered as ceftriaxone – metronidazole, ciprofloxacin-amoxicillin and clindamycin-ceftriaxone. Combination therapy of amino glycosides or vancomycin with beta-lactam was often prescribed in the hospital and could not be defined from the drug data recorded. However, antibiotic combinations between aminoglycosides and beta-lactam such as benzyl penicillin-gentamicin and ampicillin-gentamicin were discretionary to be written by the clinicians based on the manual guide of Hospital Angkatan Tentera Tuanku Mizan for the use in the hospital.

3.1. Broad-Spectrum vs. Narrow-Spectrum Antibiotics

Infectious disease can be prevented from spreading by the use of antibiotics. When primary care providers are selecting an antibiotic in response to an infectious disease, they can use broad-spectrum antibiotics while waiting to culture to find an exact antibiotic choice (V. Sanchez et al., 2014). While waiting for a culture on an infectious disease, broad-spectrum antibiotics are often used. Broad-spectrum antibiotics are effective against a wide range of bacteria and narrow-spectrum are effective against just a few types. A sickness is treated with broad-spectrum antibiotics, like amoxicillin, clindamycin, co-amoxyclav, cefdinir, ciprofloxacin, doxycycline, ampicillin, piperacillin, and azithromycin, on a regular basis. Alternatively, narrow-spectrum antibiotics, like sulfonamides, penicillinase-resistant penicillin, first-generation cephalosporins, rifampin, and vancomycin, are also used to treat an affliction. However, sometimes an improper antibiotic is selected. An infectious disease requested a broad-spectrum antibiotic, nitrofurantoin, from the pharmacy department in a clinical vignette. The selected narrow-spectrum antibiotic was sulfonamides, compounded on the premises. The improper product was not dispensed. Instead, information was gathered about the different types of antibiotics. On a visit the next day, the correct prescription will be dispensed.

Table (12-2).This table compares broad-spectrum and narrow-spectrum antibiotics based on their characteristics, advantages, disadvantages, and examples.

Criteria	Broad-Spectrum Antibiotics	Narrow-Spectrum Antibiotics
Definition	Effective against a wide range of bacteria (both Gram-positive and Gram-negative).	Effective against specific types of bacteria (either Gram-positive or Gram-negative).
Advantages	Useful for treating infections when the causative bacteria are unknown; effective against multiple pathogens.	Minimizes disruption of normal flora; reduces the risk of antibiotic resistance.
Disadvantages	Higher risk of antibiotic resistance; may disrupt normal microbiota and cause secondary infections.	Less effective if the exact pathogen is unknown; requires precise diagnosis.
Examples	Tetracyclines, Fluoroquinolones, Chloramphenicol, Ampicillin.	Penicillin G, Vancomycin, Clindamycin, Erythromycin.

3.2. Beta-Lactams

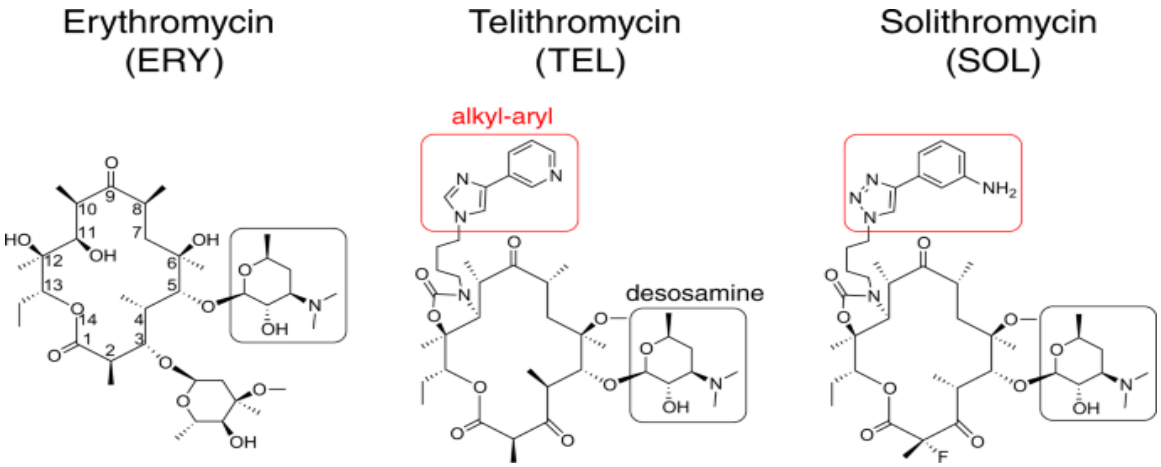
Antibiotics are produced by microorganisms or semi-synthesized and used to inhibit the growth of other microorganisms. To select which antibiotic, it is necessary to determine initial criteria. For example, it is possible to use a powdered concentrate or a solution for solution that are added to the medium cooled to 45-50°C. It is important to cool the medium; otherwise, antibiotics are inactivated. Amount and kind of antibiotic to be used depend on the strain used for transformation. The proof of the transforming activity can be the preservation of the UV sensitivity of the non-replicative forms or the obtainment of similar results with other types of drugs as mitomycin C or nalidixic acid and norfloxacin. For DNA repair studies, it is necessary to use DNA damage of a certain kind. UV treatment and nalidixic or norfloxacin intoxication do not produce primarily deaminations or depurinations stimuli.

Table (12-3).This table summarizes the main classes of beta-lactam antibiotics, including their examples, mechanism of action, spectrum of activity, common clinical uses, and resistance mechanisms.

Type	Examples	Mechanism of Action	Spectrum	Common Uses	Resistance Mechanisms
Penicillins	Penicillin G, Penicillin V, Amoxicillin, Ampicillin	Inhibit bacterial cell wall synthesis by binding to penicillin-binding proteins (PBPs), leading to cell lysis	Narrow to broad, depending on the subclass	Streptococcal infections, syphilis, respiratory infections	Beta-lactamase production, altered PBPs
Cephalosporins	Cephalexin, Ceftriaxone, Cefepime	Similar to penicillins; interfere with bacterial cell wall synthesis	Generations vary in spectrum; later generations cover more Gram-negative bacteria	Pneumonia, meningitis, UTIs, skin infections	Beta-lactamase production, efflux pumps
Carbapenems	Imipenem, Meropenem, Ertapenem	Highly resistant to beta-lactamases, broad-spectrum activity	Broad-spectrum, including multidrug-resistant organisms	Severe infections, hospital-acquired infections	Carbapenemases, porin mutations
Monobactams	Aztreonam	Targets Gram-negative bacteria by inhibiting cell wall synthesis	Narrow-spectrum, effective against Gram-negative aerobes	Alternative for penicillin-allergic patients, Pseudomonas infections	Beta-lactamase production, efflux mechanisms

3.3. Macrolides

Macrolides were discovered by in the late 1940s through the process of fermentation of the molds Streptomyces ambofaciens and Streptomyces erythreus. The term 'macrolide' antibiotic has become synonymous with erythromycin and its analogs, such as clarithromycin, azithromycin and roxithromycin . Clarithromycin and roxithromycin possess a 14-membered macrocyclic ring, whereas azithromycin is 15-membered. Erythromycin and its analogs have been widely used in modern medicine, particularly to treat respiratory, genital and skin infections.

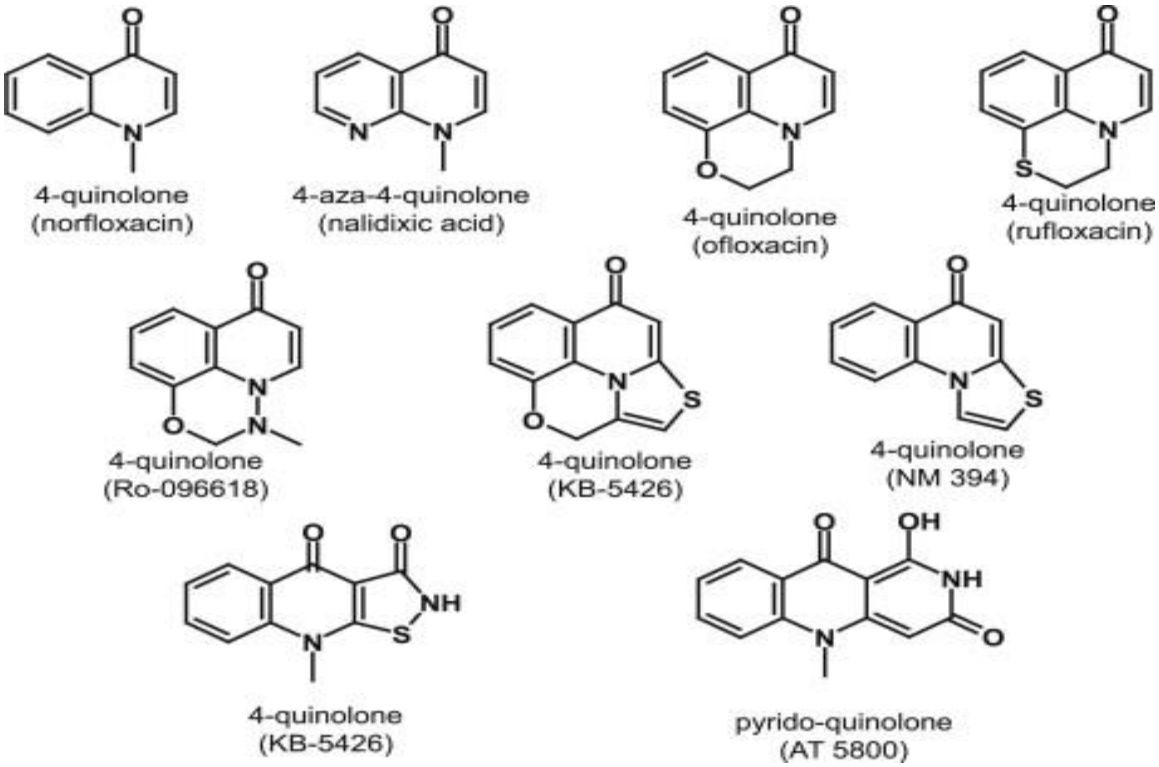


The macrolide class of drugs have many beneficial properties that have been taken advantage of, including: good oral bioavailability, high concentrations achieved in tissues and fluids, meaningful intracellular concentrations, a relatively limited spectrum of activity, a profile of being generally safe and well-tolerated. Additionally, macrolides have strong anti-inflammatory properties, providing symptomatic relief from pain associated with the plethora of inflammatory cytokines released at sites of infection. This versatile set of characteristics is what makes macrolides such a successful class of drugs, one of the most successful of all antibiotics. These attributes have made them some of the most successful of all antibiotics, with regard to being utilised in clinical practice in the hospital ward and beyond. In fact, macrolides are among the most widely prescribed antibiotics in many outpatient settings, including through general practitioner clinics and in the community pharmacy. Azithromycin is among the most well known and commonly used antibiotics in the macrolide family. Despite having a comparatively short half-life, azithromycin is able to maintain therapeutic levels of drug in plasma and tissues for five days after only a three-day course. The broad spectrum of activity of azithromycin in vitro, includes activity against a range of bacteria which are important in the pathogenesis of asthma and chronic obstructive pulmonary disease (COPD). These include species of Streptococcus, Haemophilus and Moraxella. Given the above-mentioned pharmacokinetic parameters, azithromycin has been

frequently employed as the drug of choice to eradicate these pathogens from the lower respiratory tract.

3.4. Quinolones

The founding member of the quinolone drug class was nalidixic acid, which was introduced into the clinic in the 1960s for treatment of uncomplicated urinary tract infections caused by enteric bacteria (J. Aldred et al., 2014). These antibiotics selectively target bacterial topoisomerases and are categorized as either quinolones or fluoroquinolones. Quinolones were developed first and differ structurally from other antibiotics. Despite the difference in structures, both the quinolones and the fluoroquinolones interact with their target enzymes in similar manners. Specifically, they function by stabilizing normally transient covalent enzyme-DNA interactions. This mechanism leads to the accumulation of double-stranded DNA breaks in the bacterial chromosome. Normally, double-stranded DNA breaks are repaired by the enzyme DNA gyrase, and to a much lesser extent topoisomerase IV. However, if DNA gyrase is bound to the quinolone or fluoroquinolone, then topoisomerase IV is left as the only enzyme that can repair these double-stranded breaks.

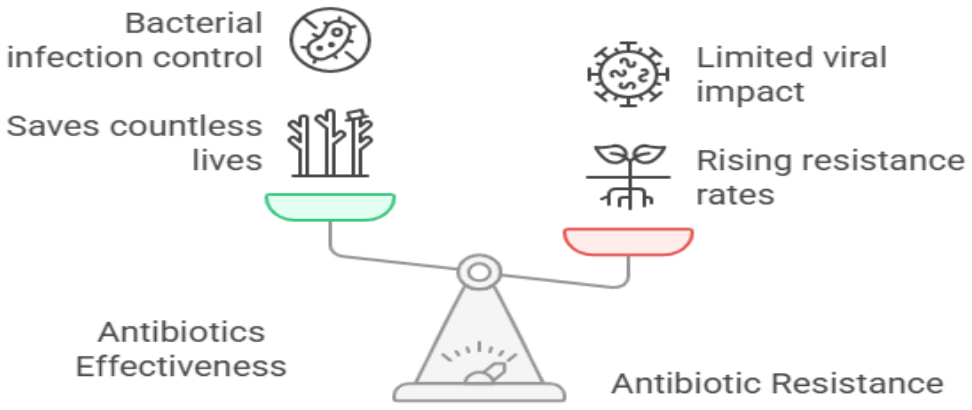


Since these enzymes make separate double-stranded breaks in the chromosome and must pass one another to reseal the nicks, a unique mechanism then arises in which the enzymes are covalently trapped on one another's DNA, effectively killing the

bacterium. Quinolones generally target the enzyme DNA gyrase and a subset of bacilli have acquired resistance to this drug class. The first quinolone-resistant strains of Staphylococcus aureus were reported in Japan in the early 1990s and were related to mutations in the gyrase A subunit. The fluoroquinolones are a synthetic derivative of nalidixic acid developed in the 1980s. The newer drugs exhibited an increased affinity for gyrase, greater penetration into Gram+ organisms, and better label properties. A class of 4 substituted ethyl ciprofloxacin was realized and is considered to be an important broad-spectrum antibiotic. This adoption has initiated a profound change in the use of this drug class as the use has amplified in use over equivalent anti-microbial agents. Tendon damage was detected in a small percentage of patients. Fluorine was added to the quinolone core to improve drug properties, concentrating on increasing half-life in circulation, improving potency, or expanding the spectrum of activity.

4. Antibiotic Resistance

Firstly, it is important to say a few things about the background of the situation. One of the biggest medical advances in history was the discovery of the antibiotic Buten in 1927. Since then, there has been a great development in drugs that fight infection. Antibiotics have been a boon in revolutionizing the fields of medicine and had saved uncountable lives. However, they are not a panacea; they are effective against bacteria but not against viruses. It is evident that over the last 25 years there have been a rising number of reports and publications on the emergence of bacterial resistance to commonly used antibiotics. Antibiotic resistance is as ancient as antibiotics themselves (Sarkar and M. Gould, 2012).



Balancing Antibiotic Benefits and Resistance Risks

In the era of indiscriminate, unsupervised usage, the question is not why antibiotic-resistant strains arose but why it has taken so long. The purpose of this will be to offer some guidance on how to ensure that antibiotics are used effectively and with the minimum risk of accelerating the development of resistance.

4.1. Causes of Antibiotic Resistance

Antibiotics are drugs or medicines used to treat and prevent bacterial infections. They are very powerful medicines that are specific in suppressing the growth of or killing bacteria. Antibiotics can delay the development of many bacterial-life-threatening diseases in developed countries and save millions of children's lives from death related to untreated respiratory infections. Despite the improvements in clinical diagnostic techniques and the better services provided by hospitals and the existence of health centers globally, the overuse of antibiotics in low and middle-income countries like Ethiopia can result in severe consequences. Antibiotic resistance is a global concern. reported that while antibiotics have greatly improved the prevention and treatment of bacterial infections, the overuse and misuse of antibiotics have contributed to increases in bacterial resistance.

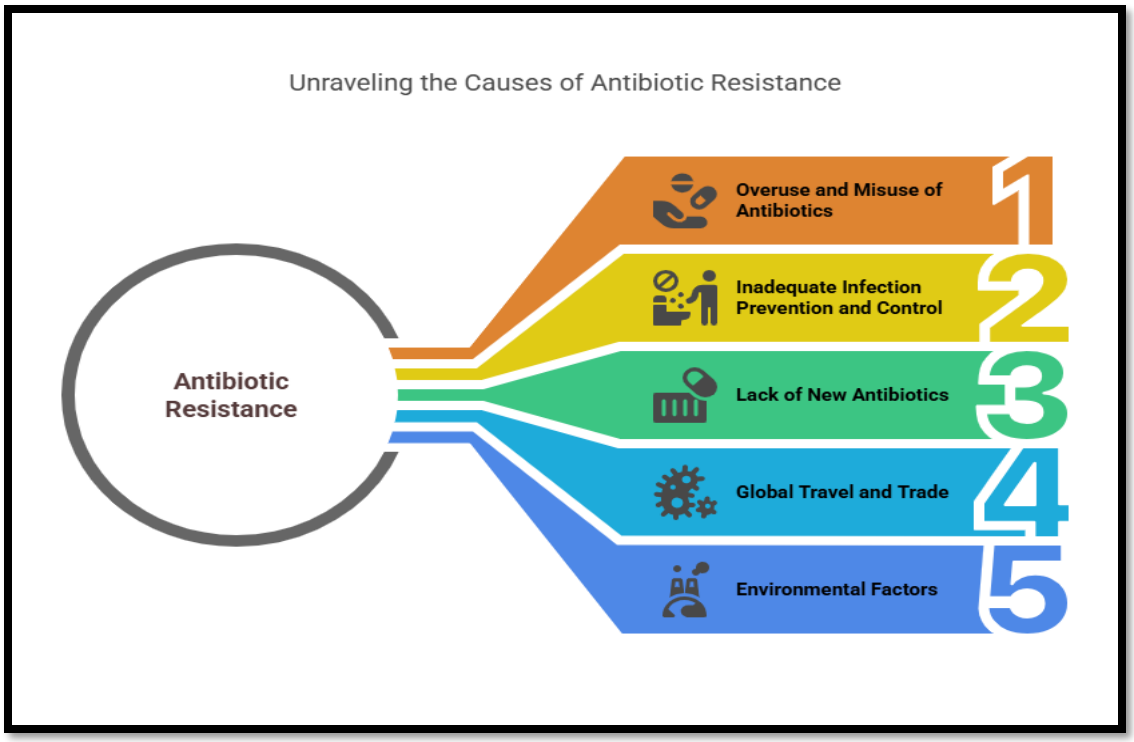


Figure8. Unraveling the Causes of Antibiotic Resistance.

This has led to the development and increase of "super bugs" which are resistant to antibiotics. Antibiotic resistance is a major public health problem in many countries. The increased resistance rates of pathogenic microbes have raised global concern

and become a common problem facing health institutions in many countries. Many antibiotics are available in developing countries without prescription and are used by the people these drugs inappropriately for self-treatment of simple febrile illnesses (Tassew, 2016). Widespread antibiotics are used in animal nutrition as well as therapeutic treatment. In addition, incorrect use of antibiotics in animal husbandry and aquaculture has resulted in the occurrence of antibiotic resistance in animals, which has become a potential threat to human health and chronic food safety risks. The emergence of antibiotics, animal pathogens, and transfer of pathogen resistance into human infectious bacteria have been reported.

4.2. Global Impact of Antibiotic Resistance

Jean Carlet and Didier Pittet opened an Alert about antibiotics in the lancet infectious diseases about access to these drugs. They are happy that the WHO Tripartite, FAO, OIE process is producing a common document and are convinced that within a few years the community has a unique opportunity to focus global attention on this. Antibiotics are among the most important discoveries of medical science and have saved millions of lives.

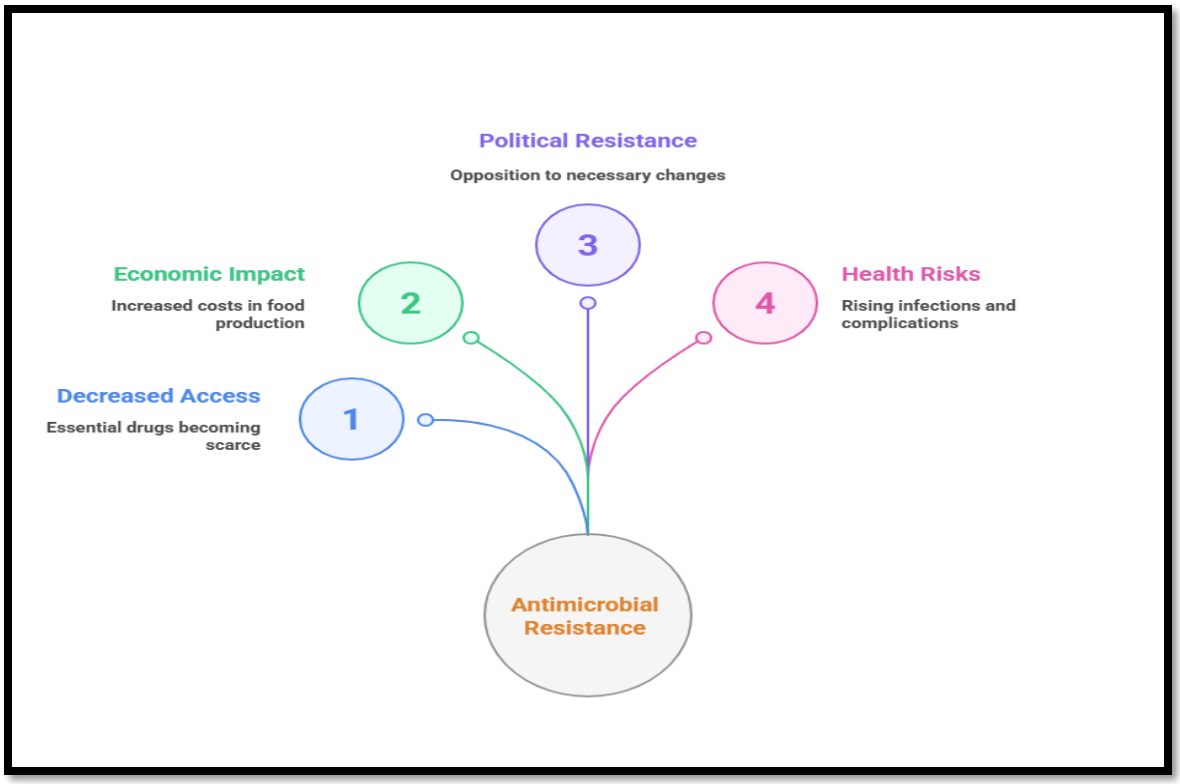


Figure 9. Antibiotic Access Crisis Threatens Global Health.

Nonetheless, it is shocking that access to these essential drugs is steadily decreasing in some countries or regions due to resistance and an inadequate development of new antibacterial drugs and that is seriously threatened by the recent withdrawal of parke-Davis and Eisai & co. from this therapeutic field. It takes 10-20 years for a new drug to make it to become a marketable product. Most developed countries have robust systems in place to restrict the prescription of antimicrobials in food animals except to treat, control, and prevent infectious diseases. Antimicrobial resistance (AMR) increasingly threatens humanity around the planet. Until now, this has not hampered the spread of the drugs in the course of the worldwide success of western medicine, but the tide may be turning. This Tripartite Expert Workshop sought to consider the widespread impact on animal health and food production—an impact that is likely to cause political resistance to the necessary changes in antimicrobial use in animals in many countries (Sarkar and M. Gould, 2012). AMR imposed severe IC pathogen-related costs. Decelerating the spread of AMR will favor the international trading of produce and livestock, as well as the spread of expertise. The main goal of the meeting was to review the current state of the risk posed by antimicrobials used in aquaculture. This risk is due not so much to residue, but to their joint application with other drugs. There was a possibility of producing a document to guide analysis of the matters, which are under consideration and need to debate and investigation.

5. Guidelines for Antibiotic Use

The appropriate use of antibiotics has become a worldwide priority (Elias et al., 2017). As the adversarial selection for bacterial strains resistant to antibiotics treatment rises, the appropriate selection of antibiotics is even more important. The use of antibiotics is everywhere, often used to treat disease livestock, promote growth, prevent post-harvest fish and farm animals, and protect human health. Many other veterinary drugs are effectively hormones, coccidiostats etc., with antibiotics being the most common. Drug administration is not standardized, and even if it is indicated that it is ineffective, many animal owners and veterinarians do not care (Irwin et al., 2003). Inappropriate prescribing, over-the-counter sales of antibiotics, and high consumption are known to contribute to an increase in bacterial selection pressure. One response to this problem is for farming practices to regulate antibiotic use. Reports indicate resistance is emerging to camamycin, quinolone, third-generation cephalosporins, extended spectrum penicillins, and ESBL. The overuse of antibiotics in agriculture is recognized as a critical factor threatening public health of humans. Generally resistant bacteria share their resistance, by conjugation, to other bacteria, allowing them to gain resistance to antibiotics they were not exposed to. Because antibiotic use is so widespread, and

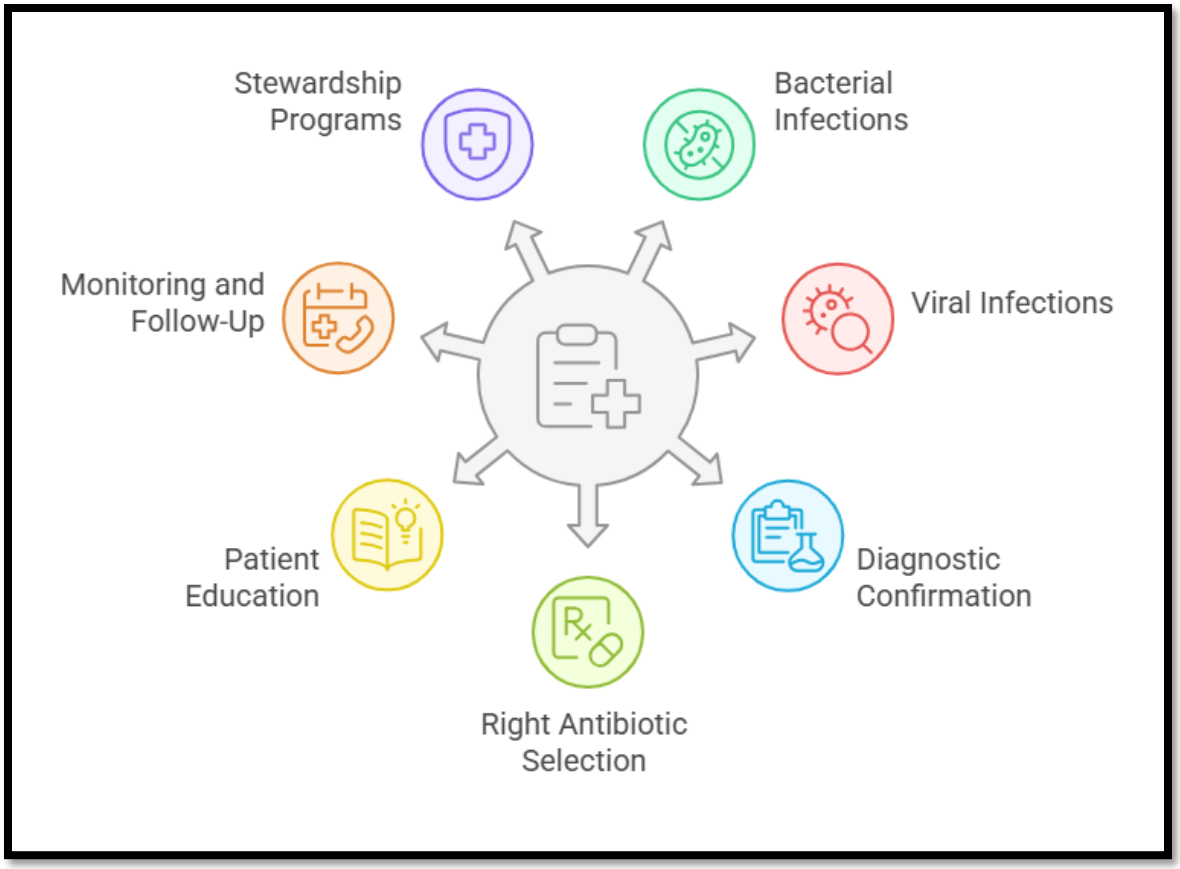


Figure 10. Antibiotic Use Guidelines.

there is a commensal relationship between humans and some animals increased bacterial resistance to antibiotics in food would occur. The same occurs among the intensive farming animals themselves. They have access to each other's waste, feces, bedding and accompany the movement of humans and other animal species. When antibiotics are used they often become environmental contaminants and are therefore easily taken up by the different animal species that might live in close proximity. Overall, if antibiotics are not used appropriately the opportunity for resistant bacteria to evolve needlessly increases. A significant clinical factor is the possibility to compromise future antibiotic therapy of illness induced by antibiotic resistant bacteria, therefore spreading the bacterial population diversity and moving it far away from the few control therapeutical options that still exist.

5.1. Principles of Antibiotic Stewardship

A group of clinical pharmacists and clinical pharmacy technicians, hereafter known as pharmacists, practicing in the public sector who are members of the SASOCP met to address a need that has been recognized for some time in promoting the responsible use of antibiotics to help prevent rising antimicrobial resistance.

(Schellack et al., 2018) found that a multifaceted approach including the use of educational materials and guidelines, participation in infection control education, auditing processes and feedback on healthcare-associated infections has been proven to improve infection control practices notably in hand hygiene. Antibiotics, once considered a miracle of modern medicine, have now become one of the most commonly prescribed agents and the most frequently abused class of therapeutic agents worldwide. Unfortunately, this abuse has led to primary, secondary, and collateral injuries. Clinically, it has resulted in serious adverse side effects and allergy reactions and has killed people. Subtherapeutic use of antibiotics is known to promote the spread of drug-resistant bacteria, including all the development of so-called 'super bugs'. Such resistance can spread to foodborne and will present a public health risk.

5.2. Empirical vs. Targeted Therapy

The patient should be receiving combination imipenem/cilastatin and ampicillin/sulbactam in addition, as this medication list is from a patient just admitted to the ICU with a Foley catheter. The other medications are unrelated. Initial therapy for infection is often empiric and guided by the clinical presentation (Leekha et al., 2011). Inadequate therapy for infections in critically ill, hospitalized patients is associated with poor outcomes, such as greater morbidity and mortality, longer length of stay, and the development of multidrug-resistant bacterial strains. Empirical therapy should be a fluid process, reevaluated in the face of new information including clinical outcomes, the response to treatment, and local susceptibilities. Medicines that require frequent dosing are more difficult to take correctly, and compliance with therapy might be suboptimal. It is important to consider in broad overview aspects of the case, the most likely offending organism given the patient's medical condition, likely sites of infection, and the specific concerns of the given case, and basic principles of pharmacokinetics and the mechanism of action of antibiotics (F. Harrison and Ouyang, 2013). Variability in the pharmacokinetic properties of different classes of antibiotics is a major limitation to generalizations but attempts to restrict observations to those of commonly used or particularly important agents have been made. Most outpatient therapy is directed at common bacterial causes of infections, but infections caused by gram-negative bacilli, including those due to extended-spectrum β-lactamase organisms, carry significant morbidity and mortality; these are more prevalent in the typical inpatient population. In this setting, empirical therapy should target these pathogens, typically involving the use of carbapenems, a broad-spectrum β-lactam antibiotic with robust activity against gram-positive and gram-negative organisms.

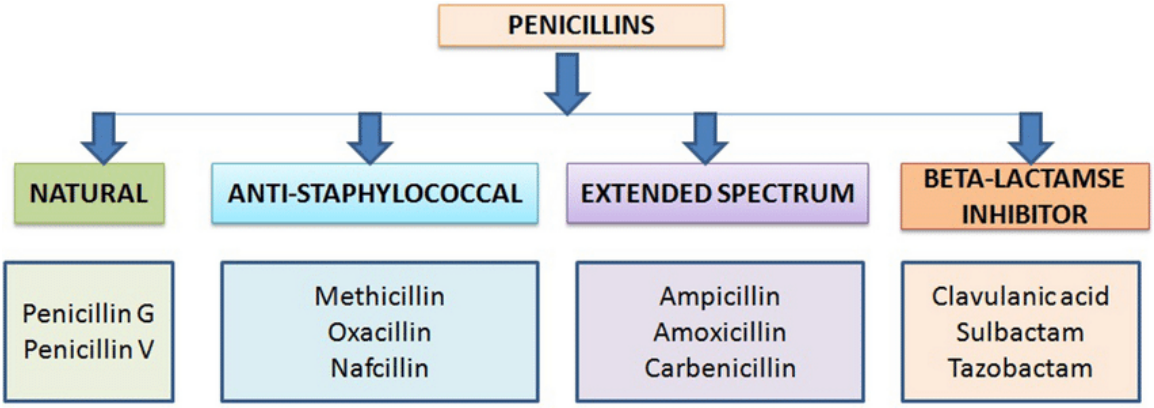
6. Common Antibiotic Classes

There are numerous antibiotic classes and each have a unique and different mode of action. When used in a hospital or clinical setting, many of these antibiotics are administered intravenously (IV), but this is an expensive and time consuming manner to administer the drug. Some antibiotics such as the fluoroquinolones also require a much larger dose of antibiotic to get adequate plasma or tissue concentrations when given orally. Some antibiotic classes may also require large volume injections or slow infusions, which makes mass-administration difficult or impractical. The majority of antibiotics used in mass-medication of large numbers of animals are administered via feed or water. This practice also ensures a uniform and consistent dose of antibiotic. It can be difficult to add all different 15-20 classes of antibiotics to feed or water, so certain classes will be more suited for this manner of administration.

There are 6 antibiotic classes commonly used for infections in food animals: ß-Lactams (penicillins), aminoglycosides, tetracyclines, macrolides (lincosamides), sulfas and fluoroquinolones. Each of these antibiotic classes has a unique mode of action. Some classes, such as the pleuromutilins are used in swine or poultry medicine almost exclusively. Each class of antibiotic has a specific concentration of active drug that must be present at the site of infection in order to affect a bactericidal result. Each class also has specific withdrawal periods after administration during which time the animal must not be slaughtered for human consumption. In particular, the tetracyclines, fluoroquinolones and sulfas have a common withdrawal of 4-5 days after last administration (Holt, 2001). Antibiotics can also have major side effects and can damage certain organs (kidney, liver, joint cartilage) as seen with the misuse of some fluoroquinolones in domestic pets. Furthermore, antibiotics can also lead to bacterial resistance and the development of “superbugs” (S Paknikar and Narayana, 2012).

6.1. Penicillins

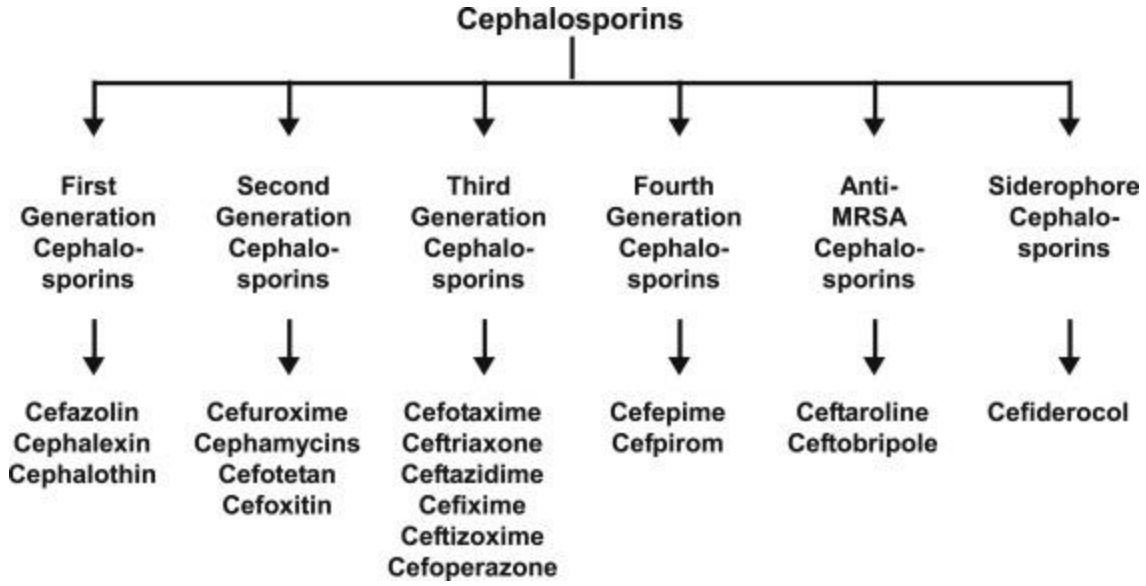
The novel coronavirus (2019-nCoV, SARS-CoV-2) causes respiratory illness in nearly all infected individuals varying from asymptomatic infection to severe disease manifesting as pneumonia and acute distress syndrome. COVID-19 behaves differently in terms of virulence and host response variability. The probability of multiple exposures to different antibiotics is timely therefore. While proper antibiotic use is advocated – secure, evaluate, and maintain policy – proper knowledge of this timely use is equally important and is based on an understanding of individual antibiotics, each of which has an environmental and microbiological profile. Possibly the oldest antibiotic group, a mature penicillin antibiotic, is good knowledge to begin with the rational use of an antibiotic group. Unfortunately,



though their precious name is used for different antimicrobial agents and anaphylaxis has been circulated on the basis of improper desensitization or testing, penicillins are often amorphous and even feared drugs and unduly rejected to be rightly described or adjudged (Lteif and S. Eiland, 2019). Narrow-spectrum penicillins (b-penicillins) specifically combating gram-positive species and an excellent system of treatment of infections caused by intact cell walls such as mild pharyngeal infections caused by susceptible streptococci, or erysipelas, furunculosis, and impetigo caused by S. aureus. Widely preferred, mainly due to their minimal reactivity and favorability are the first oral penicillin, phenoxymethylpenicillin, and propenicillin, which are not inactivated during passages under gastric acid conditions. High penicillin G levels reached after administration of these drugs for years serves as the golden standard for the systemic antibacterial agent of the minimum side effects, which noncreatinine renal clearance is free and for children is available in a variety of oral/parenteral preparations. Broad spectrum penicillins such as ampicillin, amoxicillin, and flucloxacillin, whose antimicrobial action also includes several away Gram-positive, as well as Gram-negative, Enterobacterales species, absorbing rapidly orally, achieving high concentrations in soft tissues providing their prescription with a broad indication range such as a lot of respiratory, skin and soft tissue, urinary and enteral infections.

6.2. Cephalosporins

From cephalosporin C, isolated from fungi species, the cephalosporin nucleus was originally derived. More potent antibiotics with broader spectra were prepared by the chemical modification of cephalosporins. This led to the development of 7-aminocephalosporanic acid - 7-ACA - from cephalosporin C. The modification of the 7-ACA side chains generated the first potent antibiotics of this class; cephalothin was the first cephalosporin to be launched in 1964. When the cephalosporin nucleus is appropriately altered, compounds with different properties can be obtained. This class of antibiotics was named after the structure of the chemical side chains that

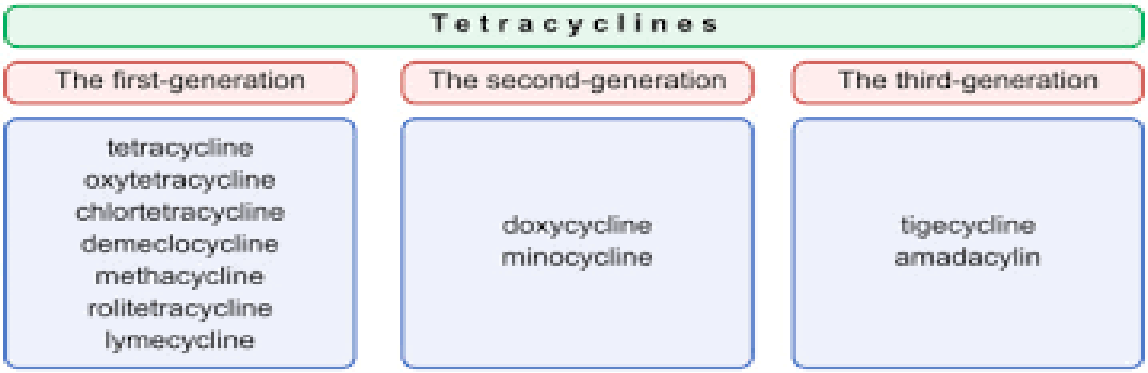


present their nucleus. Cef is the side chain while the nucleus is Cephalosporin. Despite being among the classes of antibiotics most frequently used, cephalosporins are not effective against all the different types of bacteria. Therefore, it is important to design newer cephalosporins with antibacterial activity against a greater range of bacteria, as high activity both orally and parenterally, and a favorable pharmacokinetic profile. By a broad scope of methodologies, activity and pharmacokinetic parameters of a number of cephalosporins were determined. Activity parameters were computationally derived by using topological indices. The most favorable activity profile was obtained for the combination of asymmetry of lipophilicity as a property and the presence of either sulfur or oxygen atoms precisely at the positions 6, 2 or 3 of the cephalosporin nucleus. It was observed that the increase in lipophilicity greatly enhances activity. Molecule weight less than 600 and the presence of a minimum of 10 hydrogen bond acceptors were also linked to improved activity. The pharmacokinetic parameters of cefixime were evaluated by biorelevant dissolution tests, a common compartment pharmacokinetic model, and the calculation of a set of pharmacokinetic parameters. It was found that they were in a consistent way associated with the N1–C4 fraction of cefixime, with the amount of cefixime that would be presented into the blood system post oral administration. Cephalosporins are a class of β-lactam antibiotics which are both bactericidal and exert their bactericidal action through the same mode of action as penicillins. Both these classes of antibiotics act on bacteria by interfering with the synthesis of the peptidoglycan layer of their cell wall. This inhibition is due to the formation of a stable acyl-enzyme complex between the antibiotic and the enzyme penicillin binding proteins.

6.3. Tetracyclines

6.3.1. Mechanism of Action

Tetracyclines bind to the 30S ribosomal subunit and prevent binding of the aminoacyl-tRNA to the acceptor site on the mRNA-ribosome complex (Granados-C hinchilla and Rodríguez, 2017).

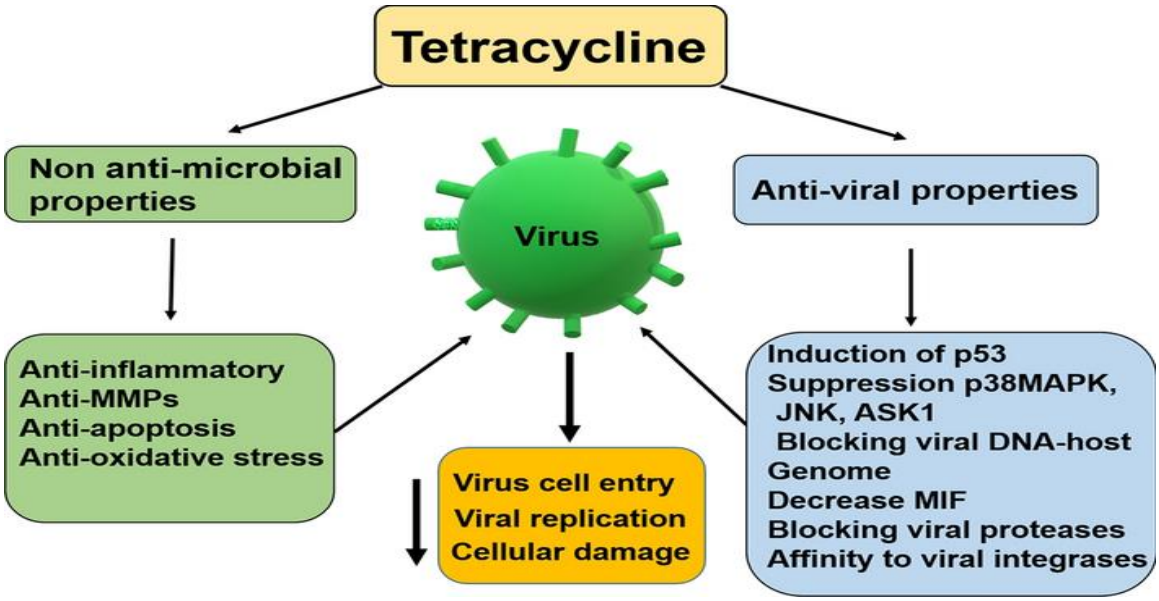


In this way, protein synthesis is inhibited and the bacteria cannot replicate. Tetracyclines are transport through the bacterial cell wall as a divalent cation-metal complex. Uptake of tetracyclines occurs through ATP binding cassette transporters in both gram-negative and gram-positive bacteria. Resistance to this group of antibiotics is mainly determined by efflux pump proteins that transport tetracyclines outside the cells preventing the action of the antibiotic. Most of these efflux pumps are energy-dependent and activate efflux tetracyclines-H+ through an antiport mechanism resulting in the alkalinization of the cytoplasm increasing the pH up to 8.5.

6.3.2. Therapeutic Effects

Tetracyclines have several therapeutic indications dealing with infections in food-producing animals and pets. Tetracyclines have been used since the end of World War II and are mainly bacteriostatic antibiotics showing a broad spectrum of activity against both gram-positive and gram-negative bacteria. In food-producing species, the classical first-generation tetracyclines are widely used, while second-generation tetracyclines are more commonly chosen for pets. The most common therapeutic indications in animals comprise pasteurellosis, respiratory infections, dermal and soft tissue infections, peritonitis, metritis and other enteric infections caused by gram-negative organisms such as Escherichia coli. For many years, tetracyclines had been used for growth promotion purposes in food-producing animals. Since the emergence of concerns about the antimicrobial resistance of

bacteria, the European countries were the first to limit the utilization of tetracyclines for growth promotion.



After 1 July 2006, the use of all tetracycline preparations that have indications for the improvement of animal growth was prohibited in the European Union. In the USA, beginning from 1 January 2017, tetracyclines will no longer be allowed for use as growth promoters and will be restricted to therapeutic use only and subject to a veterinary feed directive (VFD). Tetracyclines are broad-spectrum antibiotics showing activity against both gram-positive and gram-negative bacteria. Although mostly bacteriostatic, at higher concentrations they can kill both gram-negative and gram-positive bacteria. They also have activity against rickettsiae, chlamydiae, and some protozoa. Among the bacteria, Mycoplasma, Chlamydia, Pasteurella, and Clostridium are particularly susceptible to tetracycline action. Hautl and gill diseases of freshwater salmon, caused by Flexibacter columnaris, can be successfully treated with oxytetracycline baths.

6.3.3. Organic Relevance and Structure-Activity Relationship

Tetracyclines, including chlortetracycline, oxytetracycline, doxycycline, and minocycline, have four six-membered carbocycles forming a polyketide-derived structure. The attachment of a dimethylamine group at C4, and a pyrrole ring at C2 of the A-ring and the out-of-planarity of the D-ring conferred their unique configuration. Natural tetracyclines such as oxytetracycline or tetracycline are produced by Streptomyces species, while the derivative products such as

doxycycline and minocycline are semisynthetic products splitting 7-isobutylamino group. The antibiotics are synthesized chemoenzymatically by altering the fermentation conditions to control the alkaline pH. Currently, most tetracyclines are synthesized through fermentation of genetically engineered strains. The molecule structure of tetracycline has several function groups making it acidic and increasing the feasibility of coordination with transition metals such as Ca2+ or metalloenzymes.

6.3.4. Pharmacokinetics

Doxycycline is well absorbed orally, with peak plasma levels attained after about 2 hours. The oral bioavailability of doxycycline ranges from about 90% at a low dose to 50% at a high dose. Food and dairy products reduce the bioavailability, so it is recommended to take doxycycline 1 h before or 2 h after meals. The presence of methanol or calcium compounds in the diet may decrease the absorption of doxycycline. Doxycycline is eliminated by the renal route mainly in the form of unchanged compound. The plasma half-life in humans after oral administration of 2 mg of doxycycline is 19 h. High plasma levels are detected after oral administration in calves, being 2 μg mL−1 reached after 6 hours. The in vitro studies indicate 72.0 h half-life in liver microsomes of monkeys, 48.1 h in dogs, and 30.8 h in human liver microsomes. Tigecycline is a semisynthetic antibiotic of the glycylcycline group. It is approved for the treatment of complicated skin infections and intra-abdominal infections. Tigecycline derivatives were synthesized to combat tetracycline resistance, one of the major resistance patterns among pathogens. Tigecycline has a more rigid and bulky head and a flexible side chain. Tango mode of binding allows tigecycline to be active on pathogens sensitive to classes of antibiotics already known. Tigecycline reduced the Plasmodium berghei parasitemia 30% after 24 hours at a dosage of 15 mg kg−1. Tigecycline is the first tetracycline analog indicated in the European Union for human use. The main disadvantage is that most of the bacteria resistant to class antibacterial drugs, e.g., Burkholderia cepacia, Pseudomonas, Legionella, and Enterococcus, are also resistant to tigecycline. Since it is a relatively new antibiotics compound, in the same way as other tetracyclines, it is important that it is used in clinical and veterinary practice only in the circumstances warranted by the resistance pattern of the tested microorganisms. Tigecycline is an antibiotic active on a large group of pathogens, with enhanced properties compared to the active substance and it could be used successfully in aquaculture. There are currently no authorized tigecycline-containing products for veterinary use in the European Union.

7. Adverse Effects of Antibiotics

The main means by which antibiotics can affect or harm the host are allergic or hypersensitivity reactions of immunological origin. Antibiotic-associated diarrhea is relatively frequent, especially with certain combinations and broad-spectrum semisynthetic penicillins, cephalosporins, and clindamycin. Superinfections, development of multiple-resistant species, or the selection of certain resistant microorganisms with severe pseudomembranous colitis are the most severe kinds of colonization.

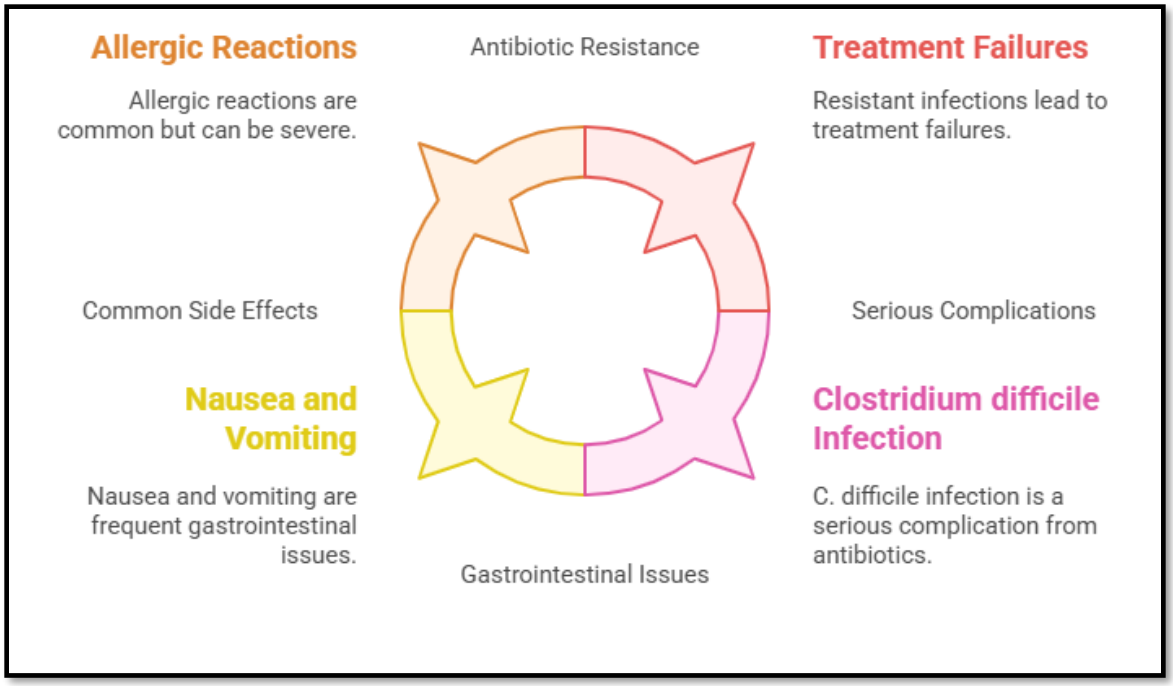


Figure 11. Adverse Effects of Antibiotics.

Toxicity is possible with most therapeutic agents and classes. The adverse effect of antibiotics may be transitory or reversible after treatment interruption, but can also cause irreversible damage or contribute to death. Pseudomembranous colitis is a rare, but serious immune-mediated inflammatory condition of the colon caused by certain necrotizing toxins. It is a direct consequence of the alteration in the microbiota of the remaining germs that protect against supercolonization. Certain antibiotics can cause hemogram changes such as anemia and thrombocytopenia, and more rarely, leukopenia. Hair hypopigmentation in children after certain treatments is accompanied by local toxicity, and after certain antibiotic intake it may cause skeletal abnormalities in children and adults, that mostly consist of discoloration and altered growth of bones, in addition to degenerative joints. There is a notable difference in the benefit–risk ratios of these drugs in favor of the

benefits with the occasional adverse effects that are usually transitory or reversible with the cessation of treatment.

7.1. Allergic Reactions

The use of antibiotics has led to a drop in the frequency of complications caused by several childhood diseases, lowering the mortality rate and vaccinating against diseases. Nevertheless, the overall use of antibiotics must be reduced due to the increasing resistance of microorganisms. Furthermore, dispensing antibiotics without a prior medical prescription should be reduced. In many cases, no prescription is necessary as antibiotics are ineffective as healing agents for infections of viral origin (Zavaleta-Monestel et al., 2024). One negative effect of the extensive use of antibiotics is the increase in the number of subjects that develop hypersensitivity to these medicines. The production of antibiotics and other substances in our country has begun to cause an allergic or intolerant stomach turn.

Many studies have been published on adverse reactions to antibiotics. Allergic reactions formed by β-lactams ranging from 0.7 to 10% of the adverse reactions are brought about by those medicines. The role of pyrazolidines and other non-steroid anti-inflammatory drugs in the development of chronic urticaria and angio-oedema and severe anaphylactic reactions that may even cause death have frequently been reported. Macrolide antibiotics are broad-spectrum antibiotics capable of neutralizing grampositive and some gram-negative microorganisms, although less efficient compared to β-lactams. Among macrolides, erythromycin and clarithromycin are frequently used antibiotics both in children and in adults. However, harmful effects have been identified in the derivation of those drugs: cholestatic hepatitis, hypersensitivity reactions, and rashes (Sánchez-Borges et al., 2013). Amoxicillin is a derivative of ampicillin and acts as one of the most used spectrums for wide-spectrum antibiotics due to the simple fact of its practicality, but it again shows that much of the administered doses remain unabsorbed. If the allergen is penicillin, ampicillin, amoxicillin, and benzylpenicillin; authorities in the field of allergies indicate that it is highly likely to happen in individuals to whom it comes phthiriasis by cephalosporins. On the other hand, cases of allergic reactions due to the macrolide antibiotic class have been recorded, although they are very rarely seen. Azithromycin, which is a necessary selenium hydroxide derivative, was previously associated with only occipital allergic decompression. However, reversible bilateral sudden hearing loss associated with low and short-term azithromycin is frequently emphasized in the literature.

7.2. Gastrointestinal Disturbances

Ingestion of an antimicrobial agent is usually accompanied by gastrointestinal upsets, such as diarrhea and disturbance of the normal gut microflora. Although many adverse drug reactions to antimicrobial agents have been reported, antibiotic-associated diarrhea, including effects on the gut microflora, has received considerable attention. It usually occurs shortly after commencing treatment with an oral antibiotic and ceases shortly after discontinuance; however, in many cases it can persist long after the antimicrobial treatment is ceased (Rafii et al., 2008).

The term "antibiotic-associated diarrhea" is often used to describe milder forms of diarrhea, with "pseudomembranous colitis" (a more severe form of antibiotic-associated diarrhea) used to describe a more severe condition. Antibiotic-associated diarrhea usually is due to a decrease in the number of normal gut microbes, making it easier for pathogens (harmful microbes) to colonize and cause diarrheal disease. The diarrhea may begin several weeks after starting antibiotic therapy, and normal gut flora may take some time to return (usually 1–2 months) to the pre-antibiotic condition. Pseudomembranous colitis occurs most commonly following use of broad-spectrum antibiotics, which, in addition to the target bacteria, also decrease the numbers of the normal gut bacteria that prevent toxic metabolites (usually enterotoxins) from Clostridium difficile (by out-competing the pathogens for nutrients and creating a physical barrier to C. difficile colonizing the gut). Treatment of antibiotic-associated diarrhea usually involves stopping the test antibiotic or replacing it with a different antibiotic. Two drugs have been approved for the treatment and prevention of severe cases of C. difficile: metronidazole and vancomycin. There are many different drugs and vaccines being researched in order to combat C. difficile, but no definitive treatment has been found. Opioids are sometimes used in cases of extremely severe diarrhea (Rochegüe et al., 2021).

8. Future Directions in Antibiotic Development

This is the Post-Antibiotic Era, with pathogenic bacteria outpacing the development of new antibiotics. Currently accepted models for the treatment of bacterial infections with a limited number of ever more expensive, broad-spectrum antibiotics are becoming less effective, and even such "last-line" antibiotics are being effectively resisted by pathogens. Infections of organs and indwelling devices have become quite challenging due to the inherent difficulty of drug delivery and removal of infected tissue in these cases. The ultimate aim of the Special Issue is to present a defensive array of technological solutions to the coming challenges in the war between antibiotics and infectious organisms, while biofilms become intractable surgical site and burn wounds. Flogosis is also associated with cancer by multiple-source feeds in immune responses.

Antimicrobial resistance is emerging as a major challenge for the future of mankind. Since the discovery of Penicillin in 1928, antibiotics have been life-saving agents for many bacterial infections. The mechanism of antibacterial action of these antibiotics is such that it selects out mutated resistant strains. A rapid increase in resistant strains is observed once the drug is introduced into the antimicrobial market. Molecular evolution is the origin of this mutation. Here, bacteria and viruses do not compete with each other, rather they cooperate and help in increasing the population so that their chance of evolution will be faster than the limit. Hence, new unconventional methods have to be thought of against the supposedly pan-resistant superbugs which are appearing on the horizon. (Mantravadi et al., 2019) Shivering energy innovative approach laid the puzzle for microbial deaths using shivering energy, imparting oscillations in molecules forming into large empires. Antibiotics started failing due to mutations in bacterial and viral genome sequences on a long run and persisting a challenge for humans to overcome resistant to antibiotics. pstmt tech innovated a simple way of using cost-effective plant extractions which will undo the mutation DNA sequences of bacteria and virus thus destroying superbugs.

8.1. Novel Antibiotic Classes

The need for novel antibacterials has been greater than ever in the face of increasing resistance to the older ones. This resistance has been seen even in the "last-ditch" last-generation drugs implying that the development of resistance now outpaces the discovery of new drug classes. From a historical perspective, three classes of drug-resistant bacteria are of major concern; MRSA, Staphylococcus aureus bacteria that are resistant to Methicillin and a number of related penicillin antibiotics, a class discovered in the late 1950's-active for about ten years and the first major harbinger of the problematic realities of antibiotic resistance; since those early days, apparently, strains of S.aureus resistant to all older drug classes have arisen; a second class of concern is MDR and now PDR (pan-drug-resistant), gram-negative bacteria, it took these bacteria many years to acquire resistance to the older beta-lactam antibiotics and their derivatives and they are a scourge in hospitals in particular; recently, strains of A.baumannii, E.coli, K.pneumoniae, and P.aeruginosa have emerged which are resistant to all known antibiotics, leaving clinicians with no treatment choices for these infections; the third class is a bit unusual, comprising of MDR and XDR strains of M.tuberculosis. Similar to gram-negatives, it had historically taken these bacteria unusually long to acquire resistance to the older drugs, so TB was very treatable with cocktails of rifampicin, isoniazid, ethambutol, pyrazinamide and streptomycin. However, the emergence of MDR-TB (resistant to at least rifampicin and isoniazid) and now more recently of XDR-TB (in addition also

resistant to second-line drugs) has led to an upsurge of potentially-contagious untreatable TB, particularly in Eastern Europe and Asia.

Antibacterials approved for clinical use since the year 2000 are reviewed here, as do those in clinical trials and especially those in the later phases. These drugs include a number of compounds with novel modes of action not previously seen chemically as drugs, such as Fosfomycin (a cell-wall biosynthesis inhibitor; approved in 2009 as a 15 g single dose for UTIs), Tigecycline (the first drug in a new class of glycylcyclines; an IV drug approved in 2005).

8.2. Combating Antibiotic Resistance

Antimicrobial agents have been important cornerstones of clinical medicine since the second half of the 20th century. However, the last decades have witnessed the emergence and spread of antibiotic resistance in pathogenic bacteria worldwide, leading to the failure of antibiotic therapy and significant deaths. The increase in resistance rates of pathogens including methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant Enterococcus (VRE), and multidrug-resistant Pseudomonas aeruginosa poses a serious threat to public health. Extended-spectrum beta-lactamase (ESBL)-producing pathogens and MRSA are endemic in many hospitals. Carbapenems are the last defense against certain pathogens and "new Delhi-type" metallo-beta-lactamase-1 (NDM-1) or AmpC beta-lactamase-producing pathogens are spreading. The increase in carbapenem or fluoroquinolone resistance will be a major threat. Moreover, tuberculosis control is hampered by multidrug-resistant (MDR) Mycobacterium tuberculosis. The decline in novel antibiotic discovery further complicates the problem. Therefore, effective strategies are required to address antibiotic resistance.

The selection and use of antibiotics are the main factors affecting the emergence and rapid spread of antibiotic resistance. Indiscriminate use of broad-spectrum antibiotics and inadequate regimens have been implicated as important factors contributing to widespread resistance. Concomitant measures include public education, developing appropriate guidelines and regulations for antibiotic use, surveillance of antibiotic resistance, and epidemiologic studies of causes and outcomes of resistance. The selection of antibiotic agents determines 3 main factors of resistance emergence; (1) the ecological pressure induced by the selection of resistant mutant strains, (2) the population dynamics which limit competition among bacteria owing to the erased sensitive population, and (3) the resistance gene acquisition or mobilization (transfer of genetic resistance determinants primarily mediated by mobile genetic elements).

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Chapter Eleven

Antifungal Agents

1. Introduction to Antifungal Agents

Fungal infections are a continuous threat to human and animal health and jeopardize entire ecosystems. Undergoing consolidation of the seed in a single (fungal) organism, fungi have evolved intricate and powerful organellar structures as well as metabolic pathways that allow them to modify their environment in the widest variety of ways possible. In humans, fungi cause a range of infections from harmless superficial maladies to highly invasive mycoses with chronic or subacute manifestations that can be life-threatening without treatment. Although these infections can affect any tissue, the vast majority (approximately 80–90%) of mycoses in humans are localised in the skin, nails, hair or vagina. Candida species and dermatophytes account for the majority (80–90%) of all superficial human mycotic pathogens.

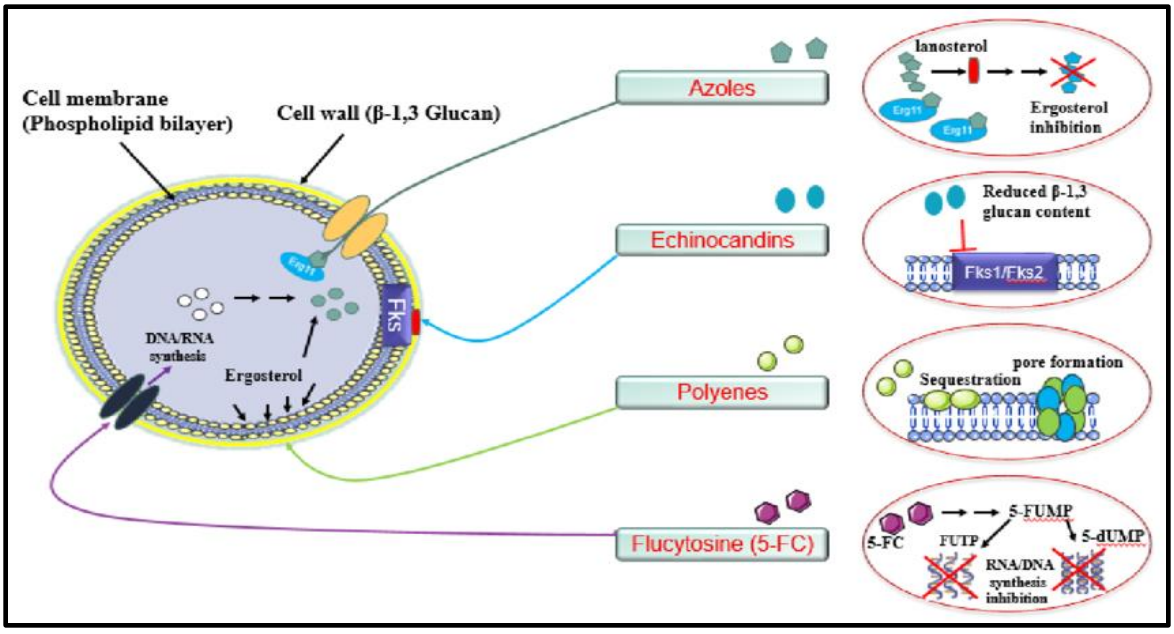

Figure 1: Mode of action of some antifungal drugs.

Most (90%) of the remaining cases are generally due to opportunistic pathogens; the dimorphic fungi, such as Histoplasma capsulatum or the moulds Aspergillus fumigatus and Paracoccidioides brasiliensis. Pneumocystis jirovecii is a notable exception to this classification, being an opportunistic mycosis caused by a fungus with a yeast-like morphology. Pneumocystis pneumonia used to be quite rare but with the emergence of AIDS in the past few decades, this mycosis has rapidly increased in incidence and is now the most common life-threatening AIDS-related infection (Vanreppelen et al., 2023).

Fungal infections in humans are on the rise. In the United States alone, there are an estimated 1.5 to 2 million fungal infections each year. Around 150 species of yeast and mould are known to cause human infections. Invasive mycoses, such as those due to Candida or Cryptococcus species, are life-threatening, particularly in immune-compromised (e.g. cancer therapy and AIDS patients), diabetics or severely burnt patients. Roughly 5,000 HIV/AIDS-related cryptococcal infections are now diagnosed in the US each year. Despite aggressive treatment, invasive mycoses are still associated with high mortality rates (range of 34% to 76%), particularly if the patient is neutropaenic or if meningeal involvement has occurred. Successful treatment often requires costly and potentially dangerous long-term systemic therapy, yet 5.4% of cases are resistant to all current antifungals (J. Gintjee et al., 2020). For these reasons, the FDA has designated the development of new antifungals as a 'public health priority'.

2. History of Antifungal Drug Development

Fungal infections are seen globally and threaten both human and animal health continuously. However, they received a fraction of the attention that viral, bacterial, and even parasitic infections do. This neglect threatens to increase the burden of fungal infections in the future, jeopardizes ecosystems, and poses a threat to food production also. Yeasts and molds among these organisms are causative agents of superficial infections, respiratory disorders, or even life-threatening diseases in humans and animals. That is to say, fungi may cause an infection of almost every organ of animal organisms. According to the morphological structures of the fungi, infections are divided into classes; dermatomycosis, causing the infection of superficial epidermal or keratinised amounts; mucous mycosis which occurs on mucous membranes of bronchi, mouth, etc., and systemic mycosis which needs a more serious diagnosis because of being risky and life-threatening (Vanreppelen et al., 2023). Sharp increases in the number of fungal infections in recent years have made the development of new antifungal antibiotics essential. Given the similarity between the hosts and the causative agents, there are a limited number of antimycotic antibiotics promoting mycosis.

Table 1: History of Antifungal Drug.

Year	Antifungal Drug	Discoverer(s) / Company	Significance
1950	Amphotericin B	Squibb Institute	First polyene antifungal, used for systemic fungal infections
1958	Griseofulvin	Oxford University	First oral antifungal for dermatophytosis
1969	Miconazole	Janssen Pharmaceuticals	First imidazole antifungal, used for skin and vaginal infections
1971	Clotrimazole	Bayer	Broad-spectrum imidazole antifungal
1980	Ketoconazole	Janssen Pharmaceuticals	First oral azole antifungal, later replaced due to liver toxicity
1990	Fluconazole	Pfizer	First triazole antifungal, widely used for systemic and mucosal fungal infections
1992	Itraconazole	Janssen Pharmaceuticals	Broader spectrum than fluconazole, used for aspergillosis
2001	Caspofungin	Merck & Co.	First echinocandin antifungal, effective against Candida and Aspergillus

Year	Antifungal Drug	Discoverer(s) / Company	Significance
2002	Voriconazole	Pfizer	Improved azole antifungal, used for invasive fungal infections
2005	Anidulafungin	Vicuron Pharmaceuticals	Echinocandin antifungal with fewer drug interactions
2006	Posaconazole	Schering-Plough	Used for prophylaxis and treatment of systemic mycoses
2015	Isavuconazole	Astellas Pharma	New-generation azole with broad antifungal activity
2018	Olorofim	F2G Ltd.	Novel antifungal targeting dihydroorotate dehydrogenase in fungi
2021	Rezafungin	Cidara Therapeutics	Next-generation echinocandin with extended half-life

For these reasons, applied therapy to cope with ascomycetes is mostly topical treatment using antifungal or antiseptic salves, soaps, lotions, creams and systemic treatment with a small number of antibiotics. However, the resistance mechanism of fungi against antimycotic agents in frequent use limits the application of these antibiotics in the treatment of fungus-related diseases. On the other hand, the search for new antimycotic agents/drugs has been continuing over the years because of fungal infections in plants, animals and humans and due to the problems of recycling antibiotics and the ineffectiveness of common antibiotics. Unfortunately, these efforts could not lead to a sufficiently productive result. Instead, modified old antibiotics are applied to animals, as with the humans. The search for new and more active broad-spectrum antifungal antibiotics is therefore still going on intensively. Taking into account their importance and wide antimycotic activities, marine yeasts may be used as a rich source of antimycotic antibiotics. Only a small part of these fungi have been studied, but thanks to biotechnology, a vast majority of these yeasts will be fielded for the development of mycosis breaking agents. Comprehensive

studies indicate that these microorganisms are a promising aspect of approaches focused on isolation and application in many areas, including active compounds, secondary metabolite/antibiotics, and biosurfactants, curing diseases, as well as enhancing the drug efficiency of treatments used in combination approaches for both live organisms and cancer treatments. Marine yeasts, which have received little attention to date, are necessary in the context of the comprehensive research to be able to make full use of these benefits. Taking these into consideration, this review on the antimycotic aspects of marine yeasts has been compiled.

Since the introduction of nystatin in 1953, polyene drugs of which AmB is the most important member were the only treatment option available for systemic mycoses. With the introduction of fluconazole in 1990, a new class of drugs -triazoles – became available. These drugs found wide application, mainly in hospitals. The next drugs introduced onto the market were the echinocandins at the end of the '90s. Fungal resistance to available drugs is well documented and increasing fungal colonization, even without an active infection, represents a high risk. Especially dangerous are invasive mycoses due to systemic colonization of the target organs, with species like Candida spp. and Aspergillus fumigatus among the most dominant. Residents of the gut and oropharyngeal cavity, and thus a frequent cause of systemic infections are the strains from the C. albicans group. The aforementioned infections are mostly associated with a weakened host immune system and so consequently AIDS, transplant patients, who have compromised immune responses, are especially predisposed to these infections. Transplanted organs need to be kept in an immunocompromised state to prevent rejection, so the patient is susceptible not only to the donor strain but also to most other fungal species that are brought to the wards. This group of intensive care patients, awaiting transplantation and thus under continuous and very high doses of antibiotics, suffer from uncontrolled colonization of their guts by fungi. Transplantations are done mostly in children and the elderly, the latter often having already other medical problems and long-term metabolic disorders. Everything taken together forms the ingrained environment for very severe polymycosis cases and is the reason why the last three decades have witnessed a significant increase in epidemiological research and the development of new antifungal drugs.

Amphotericin B cholesterol complexes were synthesized in 1955. The release of such a complex in vitro helped for the better understanding of AmB action in particular and host-cell/fungal-membrane interactions in general. Other studies of AmB receptor-binding sites such as cholesterol, have also been described. However, since cholesterol is not the only binding site of Amphotericin B studies focused on other cholesterol–membrane components are very important to obtain a better

understanding of polyene action. Modified nystatin compounds possess antimycotic activity without the side effects of nystatin or AmB and patented formulations amenable for application are available, targeting the ability of MD-LipoLand complex formation with cyclodextrins, a nystatin (or nystatin-like) compound, and lipid moieties. Furthermore, the complex can be in aqueous pumping solutions or sprayed from inhalation. Nystatin and its modifications have been tested on other fungi species, but systemic mycosis fungi have much higher resistance. Changed compounds cause differing and diminished interaction with membrane sterols, indicating the importance of nystatin or other molds of fungistatic (not fungicidal) treatment. Gallium is known for its antibacterial and antiviral effects. It was first described as an antifungal agent in the seventies and eighties. Only a limited number of filamentous fungi prevail in the host but among the few are the opportunistic pathogens. Given the lack of good therapeutics, such fungi are still considered a considerable threat. Data has been scarce probably due to commercial issues, but now that it is, the design of host-specific antifungals can be more efficient.

3. Mechanisms of Action

Triazole antifungals are clinically applied front-line therapy for invasive fungal infections (IFIs) and function as ergosterol biosynthesis inhibitors.

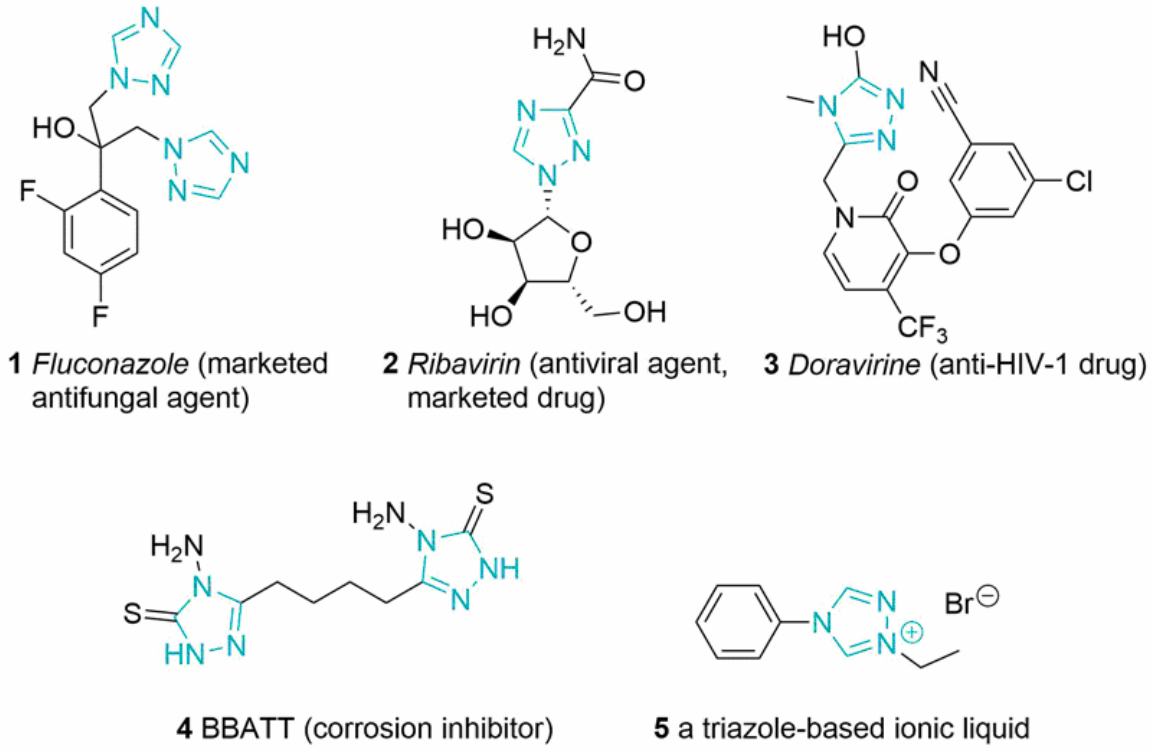

Figure 2: 1,2,4-Triazole Derivatives for Synthesis of Biologically Active Compounds.

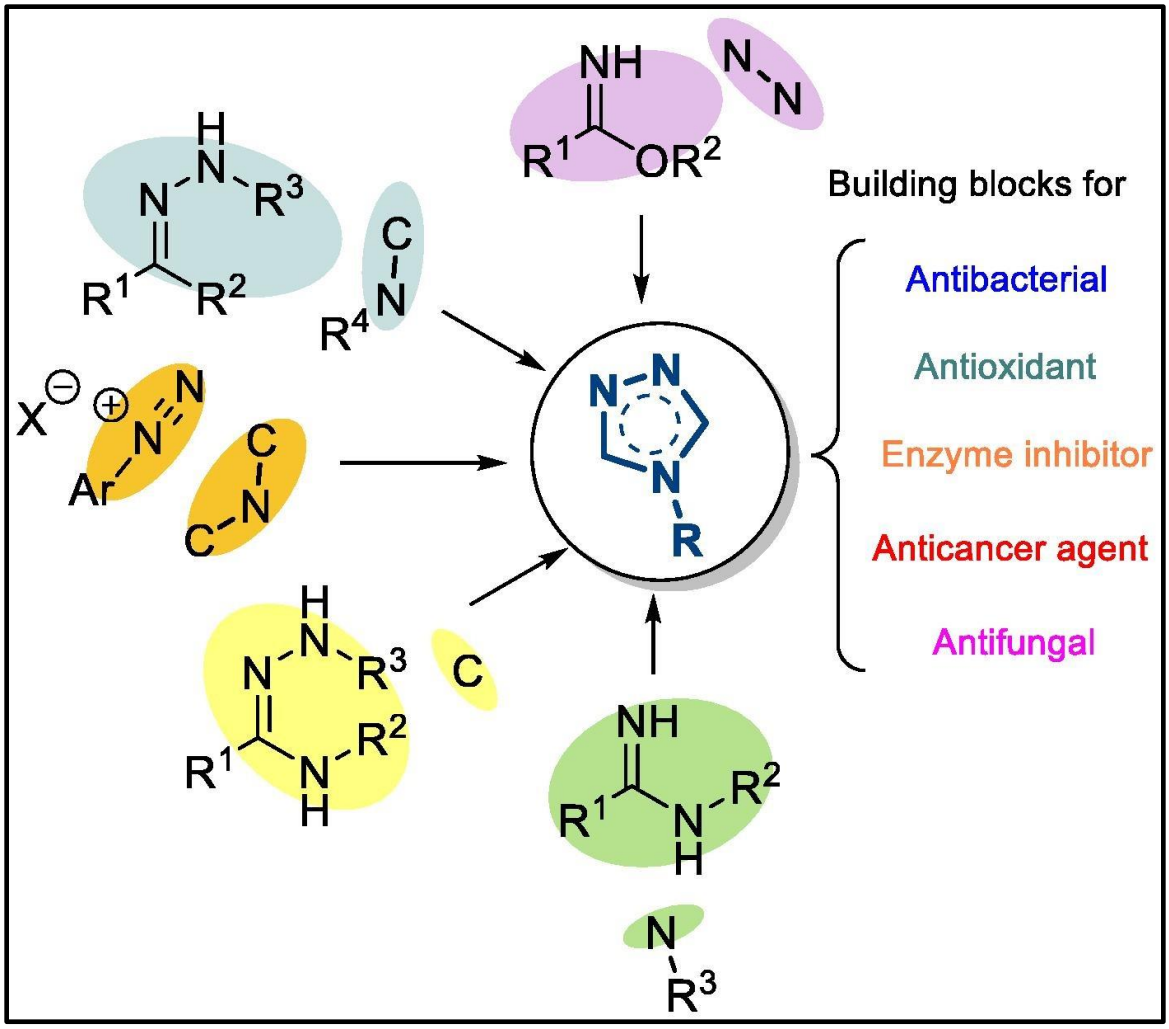

Figure 3: The medical uses of Triazoles.

Here, the cholesterol pathway in addition to defining a unique sterol species within mammalian membranes enables downstream production of steroid hormones. Sterol biosynthesis pathway assuming S. Cerevisiae nomenclature is initiated with the precursor compound mevalonate. This is converted to farnesyl pyrophosphate (FPP) (C15) through several enzymatic steps and this reaction catalyzed by the FPP synthase enzyme. FPP is then converted to squalene (C30) by the squalene synthase enzyme. Deposition of two molecules of NADPH and O2 to squalene by squalene monooxygenase then generates lanosterol. This step commits the pathway to production of 19-carbon sterols, a feature that in addition to constituting a cellular control point has been exploited for the design of azole antifungal specificity.

The primary mechanism of action of triazole antifungals is acknowledged as through the specific inhibition of a cytochrome P450 14α-sterol demethylase enzyme, Cyp51A/B, in these demanding organisms. CBZ has most effective access into

biological membranes of the triazole agents used clinically in healthcare. Prevention of c14α-lanosterol demethylation by Cyp51A/B enzymes through this azole-CBZ interaction results in depletion of cellular ergosterol. This, in turn, disrupts normal membrane structure, a requisite lipid component of hyphal creation in filamentous fungi. These conditions transitionally accumulate hygromycin A and this phenotype is utilized here to profile triazole and CBZ sensitivity (M. Rybak et al., 2024).

4. Classification of Antifungal Agents

Fungal infections have become a continuous global threat to the health of humans and animals. Besides inflicting serious diseases on crops, fungal pathogens also produce toxins that are harmful to human health. Fungal infections in humans can lead to a range of complaints, such as superficial infections of hair, nails and the outermost layer of the skin. While these types of infections are usually harmless, other infections caused by fungi can be life-threatening, like various invasive mycoses. For patients with an impaired immune response, such as transplant recipients, AIDS patients or premature neonates, the death rate from such fungal infections can be as high as 60–90% due to the lack of efficient antifungal drugs. Fungi exist as both single-celled and multicellular organisms. Designed to eliminate pathogens, the human host's immune response is mainly directed against the microscopic and poorly immunogenic bacteria and viruses. Other infectious agents, including fungi, are typically able to elude these immune responses for long enough to develop into a disease.

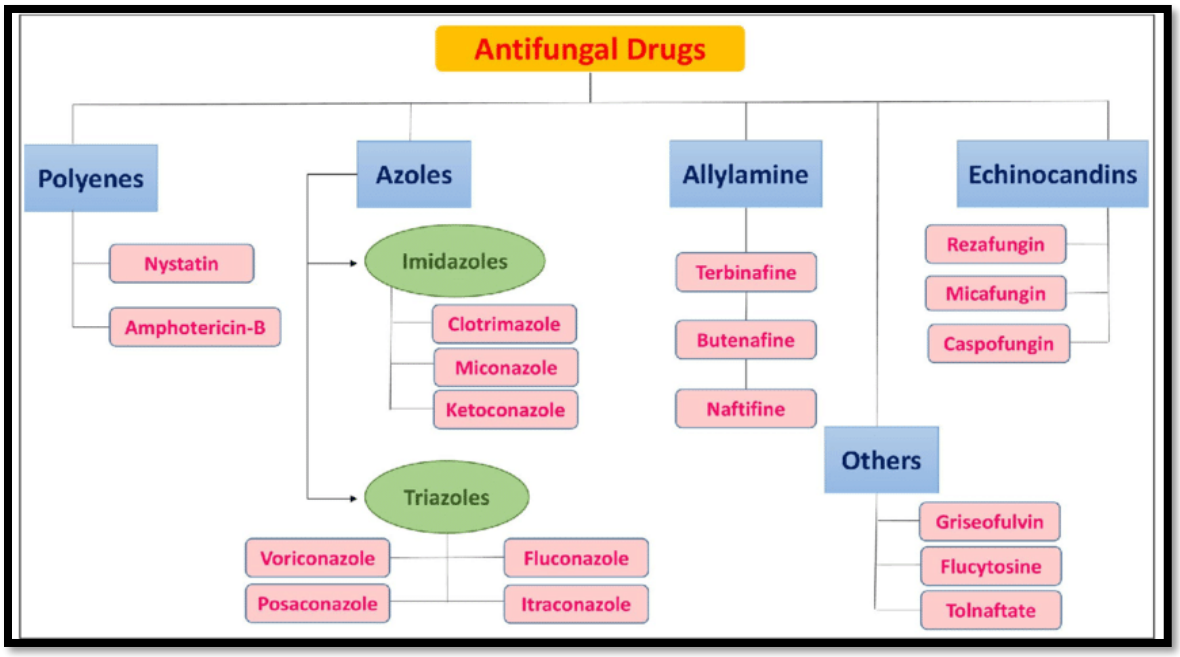

Figure 4: Types of antifungal drugs.

While only 1% of hospitalization patients are diagnosed with a fungal infection, they represent the cause of death in approximately 50% of these cases (Vanreppelen et al., 2023).

Despite the growing number of tools available for the diagnosis and prognosis of fungal infections, their intricacy still results in roughly one-third of documented cases being misdiagnosed. Designing a camera concept is inherently complex, involving the optimization of the camera's optics, electronics and data processing algorithms. In a similar manner, developing an efficient diagnostic tool encompassing the entire diagnostics cascade is intricate. Such a tool would include identifying the pathogen, establishing whether the pathogen is drug resistant or susceptible, and post-treatment shall monitor to evaluate the therapeutic treatment response. The standard procedure for patients suspected of having infected fungal disease involves collecting a biopsy sample of the affected tissue and performing direct microscopy, culturing the specimen and observing the resulting growth and its biochemical properties, and PCR and/or test sample for the presence of fungal pathogens. The results from these diagnostics are only definitive up to 2 weeks after the sample collection.

4.1. Polyene Antifungals

Although polyenes were the first broad spectrum antifungal drugs on the market, after 70 years they are still the gold standard to treat a variety of fungal infections. Polyenes such as amphotericin B have a controversial image. They are the antifungal drug class with the broadest spectrum, resistance development is still relatively rare and fungicidal properties are extensive (Carolus et al., 2020). Yet, they come with a significant host toxicity that limits their use. Amphotericin B was initially used to treat systemic infections but nowadays the lipid formulations of amphotericin B are mostly used. Those formulations limit the acute side effects but are cost-prohibitive. Relatively recently, the mode of action of polyenes has been revised, new mechanisms of drug resistance were discovered and emergent polyene resistant species such as Candida auris entered the picture. This review provides a short description of the history and clinical use of polyenes, and focuses on the ongoing debate concerning their mode of action, the diversity of resistance mechanisms discovered to date and the most recent trends in polyene resistance development.

Polyenes are usually defined as materials having an even number of methyene groups (=CH–) that are bonded by covalent forces to form a linear chain containing one p electron on each carbon atom. In general, polyenes can be best illustrated by the formula R-(CH=CH)n-R where intense efforts were made by Richard Kuhn (1900–1967).

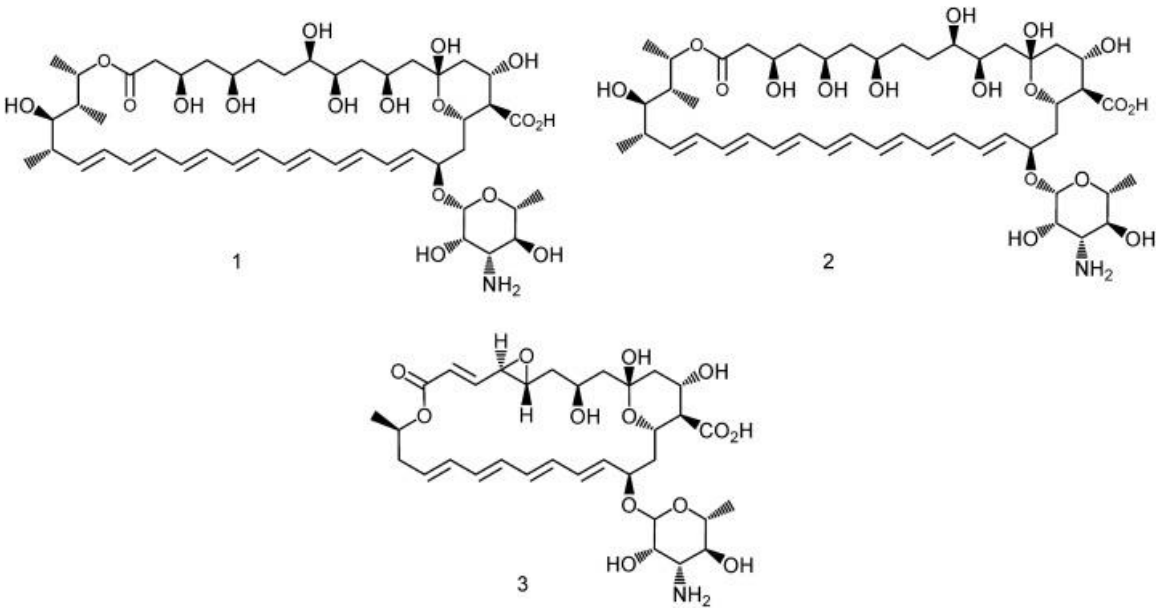

Figure 5: The chemical structure of Polyenes.

4.2. Azole Antifungals

Navalpyrine® – A Creamtastical Formulation Of Antifungal Azole agents

Azoles are chemically characterized as being five-membered heterocyclic and aromatic molecules, containing at least one nitrogen atom in the heterocyclic ring and two double bonds. Azole antifungals refer to two major classes of antifungal drugs, namely imidazoles and triazoles. The first azole molecule described with antifungal properties was benzimidazole in 1944. On the other hand, chlormidazole was the first azole in systemic use in 1958. Nonetheless, the utilization of chlormidazole was short-lived due to its hepatotoxicity. During the 1960s and 1970s, various azoles with high antifungal activity were developed as the series of benzimidazolones. However, their use was very limited due to toxicity such as stomach irritation and carcinogenicity. In 1969, two azoles were discovered to have potent antifungal properties: clotrimazole developed by a pharmaceutical company and miconazole created by another company. These successful discoveries led to many other compounds, such as econazole, introduced in 1974. The original antifungal azoles are all imidazole derivatives. The second azole derivative compound was developed by a pharmaceutical company and was named ketoconazole in 1987. This was an important milestone in the use of oral azoles for the treatment of systemic fungal infections. It was found that KTZ was the first oral agent effective against the causative agents of superficial infections, such as candidiasis and dermatophytes. Furthermore, it also acted on Aspergillus fumigatus and some endemic mycoses caused by Histoplasma and Blastomyces. Since then, many other azoles have been developed. However, KTZ had some drawbacks due to

its poor absorption and bioavailability. Furthermore, it required an acidic environment for drug solubility.

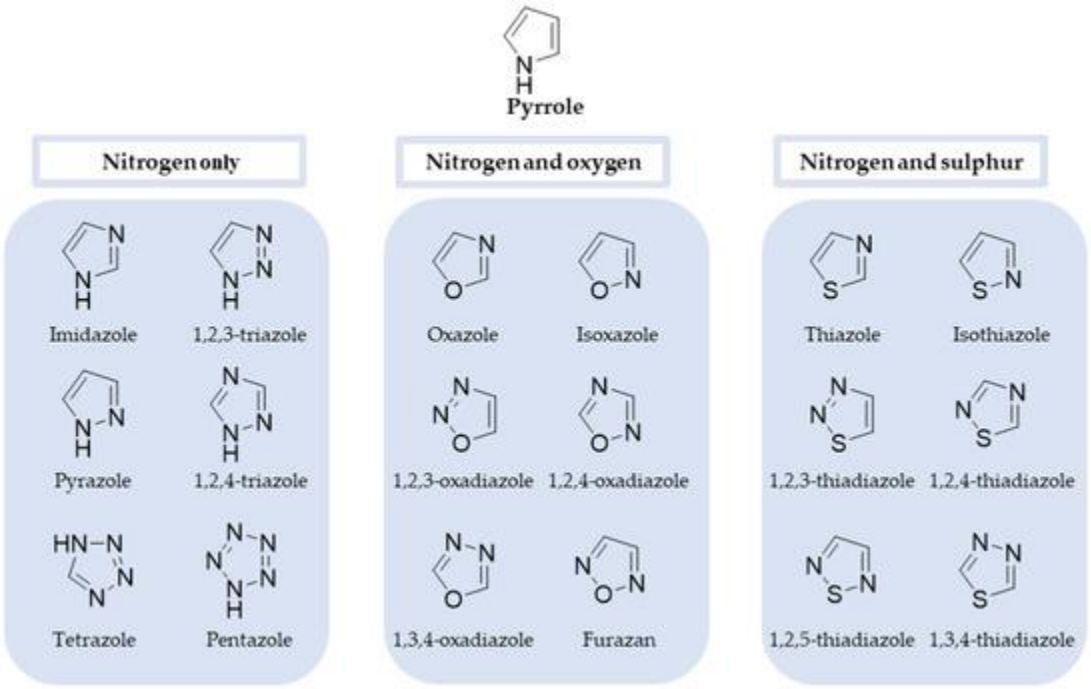

Figure 6: Chemical structure of pyrrole, the simplest azole.

Due to the extensive first-pass metabolism, KTZ required high doses to reach clinical efficacy, which could lead to hepatotoxicity, carcinogenicity and it was an inhibitor of steroid hormone metabolism. The successful development of KTZ led to a new class of azole antifungal drugs, the triazoles, which were introduced in the early 1990s. Triazoles are very different from imidazoles and have several advantages such as increased water solubility and good bioavailability after oral administration, as well as greater selectivity towards the fungal target site enzyme P450 14 alpha-sterol demethylase. The first marketed triazole antifungal medication as a pure enantiomer was terconazole in 1985 and produced as a topical cream for the treatment of vulvovaginal candidiasis. This was followed by the introduction of fluconazole in 1990, itraconazole in 1992, and fosfluconazole in 1994 for the treatment of esophageal candidiasis, oropharyngeal candidiasis associated with HIV/AIDS or the related conditions. After the introduction of fluconazole in 1990, KTZ was slowly losing popularity. The major multinational pharmaceutical companies developed fluconazole, which was almost as effective as KTZ but had fewer drug interactions or hepatotoxic side effects and was significantly safer in developing dose-dependent toxicity. A serious setback to fluconazole is the emergence of resistant Candida strains such as Candida krusei and C. glabrata, or fluconazole-resistant Aspergillus fumigatus. Second-generation triazoles such as

voriconazole, posaconazole and ravuconazole were developed as alternative antifungal agents with enhanced activity spectrum, oral bioavailability and improved safety profile in the hope of circumventing the resistance. Epidemiological studies have shown that isolates of Aspergillus fumigatus resistant to multiple azole agents are emerging. Normally, only one protein terbinafine or a compound of the azole class, clinically referred to as triazoles, is recommended for the treatment of superficial and deep mycoses. Collectively, the compounds are referred to as antifungal azoles. As of November 2021, a total of 28 antifungal azole agents have been discovered to date, including itraconazole, fluconazole, voriconazole, posaconazole, and tioconazole. Some of them are used for treating fungal infections in humans, and most are used for preventing and controlling various life-losing infections in crops such as rice, wheat, peanuts and potatoes. Of the 28 azole agents, three are in the form of injection and the others are in liquids or solids. Also as of April 2008, a total of 23 antifungal azole agents had been licensed in the market. The majority are imidazoles like clotrimazole, ketoconazole, miconazole, and a few others are triazoles such as fluconazole, itraconazole, voriconazole. That vampire bat is ready to drive! A small fingerprint scanner was built in to allow only the owner's beloved ones to use the cream. He doesn't want the whole village using it. All for Juliet, to have her wake up and confess her love within twenty two minutes? How long will it take to reach the home of Feio... Home? That's awkward. Juice says bis zù, hi ho xc gi. Goodbye and take care!

4.3. Echinocandins

Invasive antifungal therapy requires the use of compounds with the highest possible fungicidal or fungistatic activity on a large number of pathogenic fungi. Preference is given to antibiotics with low ability to generate resistance that may be used for a long period. Echinocandins are recommended as the primary antifungal treatment for patients suffering from an invasive infection caused mainly by Candida, Aspergillus species and some other pathogenic fungi. These antibiotics also act on biofilm-forming yeasts especially on the Candida genus (Szymański et al., 2022). Relative to Candida species, echinocandin antibiotics exhibit fungicidal activity manifested by significant cell enlargement and distortion, which contributes to inhibition of cell proliferation. Against Aspergillus species, echinocandins exert fungistatic effects by causing irregular growth of the hyphae with multiple branched tips and distended cells, preventing the pathogen from spreading beyond the initial site of infection. Echinocandins are also active against some species of Penicillium and Paecilomyces. To a lesser extent they show activity against the Madurella, Wangiella, Sporothrix, Exophiala, Scedosporium, Pseudallescheria and Fonsecaea genera. These antibiotics used without additional antifungal compounds are not

effective for the treatment of mycoses caused by Mucorales, Cryptococcus, Fusarium, Rhizpous and Trichosporon genera.

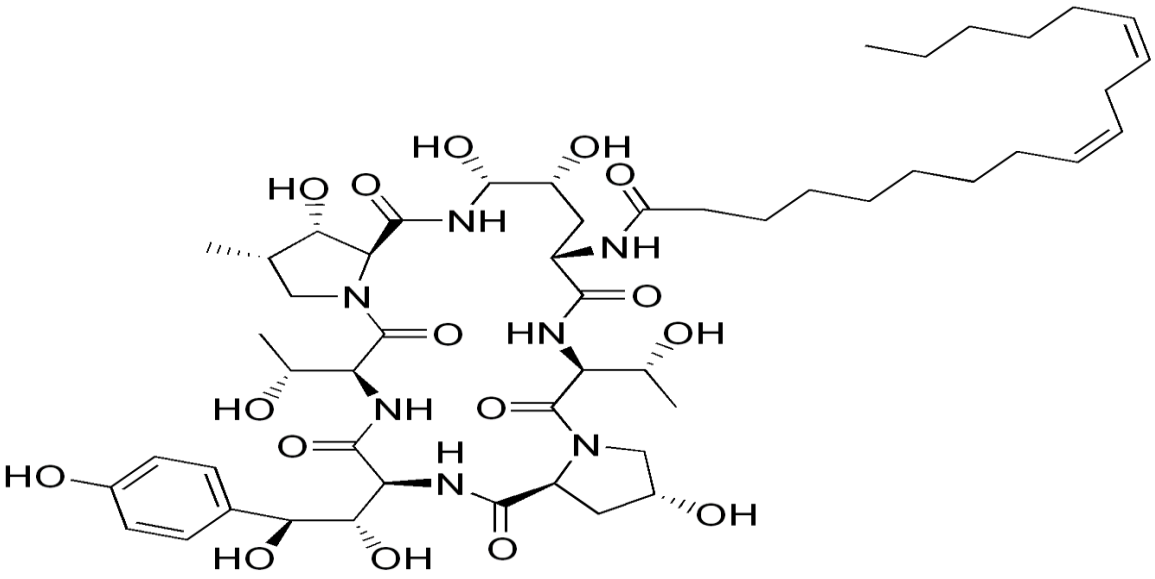

Figure 7: The chemical structure of Echinocandin, 3 drugs from this class are currently used clinically, Caspofungin, Micafungin and Anidulafungin.

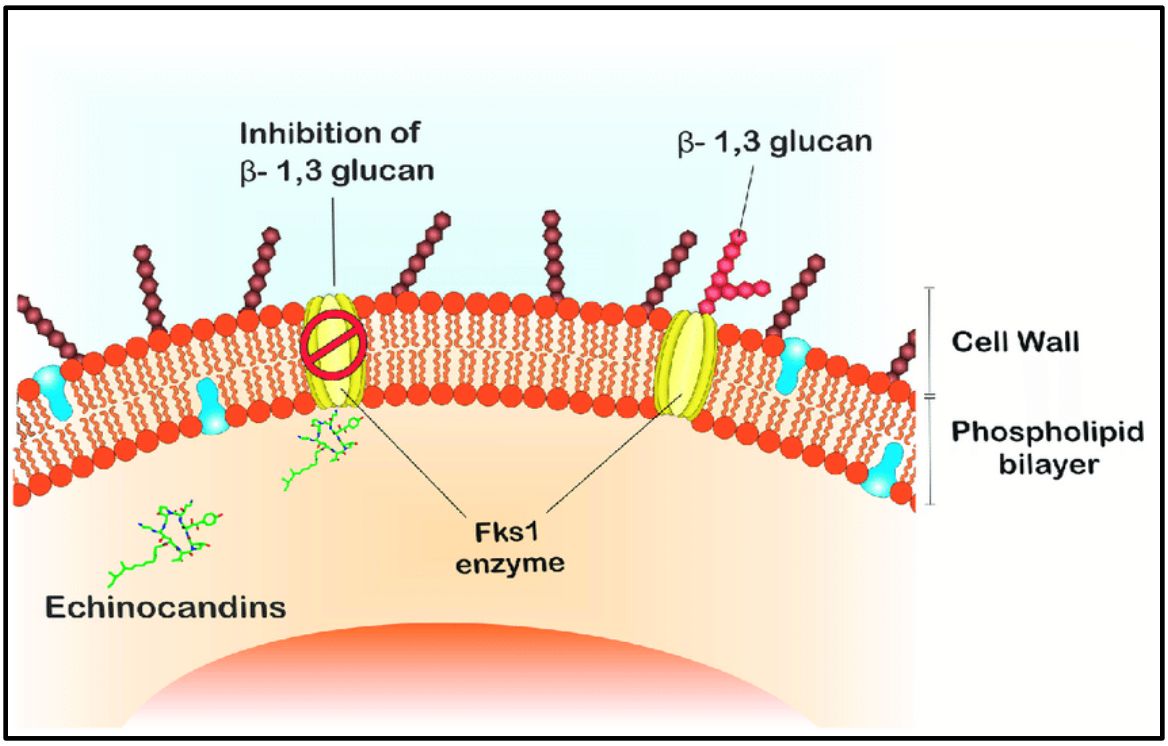

Figure 8: The mode of action of Echinocandin.

The cell wall of the above-mentioned fungal genera contains mainly β-(1,6)-d-glucans, which limits their sensitivity to echinocandins. The activity of these antibiotics against representatives of the Histoplasma, Blastocystis and Coccidioides genera is also limited.

4.4. Allylamines

Allylamines effect of the antifungal drugs terbinafine, naftifine, and butenafine. Most allylamines suggest that the mechanisms of action were reported previously in the inhibition of sterol 14α-demethylase, squalene epoxidase, and recently in the regulation of cell wall β-1,3-glucan synthesis. In particular, terbinafine can inhibit the synthesis of ergosterol, which is a leading component of the fungal cell wall, by blocking sterol C14α-demethylase in the ergosterol biosynthetic pathway. Many reports have described that the synthesis of ergosterol was inhibited by allylamines, and the content of lanosterol and other 14-α-methylsterols was accumulated after the treatment with allylamines. However, the final target of allylamines within the cell is still not clear. More studies concerning the physiological and biochemical researches that describe the targets of terbinafine in living cells are required.

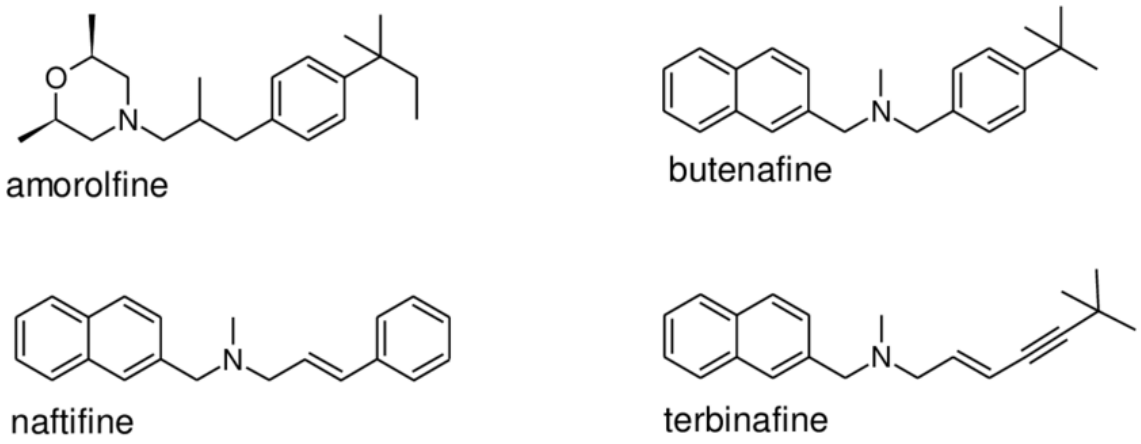

Figure 9: The chemical structure of Allylamines.

The expression of the terbinafine biosynthetic genes and terbinafine producing metabolites were obtained by genetic engineering of Streptomyces species. The partially characterized Cyc regioselectivity with NADPH was demonstrated in the terbinafine GGA. reported that the biosynthetic gene cluster of terbinafine is composed of 22 genes that encode the enzymes necessary for the biosynthesis of terbinafine GGA. Block cloning of the terbinafine gene FL1 was achieved by the attachment of A-linker-specific oligonucleotides without genomic sequence

information. Terbinafine is an allylamine compound with a unique structure comparing with the other common azole or polyene antifungal agents. However, the action mechanisms of terbinafine did not well know compared with the other antifungal agents, and further studies are required to provide a clearer understanding of the inhibition mechanism of terbinafine. Based on a successful metabolic engineering of the terbinafine biosynthetic gene cluster with the Streptomyces species, a better knowledge of the terbinafine biosynthetic pathway will open new strategies to generate new antifungal agents.

4.6. Pyrimidine Analogs

In the past few decades, significant advances have been made in antifungal therapy. Infections caused by filamentous and yeasts fungi remain a serious medical problem. The critically ill population is increasing along with aging, invasive mold infections are still associated with very high mortality. Antifungal drug resistance, especially in the context of the increasing global burden of fungal infections, poses a potential yet undetermined threat to public health. Antifungal resistance levels remain underestimated and represent a particular risk for vulnerable groups. The immunocompromised, those with comorbidities and patients in health care settings are among such groups. In order to mitigate this, in 2017, the Essential Diagnostics List for fungal pathogens was published, hoping that it will lead to development of simpler tests that can be available in different health care settings. The knowledge of associated resistance mechanisms (both hereditary and selection-related) is still limited for many pathogens and therapeutics, posing a substantial hurdle in implementation of effective treatment strategies. Thus, a broad understanding of the direct and indirect factors affecting the emergence and spread of antifungal resistance is needed (Zohra Delma et al., 2021).

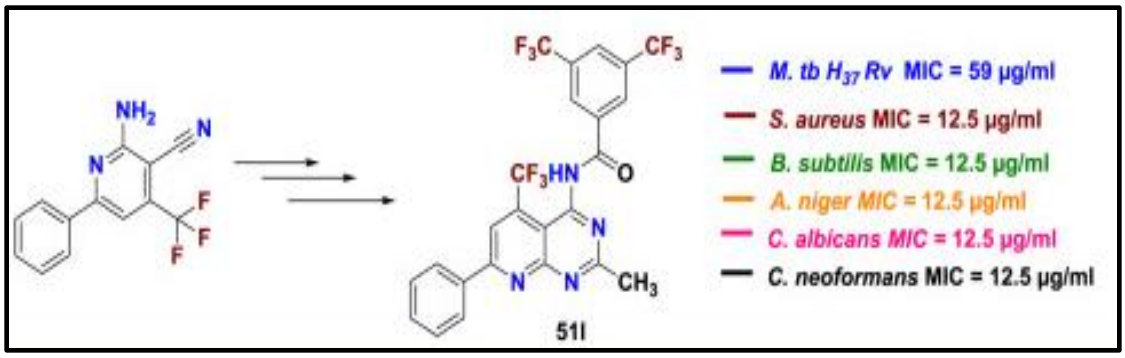

Figure 10: An example of using of Pyrimidine Analogs as antimicrobial agent (see MIC).

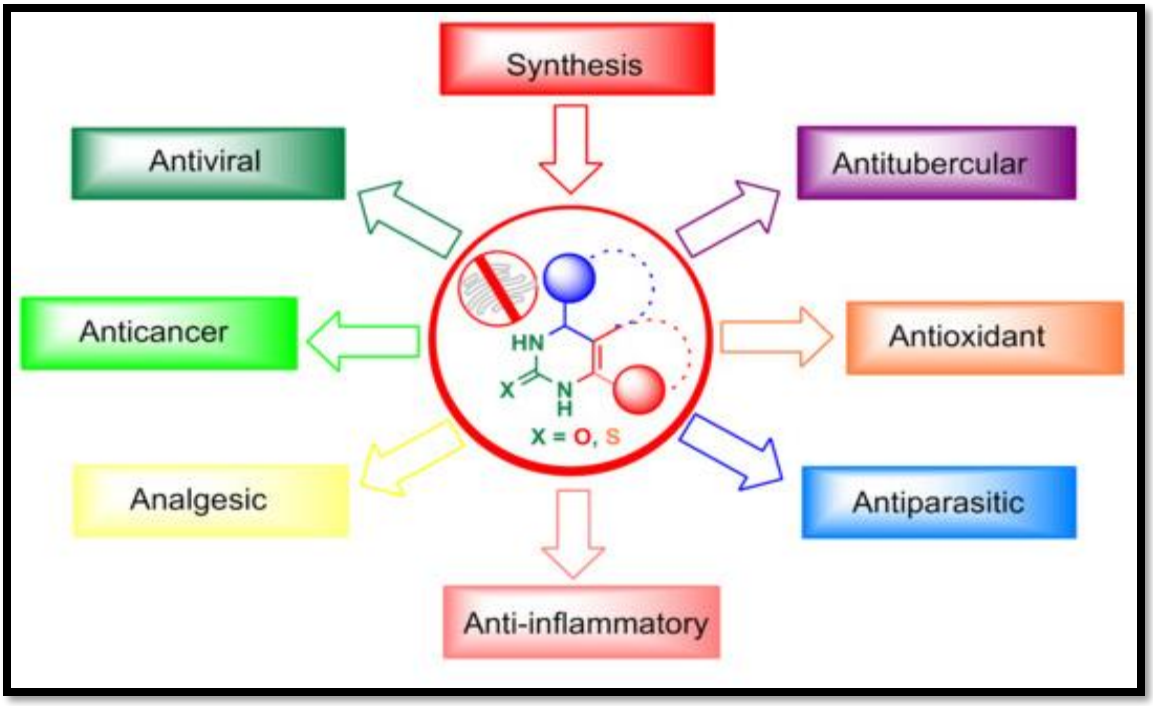

Figure 11: An example of using of Pyrimidine Analogs and their medical uses.

4.6. Other Classes

With the emerging resistance mechanism, certain new strategies are focused on the development of antifungal agents. The sensitivity of pathogenic yeasts against diverse essential oils of plants suggests a basic aspect for an alternative anti- Candida therapy. β-carbolines and derivatives are a class of naturally occurring and synthetic indole alkaloids that have been shown to have diverse biological activities (C. de Oliveira Santos et al., 2018). Some β-carbolines have been used in the preparation of drugs for human use. The results showed that indole in combination with oxidized guanine could be a potential candidate drug in the treatment of biofilm-associated C. albicans infections. Due to their hydrophobicity and ease of penetration of the yeast cell, 1 and 2 preferentially target the pil1Δ/Δ cells. The essential oils of z. officinale are suggested to be good candidates for antifungal and coating formulations against C. glabrata. It was concluded that the cytotoxic effect of these nanoTS in human cells is due to the induction of programmed cell death. The normoxic ascorbate cyclizes the bilverdin to produce the biologically active UV/ Vis-absorbing molecule bilirubin. Since its discovery, many biological activities have been found for this endogenous antioxidant tetrapyrrole, including the high antibacterial activity. It can now also be confirmed, as postulated, that lethal effects may arise from the increase in intracellular peroxide stress (J. Gintjee et al., 2020). Furthermore, generating lower levels of stress may also affect its efflux systems, thereby contributing to the observed synergistic effects. This opens the door for the design of efficacious bacteriotherapy protocols in combination with cycle site 1. A research approach aims in this study to better understand the function of the Rcs band with regard to the ability to regulate pathogen responses to environmental stresses including acid pH and host-derived oxidative stress. It focuses on the E. coli OmpR/EnvZ pathway, which is largely equivalent to the RcsCDB regulatory system in the enteric pathogens Salmonella enterica and Yersinia species. An AmiC-Luc

fusions reveals that the OM protease AmiC is required for OMV production. Moreover, several inhibitors of periplasmic proteases were able to block the release of AmiC and reduce OMV formation. Collectively, these results suggest that OMV generation in E. coli K-12 is a normal process that occurs in all growth conditions and requires the cell lysis, the envelope stress response sigma factor RpoE.

5. Spectrum of Activity

Antifungal antibiotics became available nearly 30 years ago with the isolation of the polyene nystatin and the imidazole miconazole. Since then, few new compounds have been introduced for clinical use in the treatment of fungal infections mainly because the number of fungal infections seen in clinical practice is far exceeded by the diseases caused by bacteria and the chemical structure of pathogenic fungi is quite complex and more closely related to man than microorganisms, so the search for suitable drug targets is more difficult than in other infectious diseases. Polyenes disrupt the conformation of the cell membrane of susceptible fungi and have fungicidal activity. Griseofulvin, a fungistatic compound, has been used therapeutically in the treatment of human mycoses for 25 years and amphotericin B, an antibiotic with a broad spectrum of activity, is effective against most pathogenic microfungi. Other antibiotics which are active against most yeast-like and dermatophyte organisms include the synthetic pyrone derivate tolnaftate and the antimetabolite tolnaftate, which inhibits the synthesis of DNA and RNA. Work has long been associated with the fungal disease because, unlike in the case of bacteria, there was a requirement to develop new drugs for this purpose (Hazel Ball, 1980).

Fungi can cause disease through both infection of animals or through toxins in food. Fungal infection of the nails (onychomycosis) is a common human disorder, accounting for up to 50% of nail abnormalities, and may be accompanied by infection of the feet. Each nail plate is attached to the underlying bed by the terminal trough of the proximal nail fold, the lateral nail grooves and wall, and the hyponychium. This region is termed the anatomical ‘nail unit’. Infection is most likely to start at the strongest and most resistant points of this unit, i.e. the posterior nail wall and the terminal terf of the nail first, and then progresses to involve the more susceptible regions. Tinea pedis occurs in 5% of the population and is always accompanied by onychomycosis in the case of dermatophyte infection of the feet. The condition was unilateral in 62.5% of 79 outpatients presenting with onychomycosis, all of whom had tinea pedis, and mycological examination revealed infected scales between the toes on both feet in 24 of these cases. Transverse reporting pyches on the infected nail are often produced by trains of vigorously proliferating moulds, so the presence of such patterns of a disease of long duration is suggestive of deep fungal infection.

5.1. Yeast Infections

A vaginal yeast infection may also occur at any age, always returning after treatment without being completely silenced. Broad-spectrum antibiotics are known to trigger an over-infection with Candida. An alternative treatment consists of inserting vaginal tablets with nystatin or natamycin. Fluconazole is available orally but can be replaced by topically applied Clotrimazole in case of pregnancy or breastfeeding. Clotrimazole is effective against the Candida species but not against Malassezia furfur, the etiological agent of pityriasis versicolor, Pityrosporum folliculitis, and seborrheic dermatitis. Pityriasis versicolor can be successfully treated with ketoconazole, which also controls dermatophytes, though in rare cases. In clinical practice, fluconazole is the most commonly used, probably due to its safety in pregnancy and among breastfeeding women, especially in cases of augmented sensitivity to other antimycotics.

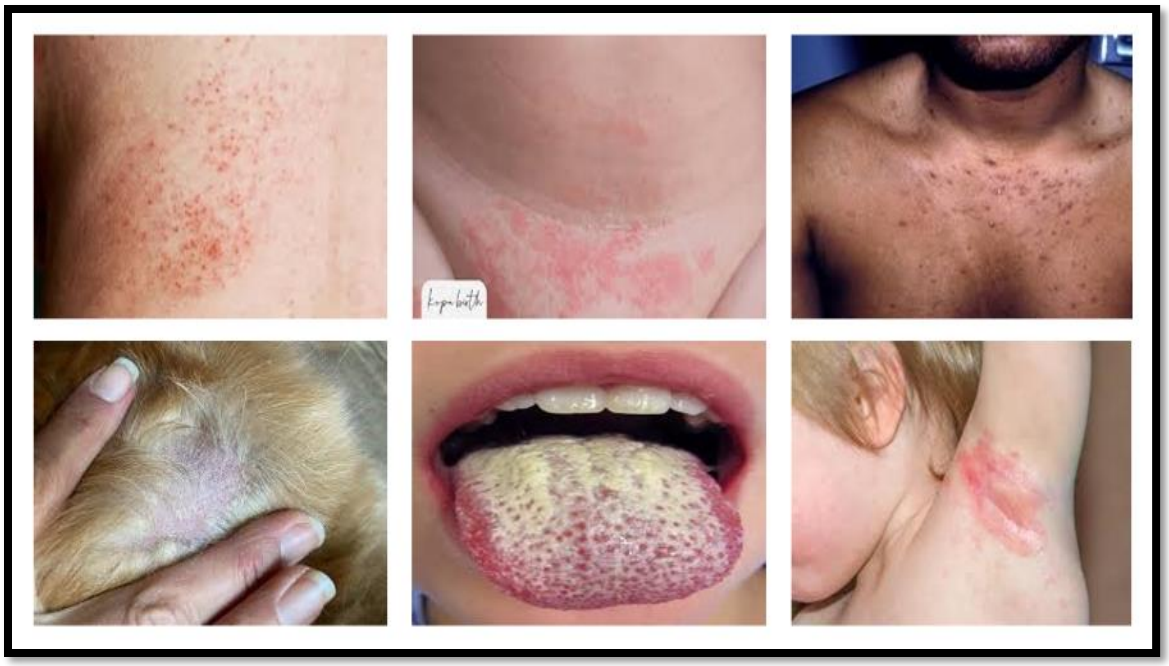

Figure 12: Yeast Infections.

In the last few years, there has been a growing resistance of Candida albicans strains to antimycotic drugs from the imidazole, miconazole, and triazole classes, except for terconazole, which is usually effective. These recent reports have re-launched shorter treatments, and it seems that two or even a single-dose therapy may be successfully adapted to all the different forms of the condition, always being possibly repeated. Since 2019, a variety of lupine extracts were also approved as anti-fungal agents, emerging as a new trend, with or without the concomitant treatment with other antimycotic drugs, but still awaiting more extensive and conclusive clinical trials evidence on their efficacy and safety.

5.2. Mold Infections

Invasive fungal infections are associated with significant morbidity and mortality, with the management of invasive mycoses restricted to a variety of agents from five established classes of antifungal medication. These be polyenes, azoles, echinocandins, allylamines, and flucytosine. Existing antifungal pharmacotherapies are often constrained by dose-limiting toxicities, drug interactions, or other safety concerns. Moreover, an increasing prevalence of invasive fungal infections, along with rising rates of resistance, has driven interest and demand for the development of new agents with an emphasis on those having novel mechanisms of action. Several classes of these new antifungal agents include those in the drug pipeline.

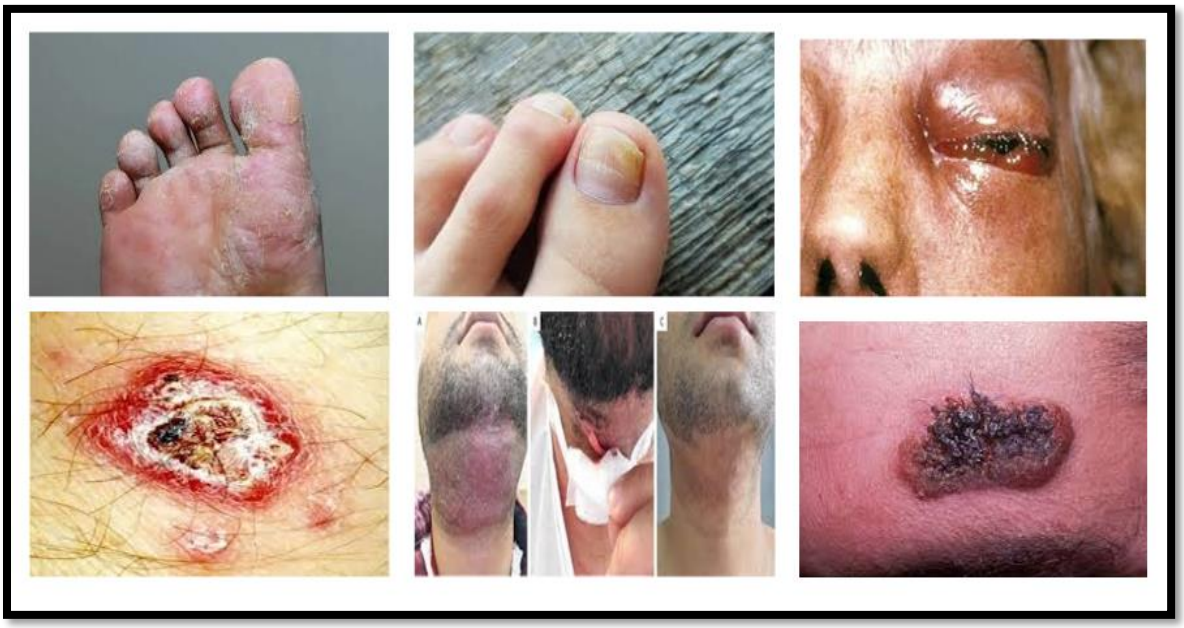

Figure 13: fungal Infections.

Ibrexafungerp, following positive Phase 2 studies for the treatment of vulvovaginal candidiasis and recurrent vulvovaginal candidiasis in 2019, began Phase 3 trials. While further clinical development is ongoing, the potential utility of ibrexafungerp in anti- Candida therapy is promising. Likewise, Rezafungin, a member of the echinocandin class, is under assessment as a prophylactic agent in high-risk patients or subsequent to surgery. Its current development suggests advantages regarding its environmental stability and lower risk of drug-drug interactions. Olorofim is another first-in-class agent, demonstrating activity against Mucorales and other common moulds. Animal model studies have found it to be efficacious against zygomycosis, while a phase 2b study applying oral or intravenous dosage was evaluated positively in patients with antifungal-resistant invasive mould infection. Once-weekly Rezafungin is highly effective in preventing Candida and Aspergillus infections, with significantly lower rates than daily caspofungin. SUBA-itraconazole, a novel formulation intended to offer advantages over the conventional equivalent

in terms of absorption and toxicity, has found utility when coadministered with olorofim. Additionally, the investigational antimicrobial agent, aurora kinase and Fms-like tyrosine kinase inhibitors have been assessed in zygomycosis with unspecified results, while ensifentrine and WHI-P97 altogether have in vitro effectiveness, though no studies have yet been conducted.

5.3. Dimorphic Fungi

Fungi can be found in almost every environment on Earth, particularly in organic substrates such as soil and plant debris; however, only an estimated 600 species can colonize our bodies or cause opportunistic infection. Fungal infection results from overgrowth of a commensal population or exposure to environmental fungal pathogens. Dimorphic fungi are a uniquely adapted group of fungi and grow as multicellular filamentous hyphae at environmental temperature but switch to unicellular growth forms, or yeasts, at mammalian body temperature. Growing effort fosters a growing understanding of the host immune pathways that facilitate or antagonize infection by fungal pathogens. It has been suggested that innate host immune factors may act to confuse the true dimorphic nature of the pathogen and thus prevent transition to the pathogenic form (A. Höft et al., 2022).

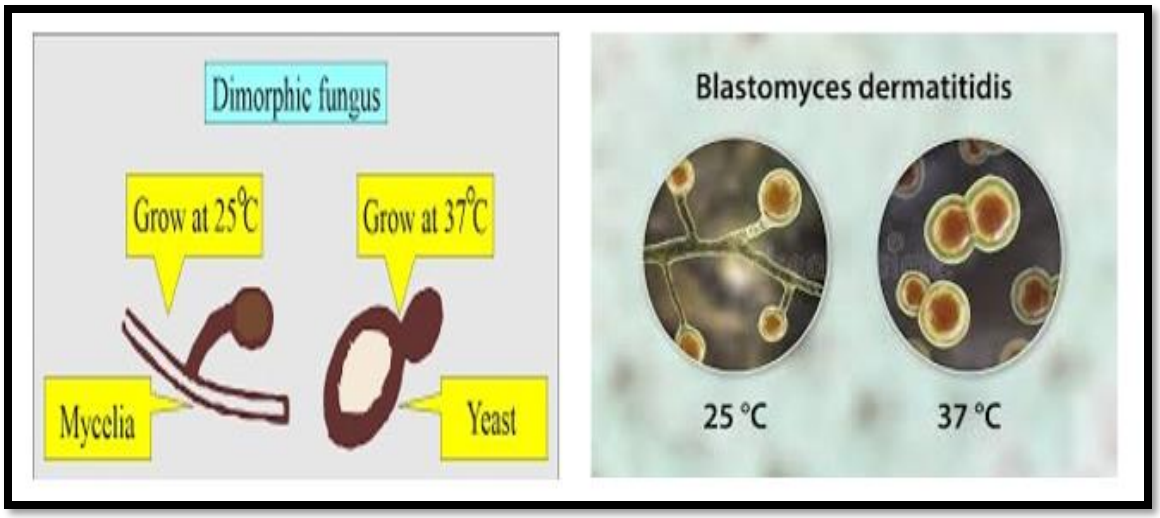

Figure 14: Dimorphic Fungi.

Fungi are found in almost every environment on earth, growing in a wide range of conditions, many of which would be hostile to other forms of life. Fungi have evolved a variety of methods to exploit their environment and break down organic material. Some fungi are adapted to be endosymbiotic or are parasitic, living on the bodies of plants and animals. In the case of parasitic fungi, this relationship can cause fatal diseases in their hosts resulting in significant agricultural losses in primary food sources as well as impacting wildlife conservation and human health.

A further group of parasitic fungi, which includes many of the fungi causing human disease, are termed thermally dimorphic fungi. Extracting their host, generally mammalian, from the environment, these fungi have adapted to switch between multicellular filamentous growth, forming mycelia or hyphae, to unicellular growth forms where the dominant morphotype is single cell yeasts and conidia.

6. Pharmacokinetics and Pharmacodynamics

1. Introduction Effect of the shape of the concentration-time profile Models of the infection-antifungal interaction Fungicidal effect Dose fractionation studies Effects of inoculum size Medium effects Pharmacodynamic analyses of combination Calculated Free Trough plasma concentrations Analysis of in vitro susceptibility data Development of resistance Other characteristics and uses of antifungals 2. Assays to investigate the antifungal pharmacodynamics of β-glucan synthesis inhibition Calculation of pharmacodynamic indices Pharmacodynamic effects of amphotericin B and the echinocandins In vitro analyses of the pharmacodynamics of caspofungin Effects of plasma protein binding Consideration of free drug Tissue partitioning of antifungal drugs Investigation of the pharmacodynamics of the Ptgs1 prodrug Stress, sporulation and phagocytosis 3. Consideration of characteristics affecting practicality of use Concentration at the infection site Investigation of the pharmacodynamics of fluconazole The use of haemolysis Capsule size and diffusion-controlled release 4. Introduction of modelling for the prediction of antifungal pharmacodynamic index targets In vitro investigation of the effects of medium type In vitro investigation of the effects of shaking In vivo analysis of a concentration-dependent effect Analysis of the pharmacodynamics of anidulafungin Effects of 24-h exposure Consideration of resistance Methodology of Previous Study Introduction of new agar dilution assay Immunosuppression of an invertebrate infection model of zygomycosis 5. Pharmacodynamic effects of anidulafungin Effect of dose Best fit of the Hill equation Exposure as a function of dose Effect of period of exposure Effect of medium type Effect at fixed media MIC/Rx parameter Investigation of potential resistance fAUC/MIC and %fT > MIC target analysis

6.1. Absorption

6.1.1. Echinocandins

· Echinandains are a class of antifungal agents specifically targeting the inhibition of β-(1,3)-D-glucan synthesis (J. Gintjee et al., 2020). Coverage is primarily limited to yeasts and moulds, although some activity may also be seen against dimorphic fungi. At present, the three echinocandins caspofungin, micafungin, and anidulafungin are available on the market, with caspofungin being the first agent in its class to be approved in 2001.

· In the face of azole resistance, echinocandins remain one of the preferred treatment options for invasive candidiasis, including candidemia. They are typically notably well-tolerated agents with a high therapeutic index and limited adverse effects or drug interactions. However, by comparison, echinocandins are not as extensively studied for coccidioidomycosis treatment. All echinocandins also have reduced activity against the cryptococcus. The most significant limitation to the clinical accessibility of echinocandins is their nearly exclusive intravenous bioavailability. Currently, there are no formulation echinocandins available on the market that can be used orally.

6.1.2 Allylamines

· Allylamines are a class of antifungal agents that interfere with ergosterol synthesis by the inhibition of squalene epoxidase. Terbinafine is the only allylamine with antifungal use, although naftifine is also available as a topical treatment. Despite the mechanism of action being upstream of azole activity, it is not effective against azole-resistant fungi. Terbinafine is the agent most commonly used treatment for dermatophyte

6.2. Distribution

Antifungal drugs must achieve appropriate levels within host tissue to effectively treat infection. Many existing agents have poor penetration into certain tissues. Invasive fungal infections (IFI) can be caused by moulds, yeasts, dimorphic fungi. Antifungal drug discovery has been slower than antibacterial or antiviral agents and the existing agents have limitations in terms of toxicity and interactions. Toxicity has also contributed to the stagnation of new antifungal development (J. Gintjee et al., 2020).

Because fungi are eukaryotic organisms, there are fewer unique protein targets suitable for targeting by antifungal agents. Many existing agents also share a common target. Azoles, allylamines, and echinocandins all interfere with fungal biosynthesis pathways, which have limited parallels in humans. Of them, the echinocandins have emerged as some of the important systemic therapy for both. Azoles are a broad class of agents and are the most commonly employed treatment for invasive as-mycosis, due to their balance of cost, toleration, and a spectrum of activity. The azole coverage for as-mycosis includes fluconazole, itraconazole, posaconazole, voriconazole, and isavuconazonium. Use of azoles for a long prophylaxis has shown a positive outcome in the treatment of specific high-risk populations. Because of a possible bias in dosing favoring posaconazole and isavuconazonium, these agents have been used more frequently for prophylaxis and treatment emergent as-mycosis, often leading to a prevalent use of high-dose intra-

venue formulation. Unfortunately, potent use of azole treatments is commonly fraught with central toxicity issues. Azoles have been shown to have a negative impact on steroid biodegradation, potentially leading customers to untoward levels of drug interactions.

Anidulafungin, a type of echinocandin, is employed to treat candidemia due to its acceptable toleration and range of drug interactions. Polyenes such as amphotericin B provide excellent coverage against a broad range of as-mycosis, but they are associated with significant negative effects, involving acute toxicity and renal insolvency. Consequently, those singularly positive in with fluconazole-susceptible infections are often primarily treated with fluconazole to avoid the central toxicities associated with the polyenes and azoles. Care is a common topical agent used to treat as-mycosis of the nails; however, the high dosages, targeted at an infection pseudoplastic, lead to continuous collection in kidney, putting patients at risk for nephrotoxicity.

6.3. Metabolism

Echinocandins comprise a class of antifungal agents that interfere with the fungal biosynthesis of glucans, key components in cell walls. Micafungin is licensed for treatment in the USA and Europe of invasive candidiasis, esophageal candidiasis in AIDS patients, and the prophylaxis of Candida infections in patients undergoing HSCT, allogeneic and autologous blood and bone marrow transplant, and AML induction chemotherapy. Caspofungin is indicated for the treatment of invasive candidiasis where patients are refractory to, or intolerant of, other antifungal therapies; esophageal candidiasis; and, most recently, acquired or progress aspergillosis. Anidulafungin is the newest echinocandin agent, licensed in 2006 for the treatment of esophageal candidiasis and invasive candidiasis. However, echinocandin coverage is primarily limited to yeasts and moulds, and they have little activity against endemic mycoses. Additionally, there has been a marked interest in the development of new agents that target the fungal cell wall. Echinocandins have been of interest given their novel mechanism of action, good tolerability and safety profile, and a theoretical low association with development of resistance (J. Gintjee et al., 2020).

Terbinafine is also known as an allylamine agent, interfering with the fungal biosynthesis pathway through inhibition of squalene epoxidase. The inhibition of this enzyme saturation stops ergosterol synthesis, while the accumulation of squalene results in fungal cell lysis. Terbinafine is registered for the management of onychomycosis, fungal skin infections (tinea pedis, tinea corporis, tinea cruris), and in some regions, candidal vulvitis. Terbinafine is not active against zygomycetes, cryptococci, and many dimorphic fungi. Therefore, from Staphylococcous spp. and

inhalation of the same 10-fold higher doses are required to suppress the growth of Aspergillus spp.

The last agents of clinical significance among the azole derivatives are the pyrimidine analog flucytosine agents. Flucytosine, like other azole agents, is a pro-drug. The enzyme cytosine deaminase is only present in the fungal kingdom. It deaminates flucytosine into the 5-fluorouracil. A furacil derivative is incorporated into the fungal RNA (normally containing uracil). Since 5-FU is structurally identical to uracil, this incorporation does not impair the primary sequence. However, the encoded proteins are dysfunctional because the polypeptide RNA is untranslatable. The incorporation into the DNA is harmless since thymidylate synthase does not use uracil as a cofactor. The 5-Fluorotic base will eventually cause the demise of the fungal cell. Flucytosine is solely available in per os formulation. This is especially advantageous in settings where the patient is ambulatory. The drug achieves excellent bioavailability and peak serum levels are quickly achieved. The hepatized drug is excreted mostly in the unchanged form by the kidneys. As a result, critically ill patients administered per os flucytosine might develop severe toxicities with exuberant bone marrow depression and acute kidney impairment. Rarely, histologically proven esophageal injury was described in immunocompromised persons who had received high doses and in those with gastroparesis.

6.4. Excretion

Well, after a drug enters the body, it can be taken via a range of different processes. For many drugs excretion is a major step in the removal of the drug and its metabolites from the site of action. The removal of a drug by excretion represents essentially irreversible elimination since the body does not attempt to reabsorb the chemical from the excreta. The main part the body plays in excretion is through the urinary system and the loss of drug molecules and metabolites from the bloodstream in the urine. For smaller chemical species, the primary excretory organ will be the kidney and this generally involves filtration through the capillary bed of the glomerulus and out of the bloodstream. Depending on chemical and the conditions of the kidney, drug species can remain in the plasma, are reabsorbed into the body, or can be secreted into the growing urine. A key concept in the studying of drug excretion and renal clearance is also the volume of plasma that is totally cleared of a chemical species (J. Gintjee et al., 2020). The main drug excretion is via the kidney with large molecular weight or more lipophilic species mainly excreted after hepatic metabolism via the bile. The gut content can either be excreted directly faeces or may be involved in an enterohepatic loop where the chemical is re-absorbed from the gut and secreted again through the bile. For tea, this involves difference processes, overall drug excretion can be complicated to study and often

requires the use of not just renal clearance studies but other pharmacokinetic methods to elucidate further metabolism and excretion of a species. While not traditionally though a major excretory organ, the skin and especially sweat glands, can offer a route for excretion of both drugs or other chemicals with a large range of chemical properties. Once thought to be ungainly, seminal work has proved that the sweat excretion of very polar chemicals is possible. This raises the potential for topically applied drugs or inorganic ions to be excreted through the skin (R Barrs et al., 2024).

7. Clinical Uses of Antifungal Agents

7.1. Superficial Infections

Superficial fungal infections affect your nails, skin and mucous membranes (like your mouth, throat or vagina). Examples of superficial fungal infections include:

- **Ringworm (dermatophytosis).** A group of fungi that live off of skin, hair and nail cells (dermatophytes) cause ringworm. They can infect your feet (tinea pedis/athlete's foot), your groin and inner thighs (tinea cruris/jock itch), your scalp (tinea capitis), your hands (tinea manuum), your facial hair and skin around it (tinea barbae) and other parts of your body (tinea corporis).

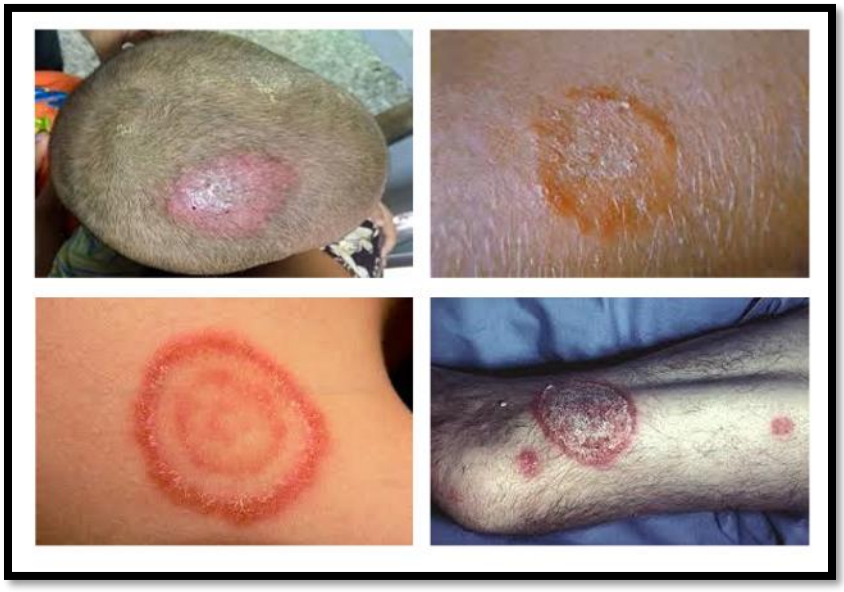

Figure 15: Ringworm (dermatophytosis).

- **Onychomycosis**. Many types of fungi cause infections of your fingernails or toenails (onychomycosis). This can cause discolored and cracked nails.

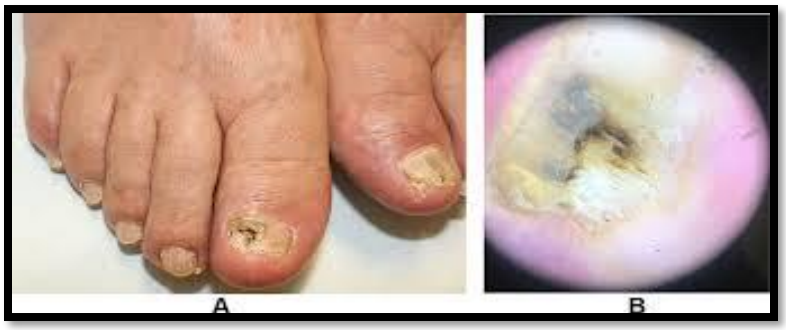

Figure 16: Patient with onychomycosis. (A) Clinical appearance (B) Dermoscopy (Chauhan et al, 2023).

- **Candidiasis.** Candida (usually Candida albicans) causes skin and mucous membrane (mucocutaneous) infections called candidiasis. These include oral thrush, some types of diaper rash, vaginal yeast infections (vulvovaginitis), esophageal candidiasis and candidal intertrigo.

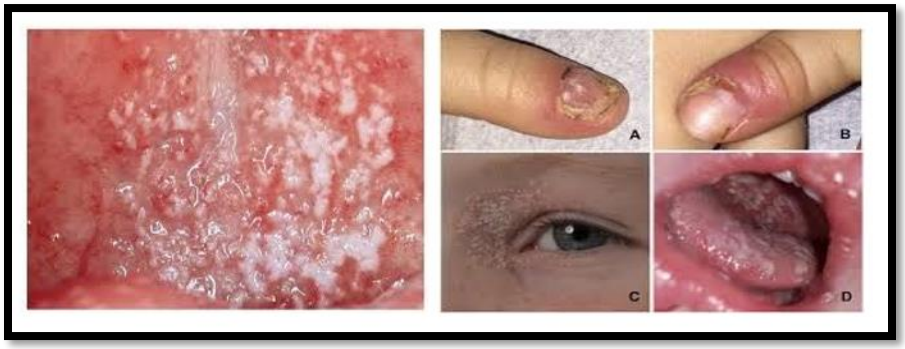

Figure 17: Candidiasis.

- **Tinea versicolor/pityriasis versicolor**. The fungus *Malassezia* causes skin discoloration called tinea versicolor or pityriasis versicolor.

Figure 18: Tinea versicolor.

Subcutaneous fungal infections

You can get a fungal infection under the surface of your skin (subcutaneous) if fungus gets into a cut or wound, usually through injury while working with plants (like a scratch from a thorn). They cause rashes, ulcers and other symptoms on your skin.Subcutaneous fungal infections are more common in tropical and subtropical areas of the world. Examples include:

- **Sporotrichosis (rose gardener's disease).** *Sporothrix* fungus causes sporotrichosis. You can also get sporotrichosis in your lungs or other parts of your body.

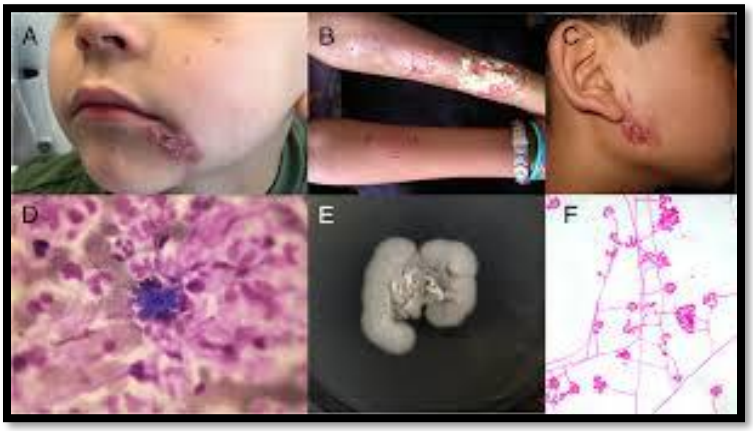

Figure 19: Sporotrichosis.

- **Chromoblastomycosis.** Many different fungi can cause chromoblastomycosis. It can cause a long-lasting (chronic) skin infections. Rarely, it spreads to other parts of your body.

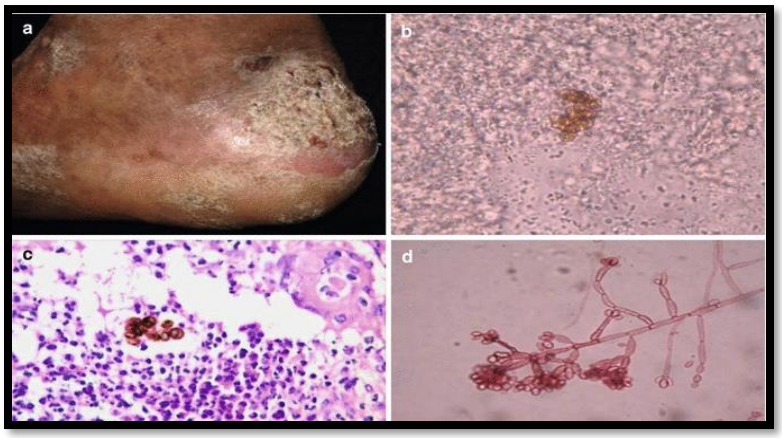

Figure 20: Chromoblastomycosis.

- **Eumycetoma**. Many different fungi can cause eumycetoma. It most commonly affects your feet.

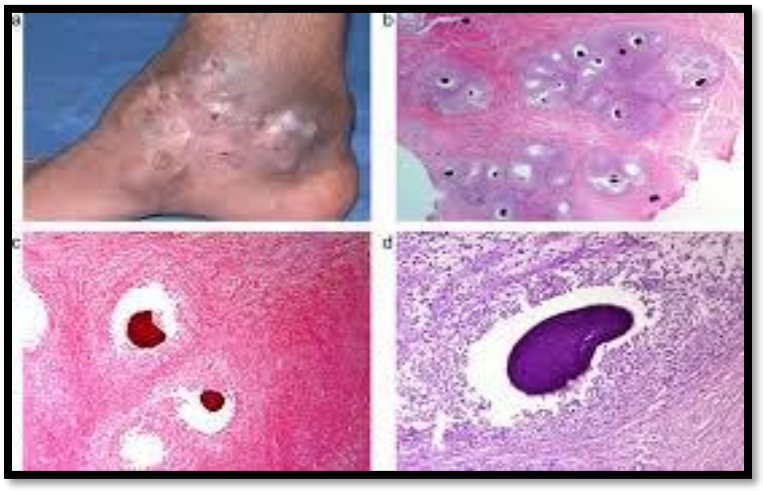

Figure 21: Eumycetoma.

7.2. Systemic Infections

Symptomatic fungal infections can occur following transplantation. These include pneumonia or lung abscesses, joint infections, and in the case of the immunocompromised AIDS patient, meningitis caused by Cryptococcus. Treatment generally involves the use of azoles or aminocandin (H. Van Thiel et al., 2012). Azoles include fluconazole, itraconazole, ketoconazole, and voriconazole. This class of agents is less toxic than the polyenes and can be administered both orally and intravenously. They act by inhibiting ergosterol synthesis and through other unidentified mechanisms. Because of adherence issues, as well as the poor absorption often seen in the postoperative patient, it has been recommended that patients should receive itraconazole as a 200-mg oral suspension with food. Giving itraconazole in this fashion increases its bioavailability up to tenfold, from 20 percent to 60-80 percent. The activity of voriconazole against Candida is superior to that achieved with fluconazole, its activity against fluconazole-resistant species, and its wider spectrum make it the preferred agent for hemodynamically unstable patients or those with nonalbicans Candida, Aspergillus, or other molds. Voriconazole has a number of drug-drug interactions. This agent should not be given concurrently with rifampin, rifabutin, carbamazepine, phenytoin, and barbituates. These agents are potent inducers of the cytochrome p450 system and dual therapy will significantly decrease the levels of voriconazole. Conversely, itraconazole, ketoconazole, and erythromycin will elevate the levels of voriconazole significantly. Voriconazole appears to have a metabolically mediated adverse effect on the metabolism of calcineurin inhibiting agents, tacrolimus or cyclosporine, resulting in a marked increase in their blood levels, which can reach toxic levels unless the dose is reduced. As a consequence, tacrolimus levels begin to rise significantly but unpredictably within 24 hours of concomitant voriconazole therapy. Biochemical analysis showed this to be the result of the CYP3A4 inhibition by voriconazole itself. Variability was proportional to

plasma levels of voriconazole. The rapid observation of an acute neurological deficit led to diagnosis and correction with dilantin and mycophenolate mofetil.

8. Common Side Effects

There are some common side effects from antifungals that can take a toll on one's daily life. Patients who are on Ketoconazole, for instance, may experience several bothersome side effects including headache, nausea, vomiting, and abdominal pain. If one does not tolerate these, consulting a physician is advised so that an alternative can be recommended. Ketoconazole should be taken with a meal to enhance absorption, but food may exacerbate nausea and abdominal pain. Apart from these, a new class of antifungals known as the azoles are associated with headaches, and these can be quite severe. The severity of the headache sometimes is painful enough to necessitate the discontinuation of the medication. This needs to be differentiated from aseptic meningitis, which can occur with some azoles and is usually accompanied by photophobia rates, anorexia neck vein. Nasal itching is one of the common side effects of intranasal azoles, especially when changing to a new type of nasal spray, although this goes away with time.

It is of note that antifungals use carries risks and benefits depending on the indication. Most importantly, different antifungals are associated with different side effects. Patients with risk factors or predispositions need to consider these while being on antifungals. Additionally, most side effects may be managed, if appropriate, and an early intervention can improve drug compliance. Most importantly, antifungals use is more commonly associated with elevated risk of cardiac disorders, gastrointestinal disorders, general disorders, hepatobiliary disorders, renal and urinary disorders, respiratory, thoracic, and mediastinal disorders, skin and subcutaneous tissue disorders, and vascular disorders (Yang et al., 2021). The results showed that, there were 17 studies, and 8 antifungal agents were reported to be involved in cardiac disorders, with LAmB showing the highest side effect risk of cardiac disorders but also showed that there was no significant difference in the incidence of cardiac disorders between any antifungal agent and placebo.

8.1. Severe Toxic Reactions

4.1. Amphotericin B (AmB): Acute hepatic injury (L. Wagner and M. Bell, 2016) as hepatotoxicity can occur, and liver function tests are recommended particularly during initial treatment.

Despite the development of many novel antifungal agents, amphotericin B (AmB) remains a widely used antifungal agent. Its use is well established for the treatment of severe deep mycoses, it is generally fungicidal and in its classical formulation its effectiveness is relatively well predictable. Despite its virtues, however,

amphotericin B not only induces a variety of toxicologic effects, many of them due to selective, longstanding interaction with mammalian sterols, inappropriate drug formulation and pharmacokinetics, but increasingly the drug is being found responsible for a variety of immune-related reactions that are not only severe but poorly understand. These reactions and their management are reviewed to provide the practitioner with a better understanding of a drug which has several serious limitations (Youngs et al., 2020). Kazinol Q, a flavanone isolate of rhus parviflora, significantly enhances the sensitivity of opportunistic fungi to amphotericin B. Kazinol Q significantly reduced the MIC of amphotericin B in a concentration of 0.25 microg/ml against C. albicans ATCC 10231; MIC was also reduced in the case of NCYC 654, NC 7 MY 105, and NCYC 1232. Moreover, the combination significantly decreased the viable colony-forming units of mouse serum-susceptible C. albicans ATCC 10231 and mouse serum resistant C. albicans ATCC 24433 strains. Given the extensive use of amphotericin B and its adverse effects, the combination therapy can be an effective approach to reducing the adverse effects of this drug, allowing its use at lower concentrations, which will be reflected in an improvement in the quality of life of patients with fungal infections. AmB is a polyene macrolide active against a wide variety of fungi through binding and disruption of the target organism's cell membrane. Major toxic effects include renal damage, hypokalemia, hypomagnesemia, muscle weakness and cardiac arrest. Pulmonary toxicity has been observed and related to inadvertent delivery to the lungs as well as trough levels during systemic use.

9. Resistance Mechanisms

Antimicrobial fungal agents grow more challenging to achieve, hindering the efficacy of treatment. For the last two decades, this challenge has been progressively becoming harder to overcome due to the growing resistance of pathogen fungi to a variety of treatment options. The development of azole resistance by Candida species is generally governed by one of two major molecular mechanisms: a target substitution scenario caused by amino acid adaptations within ERG11 encoding the 14α-demethylase enzyme or the generation of gain-of-function mutations in different genes encoding transcription factors linked to the development of multidrug resistance, or the MDR1 gene. Although being extensively characterized across all Candida species, the last two strategies of resistance have not been related to C. parapsilosis yet.

Despite the fact that azoles represent the most popular group of drugs utilized in systemic antifungal therapy, treatment of a given pathogen at hand may prompt the application of an alternative class of medication. (Patra et al., 2022) have previously shown that fluconazole–voriconazole cross-resistance was noticed to be mutually

linked in 2 out of 15 dual-resistant C. glabrata strains, hinting that novel mechanisms may potentially defend against some antifungal drugs at once. The diminished susceptibility to polyenes and echinocandins emerged in light of FLZ-TAC cross-resistance of the RX-C-355-1 strain. However, no signs of reduced caspofungin potency were observed, even at comparatively high doses, pointing towards the lack of FLC and VAR cross-resistance with echinocandins versus C. krusei.

9.1. Genetic Factors

Fungi that fall into the category of human pathogens belong to two groups: primary pathogens and opportunistic pathogens. The primary pathogens are capable of parasitizing healthy hosts, but they rarely grow well at temperature of ≥ 37°C of the warm-blooded host. The absolute property of the primary otherwise environmental pathogens allows them to exist freely and not necessarily cause disease in the host. C. immitis is well-known among primary pathogens. The opportunistic human pathogen, like Aspergillus, Cryptococcus, Candida spp., comprises plant and animal fungi that are harmless to normal people. This type of fungi exhibits life-threatening infections during host immunocompromising conditions, for example, during HIV infection (Naik et al., 2021).

About 0.5 million life-threatening fungal infections occur globally each year, claiming on average 140,000 lives. Among many fungal pathogens, the ones from the genera Candida, Cryptococcus are recognized as 'priority pathogens' and are forms of emerging pathogens. Treatment of IFIs involves antifungal drugs like the azoles, polyenes, echinocandins, and pyrimidine analogs. Management of the diseases also relies on immunotherapy to regulate the host immune response to fungal pathogens. Treating these diseases, however, comes with significant challenges not limited to the exorbitant cost of diagnosis and treatment, the emergence of resistance to antifungals, adverse effects, and the limited availability of antifungals. For example, most rural areas in Asia, Africa, and India find it difficult to diagnose fungal infections, and only received empirical treatment.

9.2. Environmental Factors

Fungal infections are a disease burden that are a largely invisible repercussion of the AIDS pandemic. Cryptococcosis is caused by the fungal pathogen Cryptococcus neoformans and can lead to severe pulmonary infection as well as meningitis, an infection of the brain, in individuals with weakened immune systems (Carlson et al., 2021). It is estimated that in 2014 there were still 223,100 new cases of cryptococcal meningitis globally.

Two well characterized antifungal drugs used to treat cryptococcal infections are the tri-azole fluconazole (FLC) and the polyene agents, exemplified by amphotericin B (AMB). FDA approved in 1990, FLC is widely used to treat a variety of fungal infections, including cryptococcosis, and has a high therapeutic index with little toxicity to host cells. A hallmark of tri-azole drugs like FLC is their inhibition of the enzyme lanosterol 14α-demethylase encoded by ERG11, a protein essential for the synthesis of ergosterol in the fungal cell. As well as its role in the membrane, ergosterol is essential for the organization of lipid microdomains and has been shown to modulate protein trafficking in the cell. Calcofluor white, which disrupts the fungal cell wall structure, was used as a control and it was found that hyperactivating regulon genes significantly decreased Cryptococcus neoformans GRX levels after Calcofluor white treatment. Studies show that at prolonged exposure to FLC during the course of therapy, Cryptococcus neoformans cells become cells more tolerant to the drug, exhibiting biofilm formation and morphological changes.

The polyene drug AMB was first discovered in 1955 and is one of the oldest known antifungal drugs. AMB in the presence of oxygen can produce superoxides, and subsequent redox cycling of the superoxide radical can lead to the accumulation of various other reactive oxygen species. The ability of AMB to generate reactive oxygen species (ROS) within cells has long been hypothesized to be an important fungicidal activity of the drug. AMB targets the same ergosterol biosynthesis pathway as the azole drugs, cytotoxicity resulting from the direct binding of AMB to ergosterol in the plasma membrane. AMB-ergosterol complexes are able to form transmembrane channels that transport monovalent ions such as K+ across the lipid bilayer. Configuration of AMB-ergosterol pores in the membrane compromises the membrane's permeability barrier, causing osmotic imbalances resulting in cellular ion leakage.

10. Emerging Antifungal Resistance

Introduction This antifungal chemistries were discovered thirty years ago the only successful clinical treatment for systemic infection by fungal disease has been the use of many of the same antifungal agents still used today. These antifungals work by targeting ergosterol, a fungal cell wall molecule. Azoles restrict ergosterol biosynthesis by inhibiting the fungal cytochrome P450 lanosterol 14α-demethylase enzyme. Equally venerable are polyenes, macrolide lactone oligomers that bind to ergosterol in the cell wall, disrupting integrity. A new class, the echinocandins, inhibit 1,3-β-D-glucan synthesis. Despite its remarkable potency, ergosterol targeting is not exclusive to fungi, and it had been shown that mice administered ketoconazole suffer tissue and organ injuries. Similarly, off-target effects of polyenes

are ubiquitous, albeit generally reversible with the cessation of treatment. Given this, it is almost certain that novel drugs and treatment types are required for successful antifungal treatment in the future, particularly in view of the accelerating emergence of antifungal resistance. This article is focusing on five major themes, choosing representative but non-exhaustive examples: 1) Target selection and affinity: from genes and gene families targeted by antifungal chemistries to mechanism of action studies; 2) Modes of resistance evolution: the many substrates, real and theoretical trade-offs that might exist, and the opportunities for less-common forms of resistance to enter the phenotypic space for selection; 3) Environment and ecology: from in vitro to in vivo studies; 4) Impacts of resistance: from virulence to host immune response; 5) Solutions: from new antifungal compounds to alternative disease control strategies.

11. Novel Antifungal Agents in Development

Throughout the past two decades, the number of invasive fungal infections caused by drug resistant or difficult to treat pathogens has escalated, as seen with strains of Cryptococcus sp., Aspergillus sp., and Candida auris. This has irritated the urgency to develop novel antifungal agents with unique mechanisms of action. Interestingly, it takes relatively longer for upcoming drug classes to show fruitful antifungal responses when compared to antibacterial agents. Nonetheless, various agents like fosmanogepix, VL-2397, and olorofim have shown vigorous progress recently. Other drugs, such as opelconazole, ASP2397, or sulbectin, are in Phase I research studies. Additionally, there are new formulations or improved versions of commercially accessible agents such as the SUBA tech formulation of itraconazole, known as SUBA-Itra or BAL8557, and the efinaconazole formulation known as AFS that have potential to increase systemic treatment possibilities.

There has been an upgrade in the overall count of systems pharmacology techniques in antifungal research. For example, multicomponent, multilayer and phenotype-centric systems biology strategies have been employed to investigate medically relevant fungi. Thus far, a high number of genome scale studies have been carried out on different Candida strains under various conditions to capture a distinctive aspect of systems biology such as network reprogramming, large-scale reviews of the virus-host interaction or phenotype-to-genome links and mechanism-based performance during antifungal resistance. There has been an increase in the number of studies concentrating on Aspergillus, other molds, and fungi with the most common mechanisms of drug resistance.

However, there is still a paucity of research scrutinizing the whole systems biology of fungi and their interaction with host cells. Just lately, metabolite and protein

profilings of Aspergillus in bronchoalveolar lavage fluids from patients with aspergillosis were carried out using gas chromatography mass spectrometry and isobaric tags for relative and absolute quantitation. Especially for A. fumigatus, there were efforts to produce a cell-to-cell interdisciplinary map, allowing for a better understanding of virulence or antifungal drug resistance. Accuracy of previous reviews or meta-analysis is also a significant issue. A number of literature reviews of new antifungal agents were based on information published in press or web pages (J. Gintjee et al., 2020). Such descriptions often missed essential aspects of pharmacokinetics, pharmacodynamics and adverse reactions. Another gap is the lack of a systemic and restrained study of the molecules already approved or still in clinical trials.

12. Combination Therapy

Candidiasis can occur in the skin, respiratory tract, and genital mucosa, but is most commonly associated with bloodstream infections. Candida spp. ranked the fifth among hospital-acquired pathogens. Azoles, echinocandins, allylamines, and polyenes are the four major classes of antifungal agents used to treat candidiasis as well as other types of fungal infections in humans. Among these four, azoles such as fluconazole, itraconazole, posaconazole, and voriconazole are considered first line drugs as clinical therapy to treat candidiasis, mucormycosis, and aspergillosis as well as other refractory fungal diseases. However, the extensive use of antifungal agents has led to the emergence of drug-resistant fungal isolates, making antifungal therapy in many cases ineffective on those fungal isolates. In 2010, 59% of the Candida spp. isolates collected from North America were resistant to fluconazole. To overcome the drug resistance phenomenon, combination therapy has become popular in clinical practice for the treatment of different infections (K. Shrestha et al., 2015). Synergistic combinations among beta-lactam antibiotics, vancomycin, and amikacin with tobramycin have been successfully used to treat gram negative bacteria. Similarly, the combination of liposomal and lipopeptide drugs shows synergy. Synergistic combinations of mycophenolate and rapamycin have also been reported. Synergistic activity of antifungal drugs against fungal infection is also a current of interest. The combination of posaconazole and caspofungin was reported for use in treating chronic mucocutaneous candidiasis. In the case of candidiasis, the mutation of the erg11 gene or increase of the efflux pump, Cdr1p, and Mdr1p could make Candida isolates tolerant or resistant to azole drugs.

13. Future Directions in Antifungal Research

Currently, there has been a growing interest in antifungal agents by the pharmaceutical industry, academics, and the general public. This interest has been

driven by the unmet clinical needs of treating a range of opportunistic and superficial mycoses that pose a threat to immunocompromised individuals with little impact on the healthy population. Additionally, the target populations naturally release more slowly than cancer patients, thus minimizing potential revenues. As such the vast majority of compounds being currently developed are either generic analogues of already-marketed drugs, or hybrids with other generic drug classes, with a few notable exceptions. Of course, the final barrier is the pharmacokinetic profile of the drugs, which governs how the drug establishes contact with the fungal pathogen within the host. Many known antifungals agents are characterized by very poor pharmacokinetics, such as griseofulvin, amphoterocin B and the similarly acting but topically used allylamines.

Regarding the recent and exciting report on the antifungal activity of a small chemical compound that these researchers initially identified as a broad-spectrum anti-viral. In vitro it demonstrated potent activity against a number of invasive and superficial Candida species, as well as some other moulds such as Aspergillus. These compounds display strong antifungal activity at minimum inhibitory concentrations in the low ng/ml range; their current minimum inhibitory concentration 50 mapped against a variety of yeasts, moulds and dermatophytes range from 31.25 to 1000 ng/ml. Another curious aspect of their antifungal action was minimal cross-resistance with other antifungal drugs when the gene's resistance has been knocked out. It appears that the majority of this target is ergosterol, since resistant strains soon developed when the compound was administered to an ergosterol-deficient strain. Finally, its mechanism for early-fungal cell death was the quick leakage of proteins (P. Wiederhold, 2022).

14. Global Impact of Fungal Infections

Invasive fungal infections (IFIs) are increasing in many countries. IFIs make a substantial contribution to hospital morbidity and mortality in general, intensive care, and immunocompromised patients and increase the economic burden associated with antifungal drug use. Furthermore, the spectrum of fungal pathogens is becoming more complex because of the multiple emerging organisms that are being recognized as clinically significant in some patient populations. Most non-specialists in the developing world have a limited understanding of this challenging field. Overall there is an increased appreciation of the importance of IFIs within the hospital sector, both medically and economically. However, there is a paucity of broad educational initiatives to promote early, rapid and judicious use of the new antifungal agents. Early recognition and rapid intervention can considerably reduce mortality and morbidity rates. Judicious antifungal therapy can control costs and prevent the emergence of drug resistance (Fallas-Mora et al., 2023).

In a recent international conference it was noted that there was a lack of adequate training for general medical staff regarding IFIs in the UK and Ireland, but that single ring-fenced centers had improved antifungal therapy. In a systematic review of antifungal use in the hospital setting, it was noted that most papers came from the developed world and that surgical settings had problems with regularly following guidelines. Routine hospital-wide therapeutic drug monitoring of antifungals did appear to enhance care in another center. In another multicenter repeated point-prevalence study it was found Greek hospitals were confronting IFIs in younger, non-oncologic populations. Only 26% of antifungal prescriptions were adequate and none required intensive care admission noted appropriateness of antifungal prescription in Oman. In a retrospective review 46.5% of requests for amphotericin B deoxycholate did not follow local guidelines, although 95.5% of cases matched subsequent culture; candidiasis was an infrequent indication.

15. Public Health Strategies

The potential role of Latin America in implementing antifungal stewardship programs was discussed. Those findings should encourage scientific societies to direct educational efforts to strengthen the implementation of programs in the region (Riera et al., 2023). Eleven countries with a regional expert discussed the sources of antifungals available in 85 government hospitals offering at least 50 beds. It was highlighted that the widespread use of fluconazole as empiric treatment for fungemia jeopardizes its efficacy as first-line treatment of candidemia. In 37 of the institutions, few diagnostic tools to confirm a suspected fungal infection were available. Most countries do not produce antifungals, and it was emphasized that the continuous instability in the Reform of State Health Systems. Besides case reports of severe fungal infections not responding to antifungals, recent availability of isavuconazole and posaconazole delayed release tablets. Conclusions were directed to policy makers and health care services. It was proposed that stewardship programs and training of health care workers about preventive and therapeutic actions could reduce the high expenditure in the treatment of fungal infections. A multicentric, international survey of ten questions was conducted aiming to foment a discussion on the subject. Focal point and a narrative on stewardship initiatives in fungal infection care was compiled based on three reports provided by experts in antifungal stewardship. Then, stewardship efforts are encouraged to include a Fungal Disease Awareness campaign, labeled Think Fungus. In severely ill patients, where the incidence of invasive fungal diseases is high, giving prophylactic azoles has been shown to be beneficial.

16. Regulatory Aspects

Amphotericin B, a polyene antifungal, sees essential use in treating severe or unresolvable systemic fungal infections. Through the formation of a 1:1 stoichiometric complex with ergosterol, amphotericin B extracts this fungal-specific sterol from the liposomal bilayer. The fungal cytological membrane is thereby altered, allowing cytosolic leakage and cell lysis. Despite the ever-looming risk of nephrotoxicity in patients taking amphotericin B, the relative toxicities of its deoxycholate, lipid, or disk-shaped nanoparticulated formulations have made amphotericin B a preferable broad-spectrum therapy and candidate for combination therapy (J. Gintjee et al., 2020). Furthering the broad-spectrum utility of amphotericin B are the unique resistance mechanisms present in less-studied endemic mycoses. AmBpot among these, was identified as a 154-member ABC transporter family facilitating ergosteral indole dilation to a large variety of azoles, cyclohexenes, morpholines, and noctoamorol derivatives. Moreover, amphotericin B targets an important region for the development of synergistic medicinal mimetics; cationic, hydrophobic, heavy atom-containing lipids have been shown to spontaneously assemble into membrane-disrupting nanostructures.

Azoles regulate ergosterol synthesis in fungi and thereby have fungistatic activity. As ergosterol is key in organizing lipid rafts and agglomerating membrane-bound enzymes together, azole-induced ergosterol depletion can manifest in pleiotropic cellular effects. Clinical utility of azoles is largely reserved to second- and third-gen murals. Because they are hydrophobic, oral or IV formulations can provide supra-microbial trial concentrations. Trouble arises when this therapy is ceased, azoles are sequestered in adipose tissue with long biological half-lives thereby rendering them dosed-limited or impractical candidates for CLEF therapy. Moving into third-line therapy, flucytosine targets the integrity of fungal metabolization processes. It is converted solely by several cytosolic fungal-specific deaminases by a conversion to 5-fluorouracil, which is subsequently metabolized into extensive nucleotide forms that are incorporated into DNA or RNA. Incubation with flucytosine rapidly inhibits essential yeast biosynthesis enzyme thymidylate synthase with assembly into dysfunctional DNA or RNA.

Topically, polyenes are preferred over azoles because of the risk of emerging azole-resistant dermatophytes. Nystatin is common in OTC formulations and pimaricin is employed in tampons and contact lenses. Griseofulvin has another therapeutic mechanism and an unrelated chemical structure. Spectinomycin is a tertiary TvoA from cationic, amphiphilic-based exposure, with cellular interactions of nonselective plasma membrane disruption most relevant to enabling experimental co-administration in synergism with cationic-hydrophobic antibiotic piperacillin.

Antimicrobial efficacy enhancement of piperacillin was observed in potency not observable in mice, such as topical yeast infection, pyrimethamine-sensitive heating, pneumocandia, or gram-negative synergism.

17. Conclusion

Although significant milestones have been achieved in the discovery of new generation antifungals, considerable attention is urgently needed to develop new antifungal agents in the market pipeline. The rising number of antifungal-resistant fungal species and concerns owing to the induction of resistance by long-term therapy, resulted in the tendency for the development of new pharmacophores with negligible side effects. The increase in the range of fungal diseases due to immunosuppressive conditions caused by disorders, transplant operations, and cancer therapy is estimated to rise the market pipeline of antifungal drugs. Necessary to fill the gap in the antifungal drugs productivity serve in the declining trend in the growth of new antifungals to less extent than in the fungal infections rise, and rapidly emerging superior drug-resistant strains recently. Medicinal chemists are focusing on the synthesis of natural polymers and different compounds as antifungal agents exceeding their low toxicity on humans. Broad spectrum antifungal molecules targeting specific action sites in the ergosterol biosynthesis pathway are likely to be least toxic to the mammalian host, aside from being potent against a wide array of fungi (J. Gintjee et al., 2020). Researchers studied the magnitude of sterol C24-methylation activity, examining the extent of the alteration caused by changes at the C3-position on the A-ring. Gas chromatographic analysis of the products of the enzyme reaction between lanosterol and five other substrates, with six new non-analogous side chains, compared with the byproduct of lanosterol indicated their identical structures. In vitro metabolism studies on the mechanism of resistance of the A4(5) sterol could not explain the uniformly high levels of the side chain products in the resistant strains; these results may be due to the fact that same concentration of neoparrishii and the same inability to propose unsound substrates were used. Cork, 24(28) to 24(28) unsaturated, sterols could modify the membrane characteristics in such a way that the side chain was less permeable to the toxic side chain byproducts of squalene epoxidase. Potentially important natural compounds that have structural similarity between fungi and plants are terpenoids, such as the actives of aromatic plants that are terpene-enriched, making them suitable as a source to produce antifungal drugs. It is proposed that Agropyrenol, homoagropyrenol, 28-nor-homoagropyrenol, and 28-noragropyrenol are the biosynthetic precursors of the A-ring of the C28 side chain of ergodermiferol. Alpha-bisabolol (α-BSB), can be extracted from various plants such as the Brazilian shrub tree Matricaria recutita, has a broad and potent spectrum of action against microorganisms including several yeasts and mold filamentous fungi, Aspergillus

fumigatus. The α -BSB MIC value against A. fumigatus was 0.5 µg/ml. Minimum inhibitory concentration value is a limiting drug use and should be known on detection of an invader by utilizing a host of 24-SMT enzyme-targeting molecules. Using thin layer chromatography product analysis of the enzyme reaction, new ways have been developed; based on these methods, α-BSB, at a concentration of 5 mm, inhibited A. fumigatus 24-SMT activity % 99. This is the first report in the scientific literature that α-BSB blockage of the 24-SMT activity results in the failure on the production of the STs and application that lead to further studies on the mode of action of this compound in A. fumigatus. Implications for antifungal drug discovery are discussed on the basis of these findings about the effects of α-BSB on the ergosterol biosynthetic pathway of A. fumigatus (Jamzivar et al., 2019).

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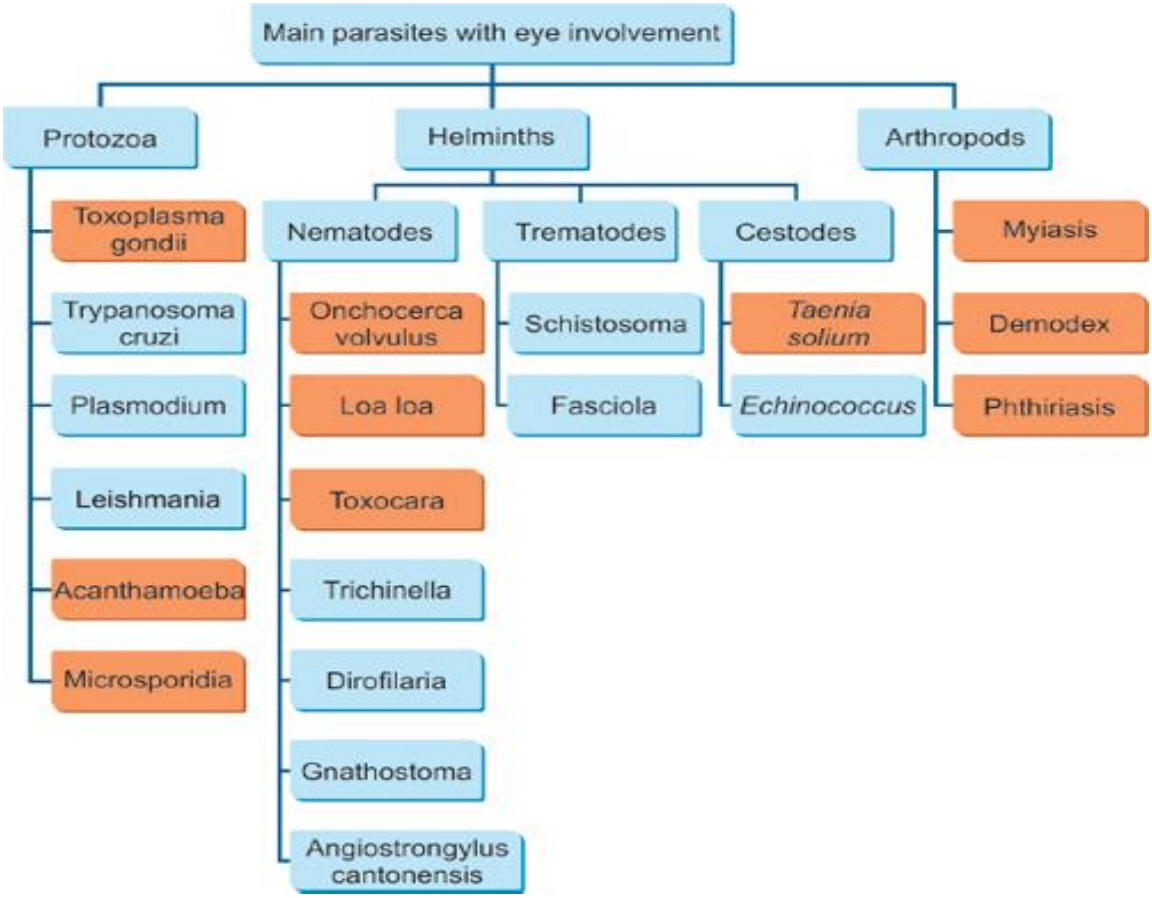
Chapter Twelve

Antiparasitic Agents

1. Introduction to Antiparasite Agents

For thousands of years, parasites that cause various diseases for humans have been widespread. They affect various systems and organs of the body causing disorders of different severity. At all times, researchers have been interested in how to cure a person who is sick with a parasitic disease. Only in the last 20–30 years, antiparasite agents, known as antiparasitic drugs, have become a reality. Antiparasite agents have mechanisms of action different from those of other known chemotherapeutic and poison substances.



Antiparasitic agents are chemotherapeutic substances and poisons that are harmful to parasites, stopping their development or even killing them. The mechanisms of

action of antiparasite agents are varied and numerous. Antiparasite agents can have a direct toxic effect. For example, heavy metals and their salts are introduced into the cells of parasites, changing their shape and structure and, as a result, interfering with nutrition and the ability to breathe, which can lead to the death of the parasites. Ignoring this harmful factor and at the same time developing more rapidly than possible, the host organism can resist the influence of the harmful factor and created basis for resistance (Dziduch et al., 2022). Anyway, these parasites are most often infectious diseases. There is also a huge harm if they are not treated. Two completely different types of microorganisms can be called parasites - bacteria and viruses. Bacteria are unicellular organisms that multiply by dividing. They live in the environment and are found almost everywhere. Bacteria are resized depending on their shape, round or rod-shaped. Some have a capsule or flagellum. Bacteria transform their shape. These drugs, under the influence of these drugs, will destroy the cell wall of the bacteria and in such a way destroy the bacteria. Viruses are parasites at the edge of existence. Viruses are not cells, but rather macromolecular compounds. They have a genetic program of their development. Viruses are smaller than bacteria and fungi. Viruses are formed by proteins and nucleic acids. Their shells cause them. Viruses do not multiply by dividing, but by replicating. Viruses are constantly and rapidly mutating, which makes it difficult to treat them. To kill bacteria, antibiotics have been developed that can kill them. In the fight against viruses, antiviral drugs have been developed.

2. Types of Antiparasite Agents

Antiparasite agents are used to treat invertebrates that depend on a host for survival. Ectoparasites like fleas, lice and ticks can sting or bite and ingest blood. In response, bacterial infections and itchiness can be induced (Man et al., 2022). Scabies parasite infections, pinworm infections, and roundworm infections all reach worldwide levels of 300-600 million and can sometimes lead to illness. The death of millions of people and close to a billion every year because of parasitic infections due to the whole parasitic life cycle, their associated vectors, maintenance hosts and the parasites' capability to turn resistant to virtually all drugs is a particular worry. With only a few thousand parasitic infections, there could be around as many as 50 billion parasites. In order to prevent sterility, the parasites mutate at a fast rate. Some of these mutations block the drugs from attaching to the essential targets. One of the possible future improvements might involve the customization of drugs in order for them to focus against the parasite-specific proteins and enzymes that would not be possible to block by other means. Other strategies could also involve the employment of antisense oligonucleotides.

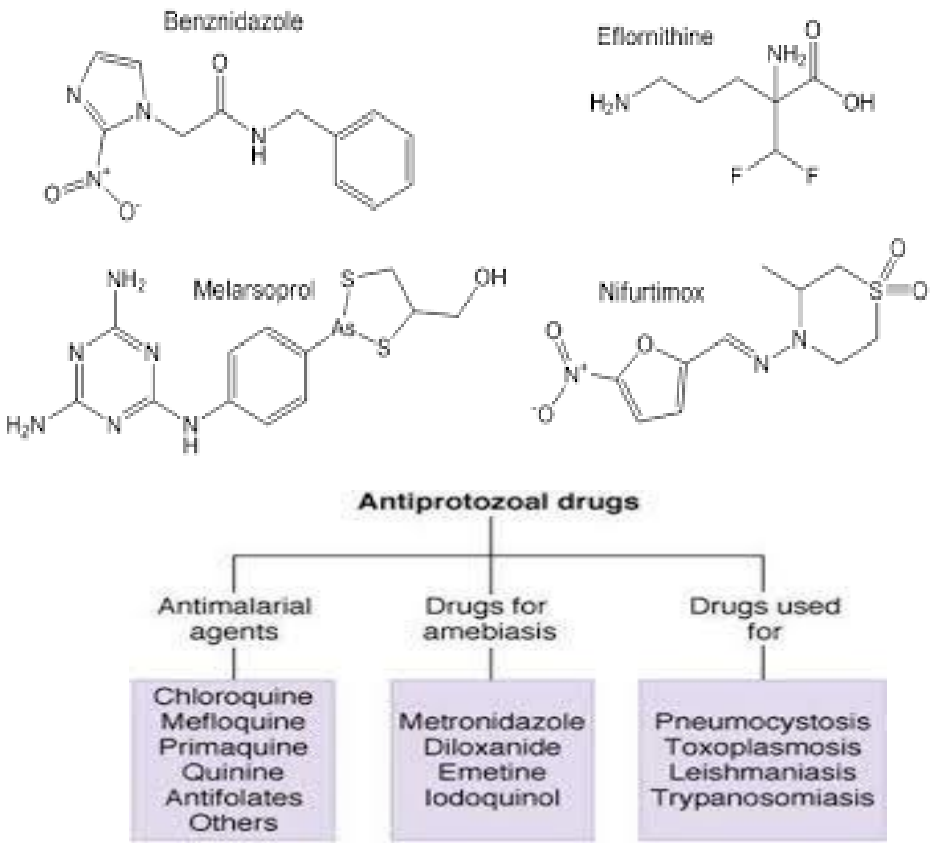
Table 1: A historical overview of the most important stages of **development of Antiparasite Agents**

Year	Type	Examples	Discoverer/Developer
1632	Antimalarials	Quinine	Indigenous South Americans
1940s	Antimalarials	Chloroquine	Hans Andersag
1950s	Antiprotozoals	Metronidazole	Jean-Marie Fourneau
1970s	Anthelmintics	Mebendazole	Paul Janssen
1975	Ectoparasiticides	Ivermectin	Satoshi Ōmura & William C. Campbell
1980s	Antimalarials	Artemisinin	Tu Youyou
2000s	Antischistosomal agents	Praziquantel	Bayer & Merck Research

These sequences of nucleotides can bind to the genetic data from the parasite and obstruct their expression. Another course of action against selectively downregulating or blocking parasite-specific gene expression would be by the use of small molecules. Drug-binding hybrid molecules might be developed which could target parasite-specific ubiquitination pathways and influence its action on proteins identified by the host and those that determine parasite durability.

2.1. Antiprotozoal Agents

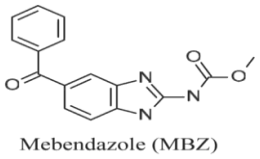
2 Materials and Methods 2.1 Antiprotozoal Agents Bulky aromatic rings linked to position 2 of the 5-guanidinobenzimidazole scaffold were identified as a common signature for the best performance of this class of compounds, as for biphenyl, 4-(3,4-dimethoxy-phenoxy)phenyl and bithiophene motifs. Docking studies disclosed the characterization of the binding mode of compound 2a to the DNA minor groove, where the guanidinium group formed a salt bridge and HB interactions with the phosphate moiety, and the NH group of the benzimidazole scaffold H-bonded to T-19. Results were further validated by SPR experiments and EMSA studies. The DNA-ligand interactions permitted a better stabilization of the DNA conformation than its apo form, therefore dampening its functional activity. Fragment-based drug discovery strategies have achieved a wide consensus in the hit generation and hit-to-lead optimization process due to their potential to increase the chance of developing more targeted lead compounds that are small in size and highly ligand-efficient.



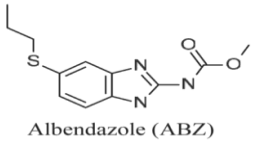
The application of this approach permitted the discovery of 5-guanidinobenzimidazole derivatives as promising inhibitors of Plasmodium falciparum apical membrane antigen 1 (AMA-1), a protein responsible for the merozoite entry into the host erythrocytes. On this basis, an FBD scaffold was selected, a benzimidazole core differing at N-1, to be optimized through synthetic investigation. Upon identification of the binding region on the target protein, a focused library of 13 final compounds was synthesized. Several compounds showed an appreciable ability to inhibit the P. falciparum 3D7 strain, acting in the micromolar range and displaying selectivity towards the HEK293 cell line. Further optimization allowed to address in 2-adamantanal series the issue of moderate variability, while retaining a good antiplasmodial effect. In particular, the replacement of the 5-methylquinoline nucleus of 2a with 4-(3,4-dimethoxyphenoxy)phenyl (2aa) led to an increase in antimalarial activity, improving the compound's IC50 value to 1.2 μM (Francesconi et al., 2024).

2.2. Anthelmintic Agents

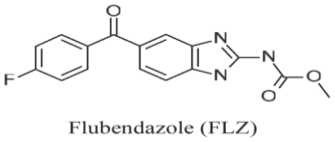
Anthelmintic drugs, also known as vermifuges or antihelminthics, comprise a class of medications that remove parasitic worms (helminths) from the body of the host. There are many classes of antiparasite agents, and they have distinct methods of action, benefits and adverse reactions, and resistance mechanisms. Classes of anthelmintic drugs include cyclooctadepsipepticles, benzimidazoles, midazothiazoles, organophosphates, open ring and spiroketal, polyphenolics, pyrimidine derivates, beta-carbolines, salicylanilides, spiroindoles, tetrahydropirymidines , and triazines.



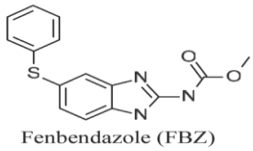
Mebendazole (MBZ)



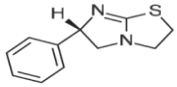
Albendazole (ABZ)



Flubendazole (FLZ)



Fenbendazole (FBZ)



Levamisole (LEV)

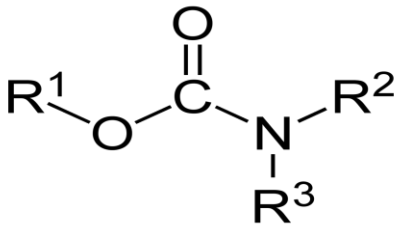
A review of helminth drug efficiency concludes that chemotherapy remains one of the most effective approaches to fight helminthic infections. That being said, helminth resistance to anthelmintic drugs is a well-known fact and deserves studies to elucidate the mechanisms of action involved. Anthelmintic resistance can surface when the dose of an anthelmintic drug is not administered or ingested in the right measurements, or continues administration to the entire livestock. With an increased number of people living on the transitory side of life, ahead of a number of unhygienic and convenient lifestyle, the transmission of diseases capable of weakening the body's immunity has been on the increase. In addition to these factors, there is change in climatic factors and greenness-associated habit, being tasked to the spreading of diseases. For example, earthworm disease is transmitted with a rate of 1.2 million individuals at the global level, every year (Fissiha and Zemene Kinde, 2021). Across the globe, about 300-600 million people from both poor countries and various groups are infected with soil related diseases; in particular earthworm disease. This is due to the reason that the people affected generally live in a poor and unhealthy condition as a result of malnutrition.

2.3. Ectoparasiticides:

Ectoparasiticides are agents that kill or repel parasites that live on the body surface of a host animal, such as lice, ticks, mites, or fleas.

2.3.1. Classes

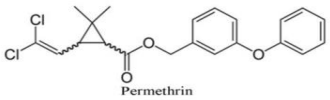
2.3.1.1. Carbamates: acting by inhibiting cholinesterase of the neurons of the parasite, leading to its killing. Phenothrin is a pyrethrin and a potent ectoparasiticide. It is used to kill a range of ectoparasites such as flies, ticks, lice, and



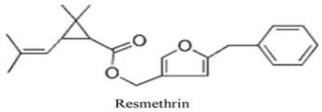
mites, and is either applied to the skin or sprayed within the air such that it is inhaled.

2.3.1.2. Chlorinated acyclic hydrocarbons: DDT accumulates and persists in the fatty tissues of animals, its residues may still be found even up to today, limiting its usage in deterring animal parasites. Hence, more integrated strategies such as non-chemical, biological controls, resistant-standard breeding, and mixtures composed of two or more antiparasite agents may be more environment-friendly and sustainable.

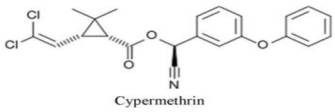
2.3.1.3. Synthetic pyrethroids: They selectively target sodium channels of the nerve cells of the parasites resulting in the parasites' excitatory influx of sodium ions into the cell leading to its depolarisation and eventual cell death. Cypermethrin is a broad-spectrum pyrethroid ectoparasiticide, predominantly used to prevent mushroom flies from damaging the strawberry fruit crop in the field.



Permethrin



Resmethrin



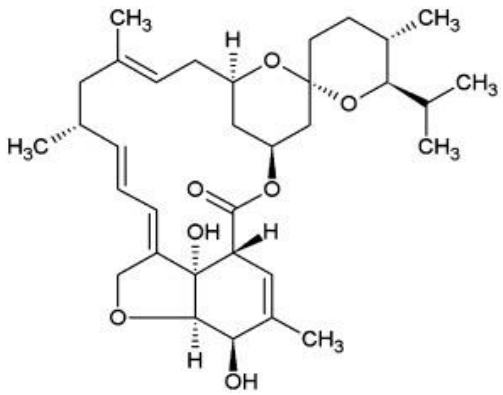
Cypermethrin

Deltamethrin is used as a general ectoparasiticide for the prevention of parasites that affect the health of poultry, including blanketweed, bitinoflagellates, and midges.

2.3.1.4. Haloether derivatives of sulfanylthiadiazole: commercialised under the name of Bayticol, is used as an ectoparasiticide in cows to kill ticks.

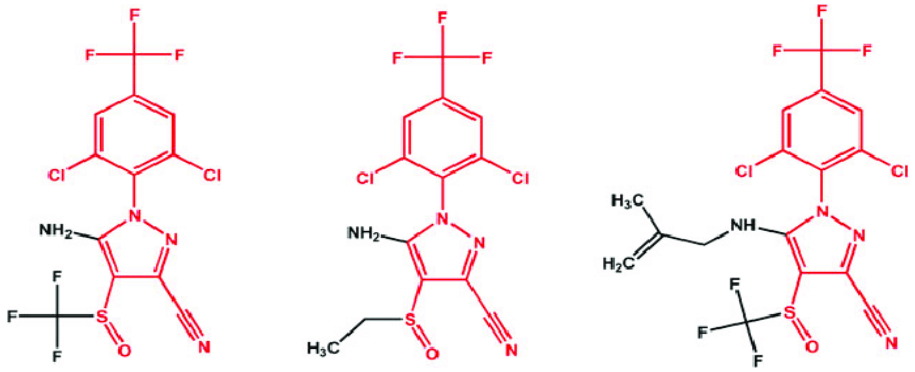
2.3.1.5. Organophosphates: Organophosphates are acetyl-cholinesterase inhibitors, antagonising the enzymes which hydrolyse the neurotransmitter acetylcholine in the synaptic clefts of the neurons and those of the parasite host animals, whilst those of the host are selectively accumulative, disrupting their neurotransmitter-controlled communication and causing snatches.

2.3.1.6.Macrocyclic lactones: Doramectin is a macrocyclic lactone ectoparasiticide licensed for use in cattle to kill various significant parasitic arthropods, including the larvae or adults of chewing lice, horn flies, house flies, mange mites, ticks, and warbles.



Ivermectin is a potent broad-spectrum macrocyclic lactone ectoparasiticide used to kill a wide variety of ectoparasites within swine, domestically kept animals, and veterinary applications on pets and treatment for human scabies. Lice-treatment forms of ivermectin are typically in a 0.5% lotion of propylene glycol. Inserted behind the site of the ear, the encompassing sebaceous glands predictably excrete the active ectoparasiticide.

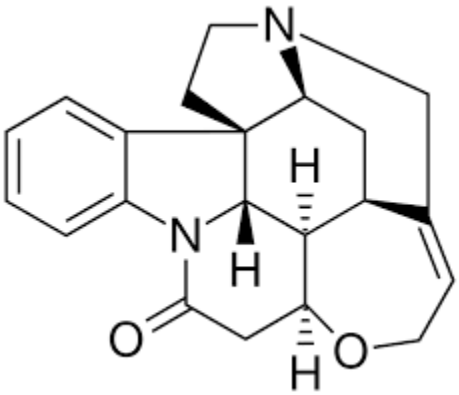
2.3.1.7. Phenylpyrazoles: It works through over-stimulating the fleas to hyperactivity leading to them falling off the animal. Alternatively, it can be slowly absorbed into the fatty tissues to kill the parasite when ingested. Fipronil is a phenylpyrazole chemical.



Fipronil Ethiprole Butene-fipronil

It works by inhibiting the pest's GABA receptor, responsible for regulating the chloride ion channels of the cells. This causes the cells of the pest to become hyper excitable leading to their poisoning and eventual death.

2.3.1.8. Strychnine: Strychnine is a very strong convulsive poison. In small doses it can be taken as a tonic; it is lethal to humans and animals in large doses. Strychnine can be absorbed by inhalation, by absorption through the skin, consumption, through a wound or the ocular area.



2.3.2. Mechanisms of action

Ectoparasiticides kill or repel parasites that live on the surface of a host. Ectoparasiticides that have mosquito larvicidal properties are effective countering the rise of mosquito-borne illnesses, disrupting the transmission vector for parasites that cause disease affecting humans and animals. Ectoparasiticides work through the following mechanisms: They kill insects (ectoparasites) via overexcitation by directly over-stimulating the nerve cells, causing them to fire constantly. Alternatively, they can fatally affect vital physiological processes when

the dose of the agent it is repeatedly exposed to exceeds a predetermined concentration, leading to its poisoning. This perturbs tissues in ways that deplete their ATP reserves or cause high levels of toxic products of the cell, resulting ultimately in damage to the parasite through the exhaustion of its viable resources right down to the atrophy of its entire cell.

2.3.3. Resistance

Resistance will arise when a proportion of the parasite population survives the exposure due to their possession of particular genetic traits that confer advantage against the mechanism of action of the ectoparasiticide. Over time, persistent exposure to sublethal doses of ectoparasiticides causes the preferable survival and reproduction of species-specific protective traits in the population of the ectoparasite, propagating the genesis and dissemination of resistance. Resistance can be either metabolic, target-site, or behaviour. It is considered crucial in slowing the emergence of resistance in parasites that infect the health of the community by employing a variety of strategies with differing mechanisms of action so that selection for resistance against one organism will be inversely selected against another organism. Care must be taken in ensuring an ectoparasiticide has systemic toxicity that is non-specific between parasites and animal hosts to reduce risks of poisoning and poisoning-through-contact.

3. Mechanisms of Action

Ceftizoxime has not only a bacteriocidal action, involving the inhibition of bacterial cell wall synthesis by competitive binding for the penicillin-binding proteins (a cephalosporin mechanism common to all cephalosporins), but also a strong interferential action with periplasmic proteins, preventing the immune bacteriocidal action, and thus assuring its action against antibiotic-resistant germs. The general route followed for the treatment of the cutaneous parasites typically involves the use of antibiotics (Man et al., 2022). In order to address the systemic and local circulation of the parasite, antibiotic use involves the per os route of drug delivery. The previous intravenous administration of 500 mg of ceftriaxone was unsatisfactory for a 13-day ulcerative form of disease in which the parasite infection was not systemic, but local flush to the areas of injuries, instead. A change to an oral treatment with a 10-day administration of 3x750 mg (1/4 of total body mass measurement), and later with a change of antibiotic to 3x500 mg of cefdinir (1/8 of total body mass measurement) and to a 3-day treatment – led to the healing of the injuries in the patients. Judgment as to the effectiveness of the earlier treatment must, then, consider the relief of the secondary bacterial infections, rather than the progressing primary cutaneous form.

The treatment of some cutaneous parasites may involve not only the general systemic oral treatment with antibiotics, but also the application of the local targeted forms of antibiotics. Because of the significant lipid and water solubility, precluding their successful application as a parenteral solution composed of an aqueous diluent-antibiotic mix, the antibiotics may also be used as a powder. In order to more effectively address the observation that L. braziliensis parasites are more directly exposed to the lymphatic bathing than to the bloodstream, the sterile intravenous administration of drugs is realised with the help of simethicone gas, which induces a rapid transformation of injectable solution into an intra-articular suspension. Due to awareness of the cefotaxime-antibiotic-mediated alteration of the blood-brain barrier drug passage, the treatment of the other parasitosis involves the intramuscular application of fortified powder consisting of the cefotaxime sodium and piperacillin/tazobactam sodium. The treatment involves the 5-day whirl treatment consisting of the instillation of the single-bolus antibiotic powder dose followed by the "magnetic" 15 s whirl of the extremity to achieve the locally proper antibiotic dosage or else a slower ivy infusion aimed at achieving the systemic dose.

3.1. Inhibition of Nucleic Acid Synthesis

Three enzymes are crucial for the survival and growth of malaria parasites in the red blood cells (RBCs). Of these, plasmodia possess none. Hence, the parasite must salvage all precursors needed for nucleic acid synthesis from the RBC cytosol. There is only one system for the salvage of free purines in RBCs – adenosine kinase (AK). In genetic engineered strains, the P. falciparum AK gene has been replaced with a human or E. chaffeensis orthologue abolishing cytosolic AK activity. The growth of the KO parasites was compromised compared to WT, and the level of intracellular ATP was sharply reduced. This was correlated with a significantly reduced level of free purines as well as other nucleotides, especially purine nucleotides and their derivatives (deoxynucleotides). Furthermore, the growth of the AK KO parasites could be rescued upon supplementation with guanosine or adenosine. All these results strongly suggest that salvage of free purines via AK is the primary source of purines for the growth and survival of the parasite.

One of four classes of antimalarial drugs exerted their pharmacological activity through inhibition of nucleic acid synthesis. The primary event during Plasmodium infection in human hosts is the invasion of red blood cells that leads to the biosynthesis of DNA and RNA of the growing parasite. A large amount of nucleic acids is metabolized, and the salvaged free bases must be reconverted to nucleotides prior to insertion into the DNA or RNA molecules, a process catalyzed by hypoxanthine-guanine phosphoribosyltransferase (HG-PRT). The opposite reaction

can be carried out by 6-oxopurine phosphoribosyltransferase (PRTase). A deep understanding of the nucleotide metabolism in malaria parasites may provide a theoretical basis for new drug discovery. Developing new inhibitors against PRTases has been difficult. However, 6-oxopurine PRTase eventually became a well recognized target for the development of competitive inhibitors that act via suicide inactivation. Since malaria parasites must salvage nucleotides owing to lack of de novo synthesis, all four nucleotides are readily available and pulsed with a drug. The successful drugs need be toxic to the parasite at the lowest dosage possible. Over 20 essential nucleotide-consuming enzymes in malaria parasites were evaluated and 13 were sensitive to the nucleotide analogs. By screening for inhibitors of these essential enzymes, potential new antimalarial drugs were identified.

3.2. Disruption of Energy Metabolism

Antiparasite agents affect parasites by a variety of mechanisms. A large proportion of effective antiparasite agents target 'frailties' in the unique biology of parasites that result from evolutionary divergence from their hosts. A number of antiparasitics interfere, directly or indirectly, with the energy metabolism of parasites by disrupting biochemical pathways. Common targets include the pathogen prokaryotic-like organelles such as the mitochondrion and apicoplast in apicomplexans, the glycosome or mitosome of kinetoplastids, and the hydrogenosome of trichomonads or chytrids. The electron transport chain regenerates ADP from ATP, which is consumed in a multitude of energy-dependent processes integral to an intricate metabolic network. Consequently, the electron transport chain has limited redundancy and is required for both motility and the cell cycle.

Learning about Toxoplasma gondii has been hampered by its evolutionary divergence from the extensively studied model apicomplexan Plasmodium falciparum and technical limitations of both forward and reverse genetics. (A. Hayward et al., 2023) developed an inducible genetic manipulation system for the cyst-forming coccidian parasite T. gondii based on the Tet repressor protein (TetR) and identified suitable promoter fragments based on the endogenous constitutive promoters sag1, sag4, sagA1, and tub1. Conditional regulation of ectopic transcripts expressed under the control of tachyzoite genes that are either up- or down-regulated in the bradyzoite stage is also reported. This enables investigation of essential bradyzoite genes beyond what is possible using population-based assays, which are currently limited to approximately 25% of T. gondii genes. Finally, a stable, inducible expression system in a closely related apicomplexan, Neospora caninum, is described. N. caninum is an important veterinary pathogen that causes

similar clinical outcomes as T. gondii in livestock animals and this system promotes therapeutic development for the treatment of neosporosis.

3.3. Interference with Cell Membrane Integrity

Single untreated cells as soon as 15min after application of temporin-SHa and confirm microscopy and survival data. Live and dead purified T.brucei (BSF Tryp MiTat 1.2) treated with (B) or without temporin- SHa (A) for 30min and stained in XTT (red: dead), Hoechst (blue: nuclei), and FDA (yellow: live). Temporin-SHa kills nonp-subergine 50% (IC50s) in less than 7.5 min. The IC50 val-sputtering 1.5 and 3uM, respectively, for purified DEAD trypanosome and Leishmania promastigoce. R.A. does not interfere with temporin-SHa antiparasitic actin (Raja et al., 2017).

4. Resistance Mechanisms in Parasites

Due to genetic mutations in the parasites, resistance to drugs can be obtained from parasites. The process by which it reacts to these drugs is quickly directed. The well-being of animals and humans has been hit by a lot of parasites that create parasitic illnesses. The struggle against these parasites is quite difficult. The utilization of drugs is the mainly used control method against parasites causing parasitic illnesses in domestic animals and humans. For a moment, the activity area of antiparasitic drugs is too extensive. For approximately 20 of 33 different drug groups utilized against parasites, resistance to parasites has already been build (Fissiha and Zemene Kinde, 2021).

The drugs used to prevent and treat parasitic diseases in poultry have different chemical structures and differences in its effects parasitic parasites. When the records in the literature were examined, it was observed that antiparasitic drugs used to prevent and treat coccidiosis in poultry were started to be in use in the early 1950s. The first generation antitumor toltrazuril is used. Antibodies are the first choice in the control of parasites that parasitize poultry hosts. Control is effectively conducted by exposing the poultry house environment to a hygienic condition, maintaining a between-run break in flock/nurturing pools, destroying feeds and watering sources. Avoid direct contact between wild birds and poultry to eliminate parasites that pose a risk to health.

Candidate drugs are added to the feed or water of the animal host, or antiparasitic drugs can be given subcutaneously or intra musculature in the form of injections, especially in animals. Toxins are secreted to eliminate these parasites, and if these methods are insufficient, members of the poultry layer are eliminated. In the literature, drug groups used in the prevention and control of invasive helminth parasites in poultry hosts are classified according to their mechanism of action.

Macrocyclic lactones, benzimidazoles, tetrahydropirimidines, imidazothiazoles, and nitroscanatel restoratives are widely used in the control of invasive helminth parasites in poultry. The drugs in these groups are believed to immobilize enzymes that are sensitive to parasites, thereby impairing their metabolism and other vital functions.

4.1. Genetic Mutations

Malaria parasites, the causative agents of infections spread by Anopheles mosquitoes, continue to be a significant cause of human mortality and morbidity. In 2013 alone, it was estimated that between 422,000 and 839,000 people died of malaria worldwide. Although there are five species of Plasmodium that cause disease in humans, P falciparum is responsible for a majority of the mortality. As P falciparum has developed resistance to all known drugs, novel antimalarials are desperately needed.

Surprisingly, there is very little understanding of how these parasites adapt to survive in the presence of drugs. There have been many studies that document the resistant phenotype, but barely any studies have investigated the genetic mutations that give rise to it. It was recently shown that point mutations must arise in the target genes before drug-resistant Leishmania lines can adapt to grow in the presence of the falcarinol-type compound. Furthermore, these mutations do not rise stochastically but, instead, must occur in a particular order. This may not be a generalizable phenomenon, and many other drugs may select for random mutations. Nonetheless, this shows that studying the genetic mutations associated with an actively drug-selected population of parasites can give important insights that are not apparent from just studying the DNA of a resistant population.

A single-nucleotide polymorphism (SNP) array was developed for P falciparum and shown to be ameliorative for characterizing genetic changes in the presence of CQ. In combination with drug testing and whole-genome sequencing of 37 CQ-resistant clones, the SNP array was used to see whether genetic changes linked to resistance were present prior to drug selection. Beyond the gene with the strongest association, a novel amplification on chromosome 12 was identified that conferred CZ resistance when present in duplicate (Cowell and Winzeler, 2018).

4.2. Enzymatic Degradation

Anthelmintic resistance is a worldwide problem and the interest of drug discovery has recently moved to also study multi-drug resistance (MDR) of parasites and the potential drug efflux transporters involved. In C. elegans it was shown that P-glycoprotein is involved in MDR of the macrocyclic lactones ivermectin and eprinomectin in nematodes harboring a mutated Ivermectin binding receptor gene

(avr-14a). Since many parasitic nematodes are agricultural or livestock pathogens the interest has expanded to such parasites. There is mounting evidence suggesting that P-glycoprotein might be involved in multidrug resistance in some permethrin selected isolates of Haemonchus contortus. This work focused on the recent understanding of nematode P-glycoproteins and expression of recombinant C. elegans P-glycoprotein 3 (Cel-pgp-3) in Lactuca sativa. However, reactivity of three anti-Pgp antibodies with Cel-PGP-3 was weak. It could be shown that in the yeast growth assay none of the recombinant P-glycoproteins tested (Lsa-pgp-1, Cel-pgp-3, Cel-pgp-4; Cel-pgp-3) conferred resistance against eprinomectin in presence of the fungal CYP51 inhibitor voriconazole. On the other hand, Lsa-PGP-1 as well as Cel-PGP-3 interacted with the anthelmintic IVM in the competition assay. However, under the conditions tested this yeast-PGP assay did not allow to detect MDR. It could be shown that the drug resistance model related expression of RNAs known to affect drug resistance in worms. However, effects were relatively low in comparison to those of xenobiotic resistance in yeast cells.

In the course of a study investigating MDR in C. elegans, it had been chosen to express some of the worm P-glycoproteins (PGPs) in yeast cells based on work done with the yeast Saccharomyces cerevisiae and the human P-glycoprotein 1 (hPGP1). The aim of the study was to express nematode (parasitic) PGP in yeast and test such cells for interaction with anthelmintics and in addition also toxicological compounds. In some parasitic nematodes P-glycoproteins were identified based on sequence comparison with C. elegans. It was intended to express the P-glycoprotein 9 (ce-pgp-9) of the parasitic nematode Cylicocylus elongates harboring a 45% amino acid identity to Cel-PGP-9 (Kaschny et al., 2015). In addition, the ce-pgp-9 genomic DNA was obtained as a synthetic gene free of yeast introns. Both the gDNA and Ce-PGP-9 were cloned just as the G418 resistance cassette. Yeast transformation was observed with a cell/yeast DNA ratio of 1x10^5:1. Fifteen cells of yeast growth on SDiQ medium supplemented with G418 and concanavalin A in the absence of the putative transformant and yeast resistance revealed the yeast dependence and expression of the G418 resistance cassette. Post transformational inhibition treatment cell sample resuspended in H2O showed up to a 60% decrease in OD540 compared to untreated or only 2x10^5 benomyl treated cells.

4.3. Altered Drug Targets

Development of drug resistance depends on a variety of biological and atmospheric factors in addition to drug pressure. It took decades to develop resistant strains to chloroquine (CQ) as those resistant strains have a significant decrease in the parasitic group currently targeted by quinolines. In contrast to atovaquone, resistance may emerge with its clinical use, as only one-point mutation in the

cytochrome bc1 complex is sufficient to confer resistance. Interestingly, such a mutation is conserved in Plasmodium from various life zones not yet exposed to atovaquone. Nevertheless, the mutation alone is not sufficient for parasites to survive drug treatment. On the other hand, it was shown that the additional mutation in accepting ubiquinone binding sites at codons 281–285 is required for significant increase in resistance, impairing the ability of atovaquone to inhibit the ubiquinone reduction and, thereby, leading to survival of treated parasites (O. Ibraheem et al., 2014).

The World Health Organization (WHO) adopted a fixed-dose combination (FDC) strategy in order to overwhelm the spread of resistance to any medicine contained in the combination. For example, no evidence primary resistance to artemisinin derivatives has emerged to date. With this strategy, the prevalence of resistance to lumefantrine remains very low with its use in combination with artemisinin. Lumefantrine is a highly lipophilic drug that becomes sequestered within fat. Drugs need to access their target sites to exert pharmacological effect. To do this, antimalarial drugs must reach effective concentration within the food vacuole of the parasite. Oral drugs need to cross at least five membranes to gain access to the cytosol and the target. The passage of lipophilic drugs, chloroquine and amodiaquine is mostly passive. They depend on the diffusion success after the drugs associate with membrane binding sites located on the food vacuole membrane of the parasite. Chemoreversal impedes the accumulation of the drugs in the sac; a complete blockage of the efflux system occurs when an excess of N-ethylamodiaquine exists in the culture media.

5. Clinical Applications of Antiparasite Agents

Antiparasitic agents are a set of compounds used to fight parasites, including protozoa, helminths, and arthropods. In the human body, these intruders can live internally or externally. On one hand, internal (ie., endoparasites) are the most widespread and can cause numerous disease states, such as nematodic, cestodic, and protozoal infections. On the other hand, external ones can settle on the epidermis or bite humans, causing cutaneous problems. These cutaneous parasites consist of occupational risks, animal bites and contacts, infestation, and stings. For treatment, antibiotics are often the initial line of approach for cutaneous parasites, which generally detach the parasites from their usual jobs. Additionally, quinolones, sulfur drinks, and sulfonylureas can also be contenders. Common routes of drug delivery for cutaneous parasites are oral, intravenous, and intralesional. For cutaneous parasites, antibiotics tend to lead the way in therapy, accounting for 94.4% of options (Man et al., 2022). These drugs either block the pathogens in their normal functioning or impede their division, reducing them to a state where they

can no longer reproduce. Nonetheless, a few antibiotics can thoroughly kill parasites. Other therapeutic choices entail ivermectin, albendazole, and metronidazole. For cutaneous parasites of concern to the USA posse, schistosomiasis is noted as a fatal microorganism that is more common than certain diseases, such as leprosy, rabies, trachoma, lymphatic filariasis, and erysipelas, which make the list.

5.1. Treatment of Malaria

Malaria continues to be a significant concern for global public health and an impediment to the United Nation's Sustainable Development Goal focusing on ending epidemics and reaching an AIDS-free generation. Malaria-related morbidity, especially in Africa, is due to the parasite's capacity to develop resistance to existing antimalarial agents. Parasite resistance to antimalarial medications remains a hindrance to managing the disease in sub-Saharan Africa (Oduro Kumi et al., 2022). In 2020, about 241 million cases of malaria were documented globally, causing 627,000 deaths. Most of the cases (96%) were in Africa. Hepatotoxicity, skin hypersensitivity, and hemotoxicity effects are common side effects of several proposed antimalarial drugs, including the natural drugs. Adverse effects of several commercial malaria treatments have limited their usage, necessitating a continued search for superior and more effective drugs for malaria patients. Pharmacologists have synthesized more than 10,000 effective antimalarial compounds, many with substantial time and money inputs. However, malaria parasite resistance to existing drugs is slowly increasing. It is urgent to discover emerging antimalarial compounds that are biologically more potent, cost-effective, and free of side effects. As a result of their simple delivery, efficacy, and cost-effectiveness, novel biodegradable drug polymer nanoparticles might have a substantial effect on the way existing antimalarials are delivered for malaria treatment. Biodegradable biopolymers are less poisonous and easier to clear from the body than their non-biodegradable analogs. Chemically conjugated antimalarial drugs to biodegradable biopolymers have several benefits. Novel biodegradable polymeric nanoparticles have attracted attention as a potential treatment option for diverse diseases. Ample antibiotic agents, cytotoxic medicines, co-factor molecules, macromolecular therapeutics, or potentially therapeutic genetic elements can all be delivered by polymeric nanoparticles. Biodegradable polymeric nanoparticles have been proposed in an effort to increase the blood plasma concentration levels of artemisinin-based treatments in the treatment of malaria. Efforts are being undertaken to bring them out of the laboratory and into the marketplace for malaria and possibly infectious disease treatment more generally. Several drug classes of antimalarial medications have been identified and are currently employed clinically. Among the classes of antimalarial drugs commercially available are: Aryl amino alcohols, such as quinine

and mefloquine, which inhibit the degradation process of heme. The artemisinins are derived from artemisia annua and are very quick acting, killing a large proportion of parasites within the first eight hours of exposure. Floating saturated fatty acid molecules agglomerate and limit parasite access to unesterified cholesterol, which the apicoplast deficient parasite cannot produce. Neuropsychiatric drugs stimulate overflow of lysoacid, which interferes with digestive vacuole function and exacerbates other drugs. When an antimalarial lipid combines with syrosadamic acid in the digestive vacuole, a lipid sourer compound is created, exacerbating the condition and inhibiting hemolytic waste efflux. Primitive nitrogen-bearing compounds meddle with glutathione on the digestive vacuole wall, stopping the reduction of hemolytic derivatives. Antifolate compounds, such as proguanil, pyrimethamine-daraprim, and WR99210, meddle with the folic acid pathway. Malaria is an A-to-Z illness that begins with a single bite from an infected female Anopheles mosquito, and goes through complicated RNA and DNA synthesizing phases on its journey to the letter Z – infected mosquitos. Upon that first bite, sporozoites are introduced into the host's bloodstream. These tiny non-reproductive cells float around for half an hour. Eventually they encounter a liver cell, enter through the lone port of call, multiply into millions of merozoites and leave to form schizonts. Merozoites are released to invade red blood cells, where they similarly multiply. At the junction of RNA and DNA synthesis, some merozoites take another path and create gametocytes to infect mosquitos, finishing the journey that eventually comes full circle. Control of malaria would require stopping sporozoite migrations in the first hour after infection, but most infected do not show symptoms for a week or more.

5.2. Management of Schistosomiasis

Schistosomiasis is a waterborne parasitic infection caused by trematodes (flukes) of the genus Schistosoma, resulting in significant human morbidity and mortality. The disease is endemic to 52 countries and territories, the majority of which are low- or middle-income areas; with an estimated 249 million individuals requiring treatment for the disease. The management of schistosomiasis is predominantly dependent on a single agent, praziquantel, discovered in the 1970s. In the last decade, it has been recommended that praziquantel should also be used for the mass drug administration of children aged ≥ 4 years living in schistosomiasis-endemic areas. As a result, the global demand for praziquantel is considerable and greater than the total number of tablets that are used to treat any other neglected tropical disease. While praziquantel is considered to be safe and effective in both children and pregnant women, resistance against the drug is a possibility, because drug pressure in the form of mass treatment is high (A. Guzman et al., 2020). In the last few years, efforts have been made to develop the next generation of antischistosomal agents.

However, as the discovery and development of new drugs can take many years, a significant body of research is devoted to preserving the efficacy of existing anthelmintics. Thus, a better understanding of the molecular mechanisms by which praziquantel kills schistosomes might reveal how drug resistance emerges and can be minimized. Nevertheless, as is the case with other trematodes and nematodes, praziquantel-resistant S. mansoni isolates have, on occasion, been obtained in the laboratory.

5.3. Control of Helminth Infections

For control of infections by nematodes or helminths, including these worms, anthelmintic chemotherapy is the primary approach. Since the first anthelmintic was discovered in 1934, there have been few new compounds, with a disproportionate emphasis on cestodes and trematodes. Worryingly high levels of resistance have been reported with the most widely-used drugs, particularly in veterinary medicine. Thus research to discover new classes of compounds must be accelerated while better understanding helminth neuromuscular pharmacology is critical to exploit new and existing drug targets. To facilitate research in this crucial area, newer techniques that are or promise to be particularly useful are reviewed.

For control of soil-transmitted nematode (STN) infections such as ascariasis and ancylostomiasis, no major anthelmintic has been introduced for around 20 years and resistance to the few available drugs is widespread. Available anthelmintic drugs for treatment of STN infections fall into three chemical groups: benzimidazoles (BZ), like albendazole and mebendazole; nematode-selective nicotinic compounds; and glycoside macrocyclic lactones (ML), like ivermectin and abamectin. Infections with STNs such as the filarials cause high morbidity and disability, and treatment is long-term with single doses given annually. A quarter of the world's population is currently infected. As zoonotic infections are acquired from animals, drugs for human consumption must be long-acting and not result in drug resistance. Administering drugs to animals is extremely difficult and expensive in the regions where these infections are most prevalent, rendering existing drugs ineffective (Fissiha and Zemene Kinde, 2021).

6. Pharmacokinetics and Pharmacodynamics

Due to the increasing emergence of drug-resistant strains of protozoan parasites, there has been a need to employ new classes of drugs and to better understand their mechanisms of action to effectively combine or sequence them for optimal therapeutic effect. Pharmacokinetic and pharmacodynamic approaches for describing and predicting the time course of drug action and the emergence and selection of drug resistance are reviewed for the major classes of antiprotozoan

drugs and their implications for the rational use of these agents (Maria Hodel et al., 2016). The emphasis is on antimalarial drugs, with a more detailed examination of the effects of pharmacodynamic properties of the drugs, of parasite growth and mutation rates, and their implications for strategies to prevent or delay the emergence of partly resistant strains. Protozoa parasites are responsible for a wide range of important diseases, including the malaria-causing Plasmodium spp. and viruses of the Trypanasomatidiae families, which result in leishmaniasis, Chagas' disease, and African trypanosomiasis. A significant increase has been observed in the incidence of protozoal infections and in the number of acquired immune deficiency syndrome (AIDS)-infected or immunocompromised individuals. With the exception of the artemisinin class of antimalarial drugs, the number of new antiprotozoan drugs being developed has declined. Moreover, resistance to the existing classes of drugs is becoming increasingly widespread. Thus, the development of combined or sequential drug therapies that use dose regimens informed by pharmacokinetic and pharmacodynamic principles has received much attention. The response to that is to review these principles for the major classes of drugs used to treat protozoan parasites and to highlight some of the critical questions that still remain concerning the emergence and prevention of drug resistance.

6.1. Absorption and Distribution

The pharmacokinetics of ivermectin have been extensively studied and essentially reflects its physicochemical properties and formulation (bioavailability) rather than a specific drug driven process. The absorption and tissue distribution kinetics of ivermectin show the influence of enterohepatic recirculation, as well as bile and tissue binding processes (Lloberas et al., 2012). The orally administered ivermectin is rapidly absorbed from the gastrointestinal tract and subjected to a high first pass effect over the liver. Ivermectin is much less bound to plasma proteins than other avermectins, and its tissue distribution is more influenced by the drug partitioning between the aqueous and the lipophilic compartments. The drug tissue binding (and elimination) is much lower compared to abamectin. Consequently, the tissue half-life of ivermectin is longer than that reported for abamectin. The drug also shows a high volume of distribution in cattle. The elimination half-life for the absorbed ivermectin in cattle is in the range of 5–10 days, which is similar to the values described in other species.

The tissue accumulation is clearly detected in different target tissues and is of particular relevance for those parasite species that inhabit the same biological compartments. The drug concentrations determined in paired liver and kidney samples of yaks necropsied 135 days after pour-on administration support the

occurrence of a strong partitioning of ivermectin. Liver ivermectin concentrations were nearly four fold higher than those found in kidney while the kidney-to-liver concentration ratio for abamectin was markedly higher. The drug tissue ivermectin binding (and accumulation) is much lower compared to abamectin and could justify the higher liver ivermectin concentrations. A low (but consistent) tissue ivermectin concentration is always measured in the spleen of cattle, yaks and sheep. Importantly, this tissue is the major target site for the indirect effect ivermectin exerts on EPG and nematode size distribution in these species.

6.2. Metabolism and Excretion

Anthelmintic drugs are applied to control parasitic worms in humans and animals. Defining the mechanisms of anthelmintic action and resistance could expedite the development of new drugs in order to cope with helminth infectious diseases. Nonetheless, this remains one of the commercially most neglected fields of pharmacological research. Different anthelmintics can affect various targets in parasites. Some anthelmintics affect the muscular system, namely paralyze pharynx and muscles of the body wall. The inhibition of critical ion channels by avermectins and milbemycins results in non-depolarizing, GABA-independent muscle paralysis. In turn, imidazothiazoles and substituted tetrahydro - pyrimidines provoke a depolarizing contraction of the muscle tissue.

Anthelmintic drugs are also considered substrates for the xenobiotic metabolism of parasitic worms. Whereas the metabolism of anthelmintics has been extensively studied in nematodes and to some extent in trematodes, anthelmintic metabolism in cestodes is under-investigated. In the case of the pork tapeworm, Conjugated UDPGT and GST activities are detected for the first time in cestodes. Subsequently, cestodes can metabolize xenobiotics by conjugation them to glucose, sulfates, glucuronic acid, and glutathione. While the metabolism of anthelmintics in parasitic worms is currently studied widely, the excretion of antiparasitic drugs from helminths has been largely ignored (Mordvinov and Pakharukova, 2022).

7. Adverse Effects and Toxicity

Ivermectin and other avermectin derivatives are widely used as endo- and ecto-parasiticide drugs for the control and treatment of invertebrate parasites in pets, livestock, and aquaculture. Ivermectin and its derivatives all have a significant down-regulation effect on nematode glutamate-gated chloride channels, but differences in efficacy were found against insects and crustaceans. Among the different insect species tested, the investigated flightless dipteran species were most strongly affected by treatment with a very low compared to the avermectin derivative dose that causes down regulation of the invertebrate glutamate-gated

chloride channels. Avermectins are a group of 16-membered macrocyclic lactones that are potent anthelmintic agents consisting of a 5-O-demethyl-22,23-dihydroavermectin B1 aglycon, aynthetic derivatives better known as ivermectins, abamectins, milbemycins, and doramectins. The target host invertebrates of these agents include nematodes (roundworms), arthropodes (arthropods), and insect larvae, along with some ectoparasites. The range of effects of these drugs on parasites is the result of multiple sites in the nervous systems of invertebrates, which are hardly tolerated by vertebrates. As the advancing knowledge of ivermectin grows, it becomes increasingly apparent that existing approval, quality control, and pharmacovigilance guidelines are inadequate to address emerging public health challenges, including the proposed mass drug administration (MDA) for Covid-19 or other forms of rapid access to the drug (Salman et al., 2022). Amplifying mass ivermectin use to the scale of hundreds of millions or billions of people holding serious public health risks.

7.1. Common Side Effects

Mechanisms of Action, Resistance, and Therapeutic Applications of Antiparasite Agents: Antiparasite properties of many agents act on enzymes that are essential to life in parasites, insects, and other invertebrates, rather than mammals; these agents can have a very high therapeutic index. Some agents do act on enzymes present in both parasites, insects, and other invertebrates and mammals. However, these agents often act on one or more parasite-insect/invertebrate-specific enzymes, whereas the equivalent enzymes of mammals are not affected by the agent. For several decades, major difficulties concerning the use of these findings have been a lack of knowledge about the agent's molecular targets, whether parasite-insect/invertebrate-specific or not, and the existence and frequency of field-evolved resistance to an agent in the parasite population. The recent advances of four groups are overcoming these difficulties for clinicians, veterinarians, insecticide developers, and agriculture's pesticide developers. (Man et al., 2022)

7.2. Severe Toxic Reactions

Severe toxic reactions involve hypersensitive or allergic reactions, degenerative reactions, secondary infection, effects on normal gut flora, or unexpected effects on nontarget organisms. The scientific literature contains numerous opinions on the mechanisms of action of plant antiparasite agents. Generally, this lack of consensus very often reflects the poor level of knowledge about these agents.

With respect to resistance, in some previously quoted reports, plant compounds are considered to have the same impacts on the parasite as some synthetic agents, e.g., by enzyme inhibition. Both are usually incorrect statements. Why? Enzyme

inhibition would require plant compounds to be specifically active on some metabolic pathway, which is unlikely except if both plant and helminth share some common metabolic pathway, and that this event leads to helminth death (a rather improbable situation). Although some parasiticidal plant principles are fast acting, there is no reason to suspect that this is due to neurotoxic effects only. On the other hand, some antibacterial plant agents are active on a wide range of bacteria, including human pathogens, due to common targets, which are not always existent in plants. On the contrary, antiparasite compounds are usually "nonspecific" since they interfere with processes different from those used by synthetic agents. On some occasions a number of compounds make the cell environment uncomfortable through hyperacidity, hyperpolarity, etc., and induce the escaping of the parasite from the host environment (and, of course, the death of the host). PAR, however, are likely to act against a wide (or a very wide) range of parasites. Given the low level of common evolutionary history among parasite taxa, it is difficult to conceive a mechanism of action involving a broad range of endoparasites.

8. Emerging Antiparasite Agents

Parasitism is a biological interaction present in nature, being one of the most successful ways to survive and develop as a species; this is particularly true for parasites of animals, in this context, protozoan parasites deserve special attention. They divide primarily into free-living saprophytes and parasites, being the internal parasites divided into commensals, endoparasites, mutually living in a shared way, with no harm to the host and the other causing damage to the host immune system (Rivera-Fernández et al., 2022).

8.1. Novel Compounds in Development

Many investigators have attempted to repurpose or rescue older drug candidates effective against some protozoa as possible treatments for toxoplasmosis. Auranofin, digoxin and nitazoxanide are but a few examples of these efforts. In the case of auranofin, its efficacy in a T. gondii mouse model is limited (Rocha-Roa et al., 2018). The development of such alternative treatments, however, has been plodding, incremental, and with only modest financial support from the pharmaceutical industry. One of the innovative findings of the present study comes from the observation that five kinase inhibitors kill both forms of the parasite, primaquine in this study was used as a control drug. Primaquine despite it is a common drug used for T. gondii infection, through its ability to inhibit the bradyzoite forms, its effect is lower against tachyzoite forms. Primaquine anti-T. gondii activity was found that inhibits 82% and 68% of the replication of Bradyzoite within and without the host cells, respectively, in contrary minimal inhibition occurs in tachyzoite forms. Then their derivatives of 4-thiazolidinone were investigated on

the other hand its in vivo anti-T. gondii activity was revealed for the first time. There are many diseases that have a high morbidity and mortality rate, in developing countries these prove tougher to eradicate as most of the drugs are very costly. Thus, there is a pressing need to find affordable drugs of herbal sources that are equally effective has the less side effect or toxicity. Many available synthesized drugs also show significant toxicity to mammals, therefore development of novel effective drugs has remained a hot research area in most of the developing countries including Ethiopia. Therefore, in the current study to increase the anti-T. gondii activity of an existing drug, the nitazoxanide, twelve new derivatives were synthesized. The nitazoxanide and its synthesized compounds.

8.2. Biological Approaches

Biological approaches are in use or in development to prevent the transmission of ectoparasites like the mite Varroa destructor that damages honeybee hives. This is highly relevant when parasitic activities interfere with agricultural or major species, like Boophilus microplus, an economically significant parasite that affects primarily cattle, buffalos, horses, goats and sheep in tropical and subtropical areas.

Parasitic activities affect a wide range of hosts from mammals, birds, fish, plants, bees and corals, including malaria, which is caused by Plasmodium parasites. The disease kills approximately 600,000 people every year, mostly children younger than 5 years old. The whole lifecycle of Plasmodium goes through two hosts, an insect vector and a vertebrate host. One of the most recent investigations on a new human malaria host, P. simium, was described in the Atlantic forest of Southern Brazil. Since Plasmodium is very genetically diverse, a defense mechanism that suppresses the Plasmodium growth would be very effective independent of the Plasmodium species and strains.

In cases of human parasites, this will need to be something that is ingestible. Approaches which may deal with poorly optimised, or totally neglected currently, human parasites can be quite general, for example attacking parasites at the point of entry could potentially apply to many different parasites.

9. Conclusion

Antiparasitic activity is integral in both overcoming current parasitic infections and in developing effective immunotherapies. There is an urgent need for advances against malaria in the discovery of antimalarial compounds (Francesconi et al., 2024). This also extends to new treatments that address the decline in antimalarial effectiveness driven by the spread of resistant parasitic strains. Advances in the understanding and treatment of neglected tropical diseases are similarly highly

sought. A great deal of effort and investment has been made towards research against leishmaniasis, one of many neglected tropical diseases. Despite this, the golden standard antiparasitic drug compounds have not yet been found, and a vaccine candidate is lacking. There are also other, less widely known neglected tropical viruses for which much work is still needed. With an overwhelmingly dense research population and regulatory scrutiny turning the focus towards immunology and cancer biology, these neglected tropical diseases have taken a backseat. As with leishmaniasis, new therapeutic advances, especially vaccines, are a necessary step to combat these insidious pathogens.

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Chapter Thirteen

Antiviral Agents

1. Introduction to Antiviral Agents

Antiviral agents are a different category of antimicrobial compounds that inhibit viral replication. A virus, similar to cellular life forms, can be considered to have a life cycle or stages in which replication occurs. Antivirals interfere with one or more of these stages, which includes: cell attachment; cell penetration; viral uncoating; viral genome (DNA/RNA) replication; synthesis of viral proteins; maturation and viral progeny release; and some other poorly defined events, such as some morphological changes during the replication of hepatitis B virus. Consequently, there are drugs that interfere with viral infections which are full validation and complete mechanistic knowledge. Therefore, antivirals can only partially avoid the viral infection, even when they are fully effective.

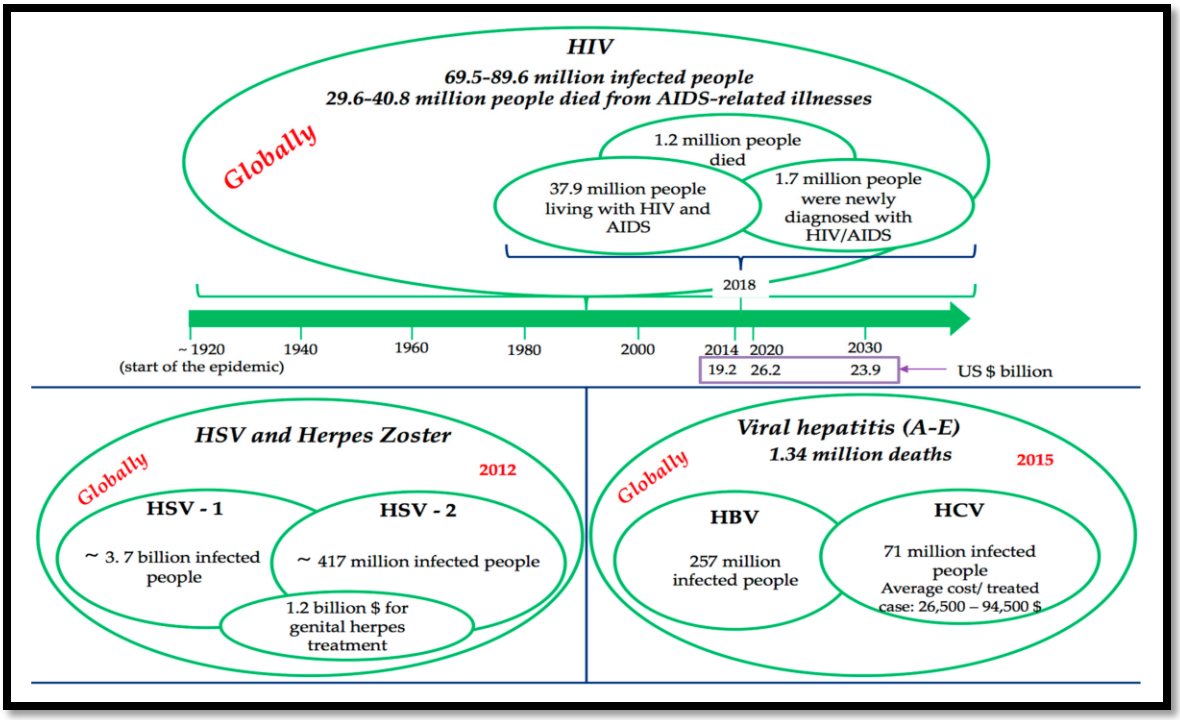


Figure 1: The spread of some viral diseases around the world

These points suggest that antivirals are frequently associated with other control strategies of viral infections, rather vaccines and vaccine-mimicking drugs. Vaccines

are capable of blocking specifically viral cell binding and penetration and avoiding infection, whereas antivirals are in general not viral isolate specific. This is a significant limitation of antivirals that their uses can eventually select the growth of viral variants not susceptible to the already drug marketed. However, vaccination and antiviral treatment strategies are synergetic in nature, as treatment with antivirals can stop severe infectious and fatal outcomes of the diseases caused by highly pathogenic viruses.

Table 1: History of Antiviral Agents and Their Discoverers

Year	Discovery/Development	Scientist(s) / Organization
1959	Idoxuridine – First antiviral drug, used for herpes simplex keratitis.	William Prusoff
1966	Amantadine – Used to treat influenza A.	E. D. Belford & W. R. Bell (DuPont)
1977	Acyclovir – First highly effective antiviral for herpes simplex.	Gertrude B. Elion & George H. Hitchings (Wellcome Research Laboratories)
1987	Zidovudine (AZT) – First antiviral for HIV/AIDS.	Jerome Horwitz, later approved by Burroughs Wellcome
1995	Protease inhibitors (Saquinavir, Ritonavir, Indinavir) – Revolutionized HIV treatment.	Various pharmaceutical companies (Roche, Abbott, Merck)
1999	Oseltamivir (Tamiflu) – Used for influenza A & B.	Gilead Sciences & Roche
2000s	Lamivudine (3TC) & Tenofovir – Used for Hepatitis B & HIV.	Emory University & Gilead Sciences
2020	Remdesivir – Used for COVID-19 treatment.	Gilead Sciences

An important advantage of antivirals is blocking viral severity without taking an into the direct immunological response generated by the host, which means that antivirals can be used also to immunocompromised persons. Also, antivirals could facilitate pathogen

eradication after vaccination this last point is especially relevant in diseases related to chronic viral infections, such as human immunodeficiency virus (HIV), and hepatitis B virus (HBV). Blocking viral replication in its early stages could avoid, in principle, the viral establishment and chronic outcome (Villa et al., 2016).

2. Viral Replication Cycle

Compared to bacteria and pathogenic eukaryotic parasites, viruses are very simple infectious agents. They have been described as 'a piece of bad news, wrapped in protein'. Essentially, viral genomes are encased by a protective shell composed of protein(s) and – in the case of enveloped viruses – of lipids. Although these protective coats and therefore the broad outlines of virology were appreciated already in the early days of bacteriology, it was only with the development of biochemical and cell biological methods that the molecular details of the replication of animal viruses could be approached. Nevertheless, some of the initial discoveries in virology are still relevant today.

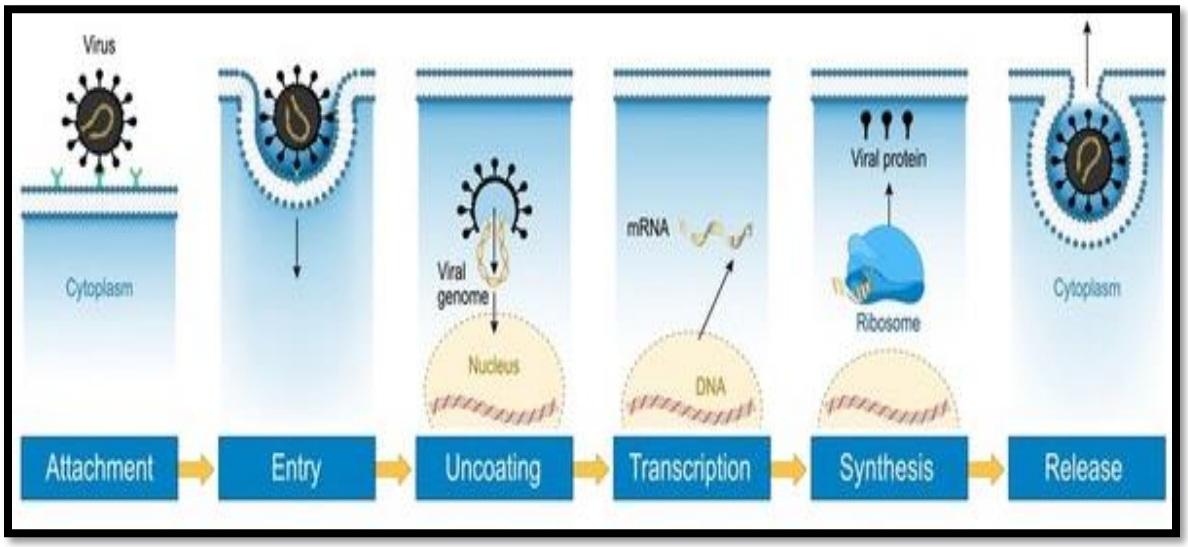


Figure 2: Steps of Viral replication.

In particular, infection studies with lytic bacteriophages led to the development of the one-step growth curve, which defined the fundamental replication cycle of a virus. The many pathogens causing massive morbidity and mortality are ascribed to representative viruses in several families. This reflects the enormous capacity of viruses to attack diverse cellular pathways and effectively counteract cellular and antiviral defence systems. Viral genomes consist of single- or double-stranded DNA or RNA and viruses have been classified according to the type of genome and the genome replication strategy used; however, the important human pathogens belong to a single-stranded RNA virus family – picornaviruses, caliciviruses, influenza viruses – and a double-stranded RNA virus family – rotaviruses that cause diarrhea.

Important human pathogens are also found in the families of herpes- and retroviruses. Among the different virus classes, some families, such as myxoviruses, papillomaviruses, polyomaviruses, retroviruses, or hepadnaviruses, are also tumor-inducing agents. Unlike multi-cellular organisms, which contain DNA as genetic material, viruses can be classified into at least six different groups of nucleic acid: double-stranded DNA, single-stranded DNA, double-stranded RNA, single-stranded RNA in the sense orientation, single-stranded RNA in the antisense orientation, and single-stranded RNA that is reverse-transcribed into DNA during its life cycle. Viruses can adopt many different genome architectures and molecular strategies to multiply, infect, and move within a host organism. Only the molecular structures of the major types of viral agents causing human epidemics and different scientific potential will be considered here. On the other hand, emphasizing the good sense of restricting the replication of the more than 200 virus families that infect humans.

2.1. Attachment and Entry

Antiviral agents targeting specific steps of the viral cycle have been described and include attachment/entry inhibitors, small molecules that block post-attachment steps, and a broad spectrum of host-targeting agents (HTAs) (Teissier et al., 2010). The prevention of the attachment and internalization of pathogens constitutes an important first line of innate defense, which is finely regulated at the host cell level by specific interphases involving lipids and proteins between the pathogen and the host. The antiviral agents that specifically block these key interactions often prevent pathogen entry, and thereby restrain rapidly the infection. Understanding the molecular basis of pathogen-host cell interactions at the attachment and entry levels is promising innovative approaches which might provide new leads for antiviral drug development. Purified SM-extracted compounds and three reference compounds targeting HCV cell entry have been compared for their effect on HCV attachment and entry, as well as on HCV pseudo particle infectivity and direct virus-to-liposome fusion. Several of the inhibitors and compounds which had been shown to inhibit EGF-induced clathrin-coated pit-mediated endocytosis effectively blocked HCVpp uncoating. Used in combination with an inhibitor of raft-independent endocytosis, further reduced HCVpp replication by about 50%, indicating that HCV may use multiple entry pathways. Inhibitors belonging to other classes used also in the study were also effective in inhibiting HCVpp infection. Co-treatment with inhibitors blocking different endocytic pathways also had a cumulative inhibitory effect on HCVpp entry. Treatment with an anti-clathrin heavy chain antibody inhibits clathrin-mediated endocytosis and reduced HCVpp infectivity by 30%.

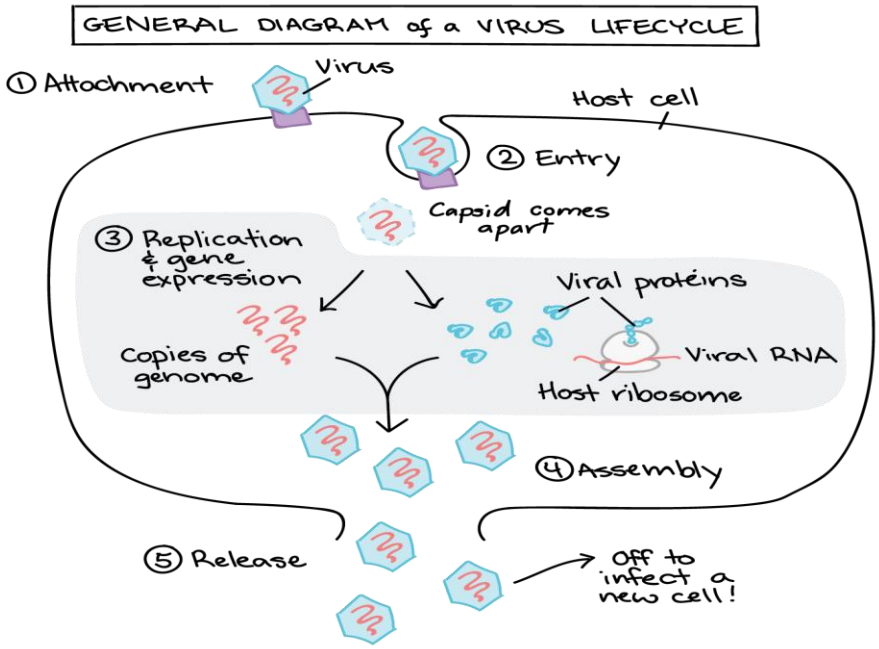
2.2. Replication and Transcription

2. Replication and Transcription 2.1. Replication Viral RNA-dependent RNA polymerases (RdRps) use versatile mechanisms to replicate their RNA genomes. RdRp synthesizes RNA using a single-stranded RNA template. To start replication at specific sites, positive-strand RNA virus replicases recognize various sequence and structural elements within the viral RNA. The second important recognition of the viral template occurs at the exit tunnel. Both reactions must distinguish viral RNAs from abundant host cell RNAs used as substrates in parallel reactions. After initiation, polymerases travel along templates and synthesize RNAs as their product. RdRps from positive-strand (+)RNA viruses deviate from clear-cut paradigms of transcription and replication operating during the infectious cycle(s) of DNA viruses, negative-strand (–)RNA viruses, and host cells. Many known cis-acting signals are located at least partly within the ORF, and replicases bind specifically to these signals. In positive strand (+)RNA viruses, the subgenomic mRNAs required to express viral genes are located at internal positions of the viral genome. (Magden et al., 2005) Despite many years of research, antiviral agents capable of specifically blocking viral replication without significantly impairing host cell functions remain limited. However, there have been some promising successes. 2.2. Transcription Viral RdRps catalyze the synthesis of –RNA or +RNA strands using single-stranded templates. Unencapsidated (free) RdRps are essentially promiscuous enzymes that synthesize RNA using alternative templates, including host mRNAs, in vitro. RdRp-mediated mRNA synthesis relies on a mechanism called internal initiation. The multifunctional development of the genomic and antigenomic viral RNA segments into mRNAs requires a mechanism for identifying the correct template of transcription and another for signaling the location of the polymerase along the template. (Paintsil and Cheng, 2009) Transcriptional promoters and promoters for replication are almost interchangeable and can also be located downstream of the syntax of the ORF. However, RdRp-promoter interactions are different in each case, and nascent RNA can loop out the polymerase at the exit tunnel.

2.3. Assembly and Release

HIV-1 and other retroviruses are included in a group of RNA viruses having two copies of positive-sense (+ss) RNA. This group also includes the Hepatitis C virus (HCV), which is a causative agent of liver inflammation and cirrhosis, as well as many other flaviviruses, such as the Zika and Dengue virus. The +ss genome is translated upon infection of a cell into a polyprotein. After proteolytic cleavage, mature viral proteins and an RNA-dependent RNA polymerase (RdRP) start replication of the genome. Except for HIV-1 and bovine leukemia virus, all retroviruses replicate through a proviral DNA intermediate using the enzyme reverse transcriptase. The +ss RNA proviral genome is reverse transcribed into

double-stranded DNA (dsDNA) by the reverse transcriptase (RT). This dsDNA is then imported into the nucleus, where it integrates into the chromosomal DNA of the host cell. The HIV-1 DNA integration is then transcribed by the host polymerase II machinery into full-length transcripts that are alternatively spliced to generate viral mRNA encoding all structural proteins for the assembly of new viral particles or the nonstructural proteins involved in the regulation of viral replication and in immune evasion (Hozáková et al., 2022).



All steps of this complex replication cycle are potential targets for antiretrovirals that currently inhibit three major enzyme activities of HIV-1: protease (PR), reverse transcriptase (RT), and integrase (IN). Furthermore, some antiretrovirals target the entry of the virus into a cell that is mediated by the interaction between the virion and the CD4 receptor and a coreceptor: the chemokine receptors CCR5 or CXCR4. HIV-1 has a complex enigmatic replication cycle that even after 40 years since its discovery is not completely understood. Several lines of evidence suggest that additional enzymatic activities of the virus are required for an efficient replication cycle, such as the nucleocapsid (NC) protein, which is required for the dimerization of the unspliced viral RNA, the Vif protein that counteracts the cellular protein APOBEC3G, which causes C-to-U mutations during reverse transcription, the Vpu protein that antagonizes the restriction factor tetherin, and possibly others. However, for the virus to successfully hijack the cellular translation machinery, a new layer of complexity is added since the ribosomes are surrounded by an mRNA

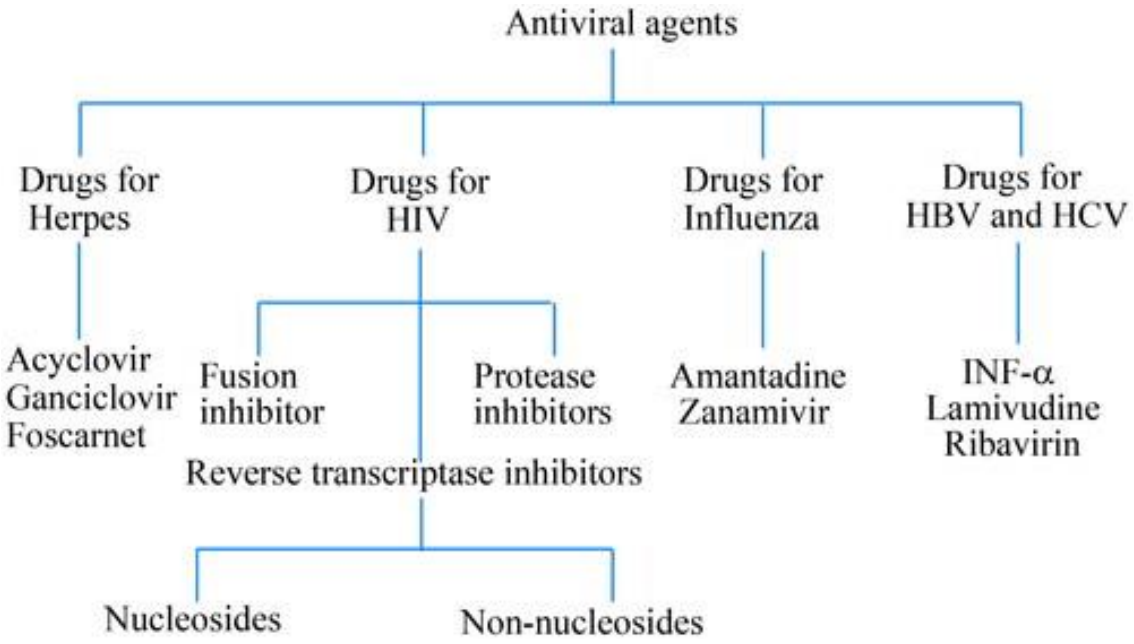
that is actively translated leading to a huge crowding effect that has been compared to a forest fire.

Several labs have been developing small molecules or peptide-based antiretrovirals with capsid-binding properties. This is supported by high-resolution structures of the CA hexamer of both immature and mature particles of HIV-1 and also more recently for the Mason–Pfizer monkey virus. The CA provides crucial interactions both during the assembly of the immature particles in the cytoplasm or the budding of the mature particles at the plasma membrane and also during the process of uncoating after infecting a cell. In the case of the immature particles, the CA forms a hexameric lattice scaffolding the Gag molecules preventing the premature disassembly of the viral core. In the mature virions, the CA forms a conical lattice that guides and organizes the viral RNA and the associated enzymes inside the particle.

3. Classification of Antiviral Agents

Antiviral agents are drugs approved for the treatment or control of viral infections; this definition includes drugs that have a specific antiviral effect, but which do not necessarily increase the host's resistance to infection such as some interferons and cellular antiviral agents (Paintsil and Cheng, 2009). Attachment of the virus to the host cell can stimulate the cell to produce interferons, which can precipitate a generalized inhibition to viral replication; these agents include drugs used against herpesviruses, certain interferons, and combinations of substances used in cancer chemotherapy. On the other hand, some substances may have a direct effect on the virus and not on the cell. This large and diverse group of antivirals includes drugs used against specifically herpesvirus (herpes, cytomegalovirus), adenovirus, hepatitis B and other viruses. Additionally, antiviral agents can also be agents that target stages in the viral life cycle. However, it is essential to consider that viruses are obligate intracellular parasites, and their replication is intricately linked with the host cell. It may be said that development of antiviral drugs is excessively complicated by the fact that viruses are small parasites that depend on the metabolism of cells where they replicate; as a result, any antiviral drug that interferes, even to a lesser extent, with host cell factors, may result in being toxic for the host. Antiviral agents and antiviral compounds are a group of medicines and chemicals which are able to inactivate viruses specifically rather than simply inhibiting the maturation and replication of viruses. Their scope of action is typically narrow, so that different kinds of viruses respond to different types or classes of agents. Modern population growth and climate change is likely to favour the emergence of new types of infectious diseases that will need new types of antiviral agents as yet undiscovered. The development of new antiviral compounds scripts

several approaches that can be seen as (Villa et al., 2016). There is an increasing need about new antiviral agents, as the vast majority of all existing antiviral substances have appeared only within the last 50 years, as modern medicine has developed. Many antivirals, especially the most ancient ones, have to date been studied from a result-oriented point of view, to assess their potency in the treatment of certain viral infections of humans, domestic animals or crops, without a detailed study of their mechanism of action and often just by serendipity, while in other cases this knowledge remains unclear. However, there still remain many known antivirals that have not yet been employed intentionally as such, or have been forgotten by the scientific community.



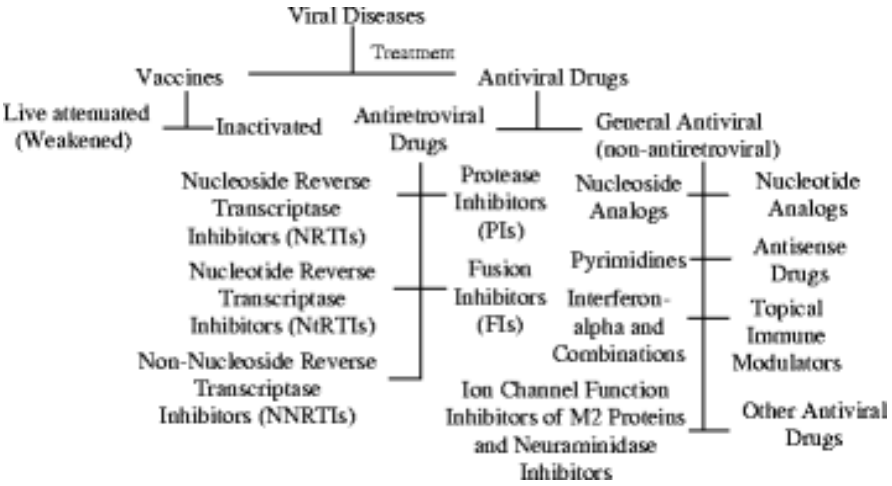


Table 2: Antiviral Agent, Target Virus and Mechanism of Action**.**

Antiviral Agent	Target Virus	Mechanism of Action
Idoxuridine	Herpes simplex virus (HSV)	Inhibits viral DNA synthesis
Amantadine	Influenza A	Blocks M2 ion channel, inhibiting viral uncoating
Acyclovir	Herpes simplex virus (HSV), Varicella-zoster virus (VZV)	Inhibits viral DNA polymerase
Zidovudine (AZT)	HIV	Inhibits reverse transcriptase
Oseltamivir	Influenza A & B	Inhibits neuraminidase enzyme
Lamivudine	HIV, Hepatitis B	Inhibits reverse transcriptase
Ritonavir	HIV	Protease inhibitor – blocks viral protein processing
Remdesivir	SARS-CoV-2 (COVID-19)	Inhibits RNA-dependent RNA polymerase

3.1. Nucleoside Analogues

The mechanism of biological activity of most antiviral drugs based on nucleosides and their analogs is associated with their intracellular transformation into 5'-mono-, di- and triphosphates under the action of viral and cellular kinases with subsequent inclusion of triphosphates into nucleic acids, which leads to inhibition of viral genome replication. The viral enzymes involved in the replication of viral nucleic acids are RNA-dependent RNA polymerase, RNA-dependent DNA polymerase, and DNA-dependent DNA polymerase. Herpes simplex viruses encode thymidine kinase, which carries out 5'-monophosphorylation of nucleosides and their analogs in the

cell. Viral enzymes are usually less specific than the enzymes of the host cell; therefore, in most cases, different nucleoside analogs are phosphorylated to triphosphates only by viral kinases (A. Zenchenko et al., 2021).

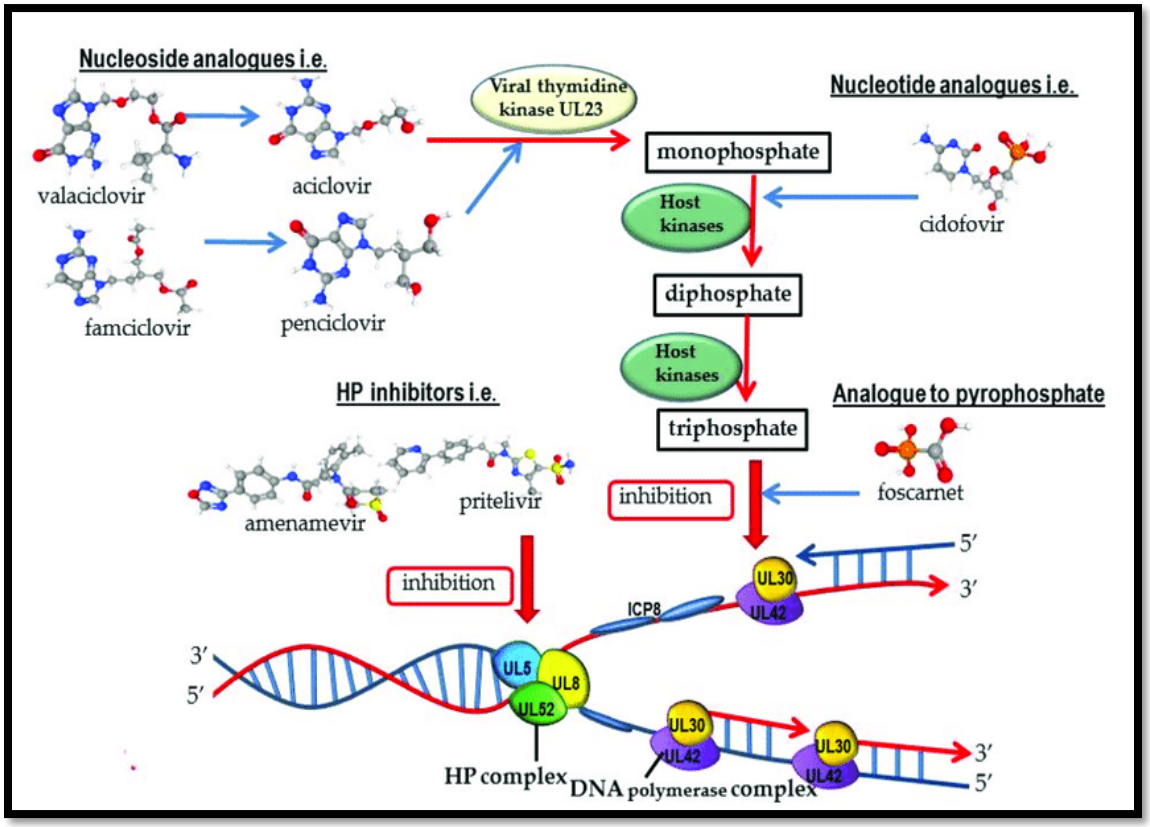


Figure 3: Nucleoside Analogues(The mechanism of biological activity)

The action of nucleosides and their analogs can be aimed at inhibiting the synthesis of viral nucleic acids, increasing the frequency of lethal mutations of viral genomes and other possible mechanisms of action. The antiherpetic acyclic nucleoside analogs penciclovir and its prodrug famciclovir inhibit first of all the replication of the genomes of Herperviridae, encoded in the form of DNA, which is synthesized on temple in DNA genome of virus and is synthesized by viral DNA-dependent DNA polymerases and amplified by cellular DNA-dependent DNA polymerases. The substrate specificity of DNA polymerases is due to the fact that they require a free 3'-OH group in the nucleic acid primer or, when synthesizing de novo, in the nucleotide triphosphate that is incorporated. Due to the absence of 3'-hydroxyl group, the incorporation of penciclovir triphosphate into the DNA chain of Herperviridae is 100-300 times more pronounced compared to its incorporation into the chain of the host cell DNA. Also, penciclovir triphosphate inhibits competitively long-chain elongation. The mechanism of action of penciclovir triphosphate and its derivatives leads to the fact that the replication of viral DNA is

suppressed to a greater extent than the synthesis of cellular DNA. On the contrary, acyclic purine nucleoside analogs acyclovir, ganciclovir and their prodrugs inhibit the synthesis of viral DNA slightly stronger than cellular DNA, as a result, it is shown that the incorporation of acyclovir triphosphate and ganciclovir is more effective into the DNA chain of the host cell compared to the incorporation of penciclovir triphosphate and famciclovir. Acyclovir triphosphate, which is 20–30 times more effectively incorporated into the DNA chain of the herpes simplex virus, compared to the chain of the host cell. This is due to the fact that the DNA polymerase of herpes simplex virus is more effective in prolonging the chain with acyclo-NTP and has a lower rate of excision of such a nucleotide. Furthermore, acyclo-ganciclovir triphosphate, incorporated into the chain of elongation of the elongation and elongation of the cell, stop the chain transcription and do not allow filling necessary infections along these fragments with acyclovir triphosphate and ganciclovir triphosphate. From the mechanism of action of the polymerase inhibitor, and the nucleoside analogs it follows that for a number of viral and cellular kinases to attack one to stosikirue c molecule.

3.2. Protease Inhibitors

Proteolytic processing of viral polyproteins and its importance in viral replication and infectivity was recognized in the mid 1980s for retroviruses, flaviviruses, picornaviruses, and more recently coronaviruses. Although HIV-1 is not the only virus for which it is essential to cleave polyproteins, the sequences of highly variable viral processing sites were utilized to demonstrate that potency could be achieved without cross-reactivity and the efficacy of an antiviral drug did not depend on knowing the sequence of the strain being treated. In addition, it was hoped that necessary broad spectrum antiviral activity could be obtained by targeting the processing sites of the oldest, most slowly evolving, and most therapeutically problematical viruses. Since disease syndromes are so highly conserved among lentiviruses, the broad spectrum approach to lentivirus therapy was considered prudent (Anderson et al., 2009). Viral proteases differ from their human homologs in structure, substrate recognition, catalytic mechanism, and allosteric regulation. This has made it possible to develop many potent inhibitors that bind tightly to viral proteases with little cross reactivity to human proteases. Some viruses (HIV-1, HCV) encode proteases that are essential for virus growth. Others simply have the misfortune to encode very efficient proteases that are subject to potent noncompetitive inhibition by small molecules. The structure of no viral protease has as yet been solved free, as a complex with an uncleaved or a folded protein substrate, or bound to an allosteric or noncompetitive inhibitor. Hepatitis A virus processing was shown to be cleaved by caspase 3, the principal apoptosis

executioner in hepatocytes and HAV-infected cells die by apoptosis, caspase 3-mediated HAV processing is an attractive target for therapy.

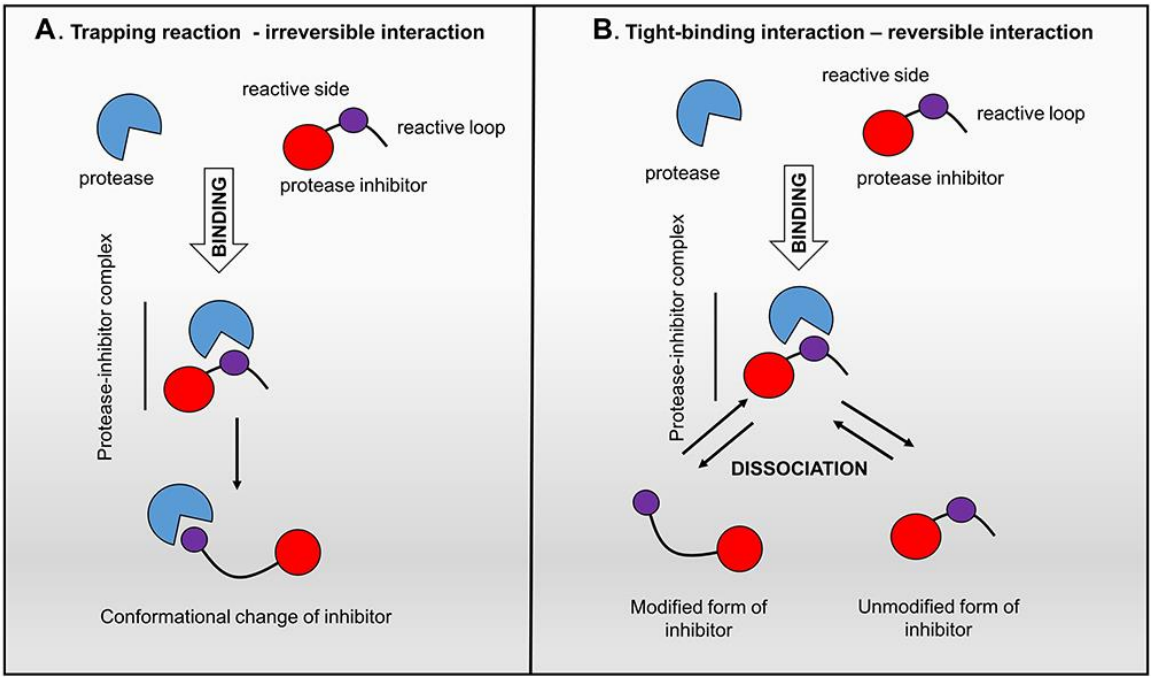


Figure 4: Protease Inhibitors.

3.3. Entry Inhibitors

Progress in the development of antiviral agents over the past 20 years is reviewed with particular attention to molecules directed against recently discovered viral targets that are currently at the investigational stage or are already approved for therapy.

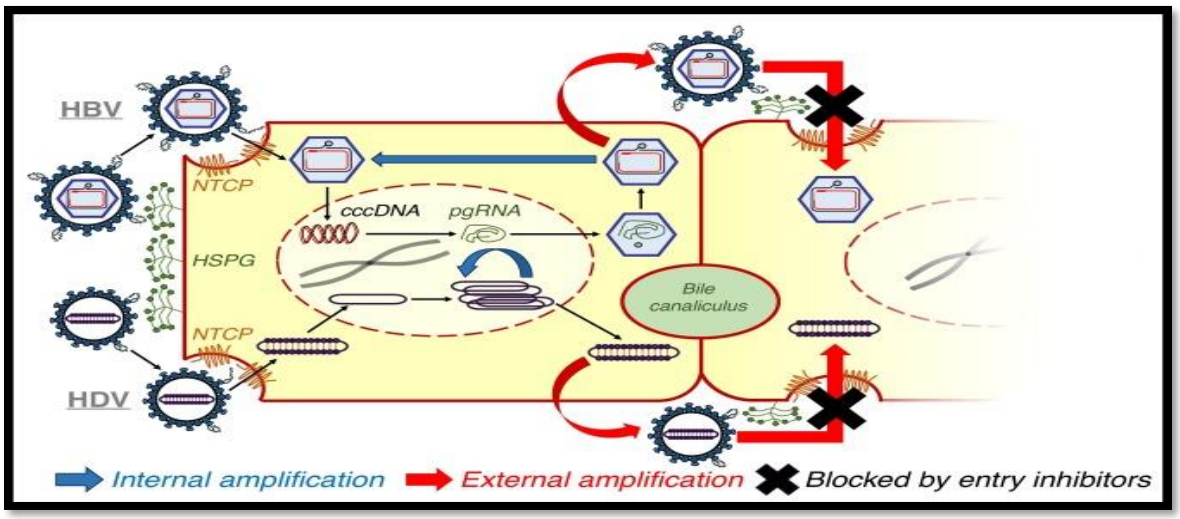


Figure 5: Entry Inhibitors.

Viral entry is the first critical step of the viral multiplication cycle, and targets at this level have been fast to develop. Currently, nine entry inhibitors are on the market: amantadine and rimantadine against influenza viruses, enfuvirtide against HIV, and the squalamine derivative AGE-7, the quinolone derivative WIN51711, and the iminosugar derivatives celgosivir, castanospermine, N-nonyl-deoxynojirimycin, and N-butyl-deoxynojirimycin. Enfuvirtide, a peptide that inhibits HIV-mediated fusion between the viral and cellular membranes by targeting the viral gp41 fusion protein, was the last of these drugs to be approved and represents a new addition to the current antiviral arsenal (Teissier et al., 2010).

3.4. Integrase Inhibitors

HIV-1 IN is an interesting target for drug discovery since it interacts with every viral DNA and has few cellular homologues. The IN proteins from the related retroviruses do not share significant sequence homology outside the zinc binding domains. MLV IN did not substitute for HIV-1 IN in the replication of IN-deleted HIV-1, and inhibitors of HIV-1 IN are not active against MLV IN.

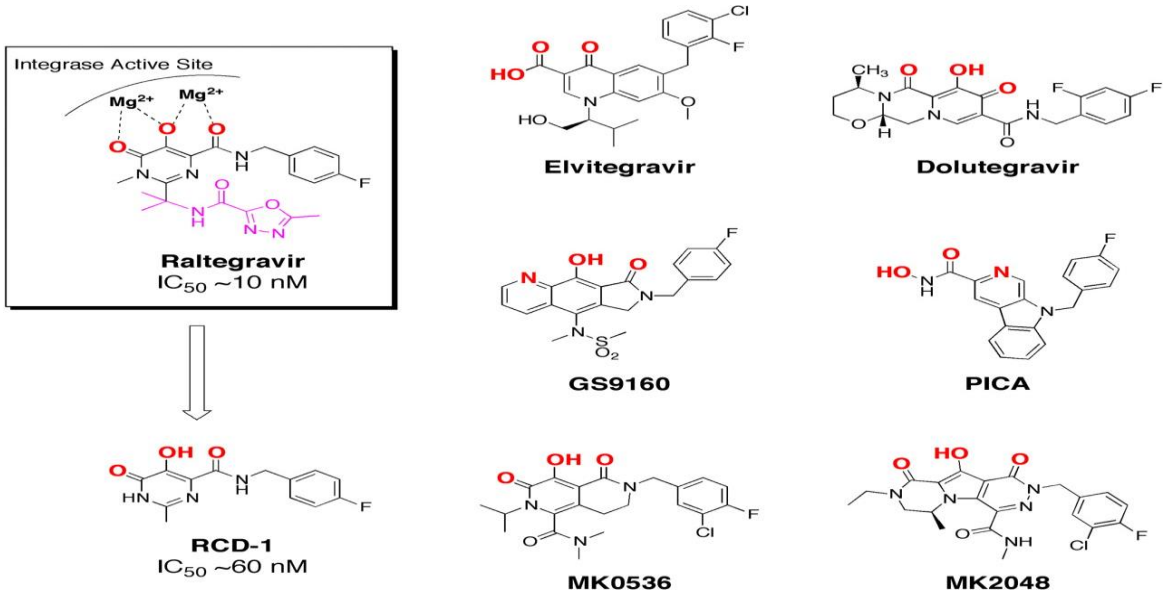


Figure 6: HIV integrase inhibitors.

Furthermore, HIV-1 IN has no cellular homologues although there has been some suggestion that LEDGF may be one putative cellular cofactor. Consequently, inhibitors of HIV-1 IN would be expected to have minimal toxicity. The combination of these properties has made IN a compelling target for drug discovery. Unfortunately, progress has been impeded, in part, by the lack of soluble, stable, purified IN protein available for in vitro activity and/or structure-based drug discovery. In response to this challenge, considerable efforts have been expended

toward the production of such reagents. It is expected that these proteins will significantly accelerate efforts and facilitate the discovery of more potent and less toxic IN inhibitors. Treatments for AIDS act by inhibiting enzymes HIV protease, reverse transcriptase, and integrase. The integrase enzyme is an essential viral enzyme for HIV-1 replication and is required for the integration of viral DNA into the host chromosome. Inhibitors of IN are a recently approved class of antiretroviral agents. Raltegravir is the first clinically approved IN inhibitor and has been shown to decrease viral loads significantly. Understanding the Epstein–Barr virus IN could help the development of drugs that inhibit its activity and may prevent or reduce the occurrence of EBV-associated cancers. Because of their structure-based design, alternative metal ions may be used as potential allosteric-site inhibitors for antiviral therapy of EBV infections.

4. Mechanisms of Action

Antiviral agents are drugs approved for the treatment or control of viral infections. The use of these drugs is limited by the rapid development of antiviral resistance (Müller and Kräusslich, 2009). Most viral infections resolve without specific antiviral treatment; a notable exception is infection with the human immunodeficiency virus (HIV), which currently cannot be cured. A large group of viruses, including a number of retroviruses, herpesviruses, and hepadnaviruses, establish chronic infections that last the lifetime of the host. Often, the virus cannot be removed, but the infection may have long asymptomatic phases and the emergence of disease is slow and may be prevented or attenuated by the host immune defense. Moreover, many viruses will remain latent as long as the immune system of the host is intact. Suppression or modulation of immune functions, as, for example, in the case of organ transplant recipients, is frequently associated with a reactivation of latent viral infections. An ideal antiviral agent should be effective against both actively replicating and latent viruses (Paintsil and Cheng, 2009). Most currently available antiviral agents, however, are effective against only replicating viruses because of the close interdependence of viral replication and the host cell. This is particularly true for small DNA viruses, such as HPV, which encode only a limited number of viral proteins and make extensive use of the host cell machinery to complete their life cycle. Hence, in the case of HPV-induced diseases, removal of the virus does not necessarily lead to complete removal of the malignant cells. This is one of the reasons why existing antiviral drugs exhibit tumor growth inhibitory but not tumor cell killing capacities. In general, no antiviral agent available to-date is capable of overcoming a persistent viral infection. After treatment cessation, virus production usually resumes and the infection will re-establish as soon as the drug concentration falls below effective levels. Communally, several lines of evidence

suggest that an adjunctive therapeutic strategy combining antiviral drugs and "mini-haart" potentially improves treatment outcome for intraepithelial neoplasia.

4.1. Inhibition of Viral Entry

The entry of enveloped viruses into their target cell is the first step of the viral life cycle and an essential prerequisite for a productive infection (Teissier et al., 2010). Entry involves several successive steps and is amenable to therapeutic intervention at multiple points, both early in the process and late after virus binding to the cell surface (Badani et al., 2014). Entry inhibitors act by targeting viral and/or cellular components and can be divided into two broad categories. The first targets steps involving protein-carbohydrate, protein-protein, or protein-lipid interactions, while the second inhibits virus infectivity by some non-specific aggregation or other direct but not sequence-specific effects on the viral particle. The latter group includes, but is not limited to, peptides with highly cationic sequences, peptides not expected to interact in a specific sequence-specific manner, or sequence-unspecified peptides or small molecules with direct detergent-like or membrane-lytic actions on lipid membrane.

For each of the two categories of entry inhibitors listed above, there are several reported instances of the inhibition of unrelated viruses by otherwise ineffective peptides or other small molecules. While it is very easy and common to find examples of inhibition of unrelated or under-related viruses by an inhibitor of interest, a broad range of unrelated or under-related viruses should be tested in efforts to find if a single inhibitor provides generic inhibition. A good analysis of this potential for peptide entry inhibitors on influenza was reported. For a comprehensive view of the efficacies of eighty-four peptide entry inhibitors against nineteen unrelated enveloped viruses, not including influenza, considering that sequence unrelated peptides are expected to be more likely to inhibit unrelated viruses by abideing trade inhibition or by generic activity.

4.2. Inhibition of Viral Replication

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4.3. Inhibition of Viral Release

In recent years, constant search has been maintained for inhibitors that can efficiently target different steps in the viral life cycle. The intention is to gain a useful therapeutic interest while minimizing possible resistance. Despite progress in drug design, development of novel medication is complex, time consuming, and often associated with high costs. Therefore, adverse effects must be considered and avoided as far as possible (Hozáková et al., 2022). To date, the most prevalent

inhibitors either affect production and maturation of new virions or interfere with the functions of the viral genome inside host cells. Inhibit assembly of newly made virions can act on important enzymes which are able to prevent genome replication. Another effective approach is to interfere with the functions of the viral genome after infection or to prevent the translation of viral proteins. Similarly, the targeting of other viral proteins, which contribute to the suppression of host cell defense, may be promising.

Viral capsid also plays an important role in the protection of viral genome from hostile cell environment combined with its function in the transport of the genetic information to another infected cell. To guarantee this, the replication of virions usually requires the suite of several capsid proteins. The inhibitor of the intact viral capsid might therefore constitute an additional group of anti-viral agents. Unfortunately, efferent inhibitors targeting this protein are still rare. Until recently, the experimental evidence of capsid protein capable of virion assembly comprises only several viral enzymes, for example nucleotide polymerases or integrases. These enzymes could be targeted by specific chemical inhibitors, but capsid structure usually contains no enzymatic center. The development of effective antivirals could otherwise be directed towards viral capsids. Capsid inhibition might feature low side effect and high therapeutic efficacy.

5. Resistance to Antiviral Agents

Viral evolution has many important public health consequences (K. Irwin et al., 2016) and is being increasingly investigated within a population genetic framework. Various parameters, such as nucleotide diversity, the strength of selection, and the effective population size (Ne), can be monitored almost in real time. Current efforts are focused on the development of methods to infer epidemiological parameters, such as the infection date, the origin, and direction of transmission of an outbreak. In the absence of treatment, such estimates can inform public health strategies to block (or facilitate) the spread of the disease.

However, within-host viral evolution is more difficult to study and is more typically investigated using sequential samples collected from a patient undergoing antiviral treatment. The evolutionary dynamics of drug resistance during untreated infection are far less understood. An increasing number of viral infections are treated with antiviral drugs that target various mechanisms of viral replication. If the treatment is not effective and some new viral genomes replicate, selective pressure may cause the virus to rapidly adapt toward drug resistance. The rate at which new drug resistance mutations appear is determined by both viral and drug properties, including the class of the drug, the specific molecular target, the viral population

size, and genetic diversity. Unlike free-living organisms, resistance development may proceed at a faster rate in viruses, as they typically exist in an infected host at a much larger population size than bacteria or fungi.

However, the spread of drug-resistant variants among hosts may still be slowed by drug treatment, providing the rationale for combining treatment with two unrelated drugs. This approach is widely used in the case of HIV, and resistant strains would have to evolve multiple resistance mutations simultaneously, which constitutes a much stronger genetic barrier to resistance development. All of these questions have motivated the recent development of experimental systems of virus evolution to use cell culture viral replication and treatment with the nucleoside analog inhibitor. Using herpes simplex virus type 1 (HSV-1) carrying a mutation in the viral polymerase non-catalytic subunit gene that confers high resistance, it was possible to routinely generate resistant clones and study their properties. The HSV-1 system was analyzed to search for evidence regarding initial resistance allele frequencies in HSV-1 populations.

6. Development and Discovery of Antiviral Agents

In spite of the significant advances in the field of antiviral drugs, there are currently limited treatment options for viral infections, such as influenza, rubella, yellow fever, smallpox, and avian influenza, among others. However, new and effective treatments for most viruses, such as RNA viruses or human immunodeficiency virus, will be available with the advent of modern technology, including molecular biology, combinatorial chemistry, and computer-aided design of compounds or protective antigens targeting various enzyme inhibitions of the viral life cycle. Vaccination is another effective method of preventing viral infections, and the global market for this is expected to reach nearly $70 billion in 2012. However, vaccine development against emergent viruses has its own sets of difficulties. Resorting to currently available vaccines has certain disadvantages. Treating infected persons with protective antigens is one alternative. Another approach is the development of antiviral small molecule compounds. Such therapies have the advantage of being able to treat viral infections even after the exposure of the host to the virus. In this review, novel approaches to antiviral agents are described including ribozymes, lyase, and RNA aptamers. Furthermore, the development of other potent agents such as viral and host protease inhibitors, as well as dU analogues, is also discussed (Paintsil and Cheng, 2009).

6.1. Traditional Drug Discovery Methods

In the struggle to develop novel antiviral agents, both synthetic or phytochemical sources remain promising. Nonetheless, continuous investigations of herbal

extracts, prioritizing those that are used in traditional medicine, might reveal further antiviral agents.

The development of novel antiviral drugs against both human and animal viruses has deserved great curiosity in the past decades. Plant-derived chemicals have been used as remedies for the prevention and cure of numerous diseases including viral infections for thousands of years, well before the universal application of the current synthetic pharmaceuticals. While the antiviral properties of only a minor portion of the plant species have been investigated, a rapid increase in reports on the activity of natural products against diverse viruses has been observed in the last two decades. During this time, more than natural chemicals from various sources, and particularly from plants, were documented to exhibit in vitro or in vivo anti-viral activity. Natural agents have been discovered that restrain viral binding, fusion, entry and replications, as well as activate host-mediated defence mechanisms. Both the direct anti-viral activity of plant-derived chemicals such as alkaloids, saponins, tannins, flavonoids, and essential oils, and the agents that many directly enhance the resistance of vertebrates against infection, such as nucleosides, nonspecific immunomodulating agents and interferon inducers have been reported. It is thought that only part of the wealthy resources of traditional medicine was examined for the development of potent anti-viral compounds. Notably, investigations into the antiviral activity of multiple agents used only in the traditional drug should be intensified.

6.2. Computer-Aided Drug Design

The antiviral activity of a compound is based on its ability to counteract the pathogenesis of viral pathogens upon cellular infection.

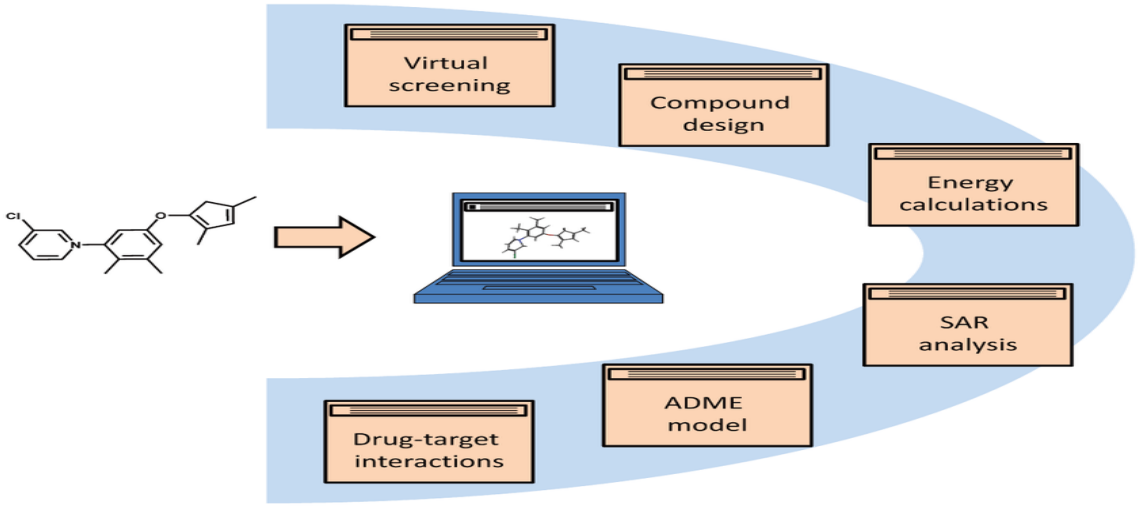

Figure 7: Computer-Aided Drug Design.

Antivirals aim to target diverse stages of the virus's life cycle and inhibit primary cellular mechanisms for the viral replication process. Table I presents antivirals acting at different key stages of the virus's life cycle and viral life cycle stages that might be targeted by antivirals with the potential to guide the design of new chemical entities (Diakou et al., 2022). Drug discovery has dramatically changed upon the development of antivirals. Traditional drug discovery is not commonly used at present and has been replaced by the emergence of rational drug design methodologies, especially by the advent of computer-aided drug design techniques. Antiviral CADD usually encompasses ligand-based drug design methods, whereas structure-based drug design approaches are mainly used to develop drugs for diseases other than viral infections.

7. Adverse Effects and Toxicity

Till the date of the writing of this chapter, about 12 targeted antiviral drugs and 2 broad-spectrum antiviral drugs have been approved for human therapeutic use. These include drugs mainly for treatment of human immunodeficiency virus, hepatitis B/C, herpesvirus, and influenza. Currently, research on antiviral agents is one of the rapidly increasing areas of study, with an increasing number of antiviral drugs under development. Most current antiviral drugs target the earlier stage of the viral life cycle, for instance fusion, entry inhibitors or reverse transcriptase inhibitors are mainly used to prevent the occurrence of a viral infection, and cannot successfully kill the infected virus-producing cells. In addition, these kinds of antiviral drugs are generally used within a wider time window, as compared to treatment of a targeted drug during the later infection stage. As a result, viruses can develop drug resistance to these drugs more easily.

Despite being studied for many years, new antiviral targets and drugs are still urgently needed. It is believed that, with advancement in the understanding of viral life cycles, there are numerous novel human-virus interactions that can be exploited as attractive new antiviral targets. Screening the small molecular drugs for compounds that functionally interact with these hosts could potentially result in repurposing existing drugs for antiviral therapies. The combination of these drugs with currently used antiviral agents might enable a broad-spectrum antiviral effect for even newly emerging viruses and are expected to exert their effects with rapid kinetics. Combinations of such drugs plus very early treatment post-infection can possibly yield a “cure” in which no latent or sleeper virus remains and in which viral resurgence post-drug cessation is deterred.

8. Future Perspectives and Emerging Trends

Over and above the conventional strategies the new era challenges and needs arising techniques and therapies to combat the threats. Despite the intensive research going on around the world, the future in antivirals relies probably on unveiling some of the most peculiar and discrete mechanisms leading to emerge for currently overlooked aspects of viruses and their long-lived interactions with hosts. Emerging trends in antiviral agents range from discovering previously neglected principles leading to states and conditions promoting a latent period, rather than replication, of invading viruses, thus buying time for the onset of an immune response, to the development of essentially novel, even nanoscale agents, attacking viruses by modes unmet before and leaving the host cells untouched. These strategies are accompanied by therapies influencing primarily hosts by tuning the enticingly complex interplay between viruses and their carriers, already tested by Nature passing on such interaction, beneficially, through the eons. The evolution of the emerging approaches is highlighted on experiments on a lynchpin pathogenic agent, the herpes simplex virus that also well illustrates ever-increasing difficulties in controlling emerging, multi-drug-resistant infections by life-long latent carriers. This study is designed as an inspirational introduction favoring wider research in probably the most intriguing and least understood domains of modern biology.

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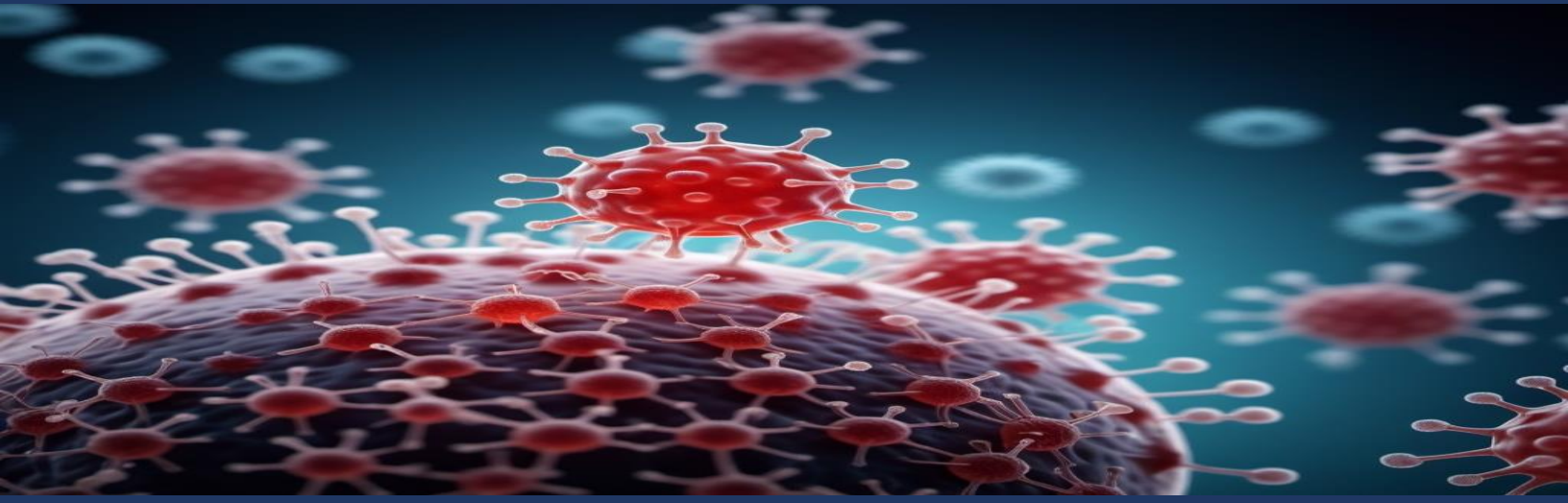

Microbiology plays a crucial role in pharmaceutical sciences, providing essential knowledge about microorganisms and their impact on human health. This book is designed specifically for pharmacy students, offering a clear and concise understanding of microbial biology, pathogenic mechanisms, and their relevance to drug development. It covers fundamental topics such as bacterial classification, antimicrobial resistance, and infection control, with a focus on real-world pharmaceutical applications. There is an urgent need to rewrite the basic principles of science from time to time. In light of the technological changes and developments we are witnessing today, we have noticed a strong need to attempt to rewrite the basic principles of microbiology, especially for pharmacy technology students. In doing so, we seek to simplify these principles and also ensure they cover all the general concepts they need in their practical lives.