UNIVERSITÀ
DI PARMA

Dipartimento di Scienze Medico Veterinarie

Corso di Laurea Magistrale in

Produzioni animali innovative e sostenibili

ANTIBIOTIC RESISTANCE AND ALTERNATIVE THERAPEUTIC PROPOSALS

Relatore:
Chiar.ma Prof.ssa MARIA CRISTINA OSSIPRANDI

Laureanda:
MARTINA AZZANO

ANNO ACCADEMICO 2022/2023

To those who always believed in me, I love you.
To myself, long story short, I survived.

SUMMARY

ABSTRACT

Antibiotic resistance is a global problem for which a solution is urgently needed. Since the 1928 discovery of antibiotics by Alexander Fleming, these pharmacologically active molecules have been increasingly used resulting in the emergence of the phenomenon also known as antibiotic resistance. This problem could lead by 2050 to more than 10 million deaths which will exceed the 8.2 million deaths given by cancer. It can be seen, in any case, that an antibiotic, sooner or later will lose its effectiveness given to the development of resistance. In addition, the problem arises from the inappropriate use of antibiotics due to the fact that, in many cases, are administered wrongly, when there is no need, or because the patient does not comply with the treatment prescribed by the doctor (be it a doctor or a vet); in developing countries, the problem arises when drugs are not available and therefore expired ones are administered or drugs are sold over the counter. In addition, the problem may also manifest from inadequate use of antibiotics in livestock animals, which, for both therapeutic and preventive reasons, leads to an increasingly critical situation as the selection of pathogens resistant to any antibiotic molecule is encouraged. Another key point inherent in antibiotic resistance is the ability of bacteria to form a biofilm, which correspond to an aggregation of microorganisms that are strategically used as a form of protection against antibiotics. This makes the work of antibiotics, especially conventional ones, more difficult; therefore, alternative therapies need to be developed as soon as possible. In addition to these, antibiotic resistance can also be combated with an approach that is called "One Health," which involves interdisciplinary collaboration and cooperation. It promotes the idea that to ensure good health and well-being of people, animals, and environment, the health professions involved must also collaborate by making more targeted and just use of antibiotics in both human and animal therapy.
To solve the problem given by antibiotic resistance, we can no longer rely solely on the use of antibiotics but must focus on the discovery of alternative therapies. Scientists have been active

in working on as many innovative therapies as possible, those that have been brought forward are those that employ bacteriophages, nanoparticles, and antimicrobial peptides. These used singly or together with the use of antibiotics could lead, if not to a resolution of the problem, at least to a breakthrough.

RIASSUNTO

L'antibiotico resistenza è un problema globale per il quale urge trovare, in tempi rapidi una soluzione. Dalla scoperta degli antibiotici, avvenuta del 1928 da Alexander Fleming, questi sono stati sempre più utilizzati con la conseguente comparsa del fenomeno altresì noto come antibiotico resistenza. Questo problema potrebbe portare, entro al 2050 a più di 10 milioni di decessi che supereranno gli 8.2 milioni di decessi riportabili a forme tumorali. È possibile constatare, in ogni caso, che un antibiotico, prima o poi perderà la sua efficacia data dallo sviluppo di resistenza (adattamento biologico del microrganismo come linea di difesa). Il problema sorge, inoltre, dall'inadeguato utilizzo degli antibiotici che molte volte, vengono somministrati erroneamente, quando non vi è necessità, o perché il paziente non rispetta la cura prescritta dal medico oppure nei paesi in via di sviluppo il problema sorge nel momento in cui i farmaci non sono disponibili e dunque vengono somministrati quelli scaduti oppure vengono venduti farmaci "sotto banco" (senza alcuna indicazione terapeutica). In più, il problema può derivare anche da un inadeguato utilizzo di antibiotici negli animali da reddito, che, sia per motivi terapeutici che per motivi preventivi, portano a una situazione sempre più critica in quanto si favorisce la selezione di patogeni resistenti ad ogni molecola antibiotica. Altro elemento fondamentale inerente all'antibiotico resistenza è la capacità dei batteri di formare il biofilm, ovvero un'aggregazione di microrganismi che viene usata come forma di protezione dagli stessi. Ciò rende più difficoltoso il "lavoro" degli antibiotici, soprattutto quelli convenzionali; per questo motivo è necessario sviluppare terapie alternative. Oltre a queste, l'antibiotico resistenza può anche essere combattuto con un approccio che viene definito "One Health" che prevede una collaborazione interdisciplinare. Promuove l'idea che per assicurare una buona salute e il benessere di persone, animali e ambiente, le professioni sanitarie (medicina umana come pure medicina veterinaria) devono collaborare anche facendo un uso più mirato e corretto degli antibiotici sia in terapia umana che animale.

Per risolvere il problema dato dall'antibiotico resistenza non ci si può più solamente appoggiare all'utilizzo degli antibiotici ma è necessario concentrarsi sulla scoperta di terapie alternative. Gli scienziati si sono alacremente attivati per lavorare su più terapie innovative possibili, quelle che sono state portate avanti sono quelle che impiegano i batteriofagi, le nanoparticelle e i peptidi antimicrobici. Questi utilizzati singolarmente o in associazione all'uso di antibiotici potrebbero portare, se non alla risoluzione del problema, almeno a una svolta.

INTRODUCTION

Since their discovery, antibiotics have proved to be a breakthrough in medicine and the treatment of infections and diseases. Unfortunately, the misuse of these life-saving drugs has led to the development, by bacteria themselves, of resistance to antibiotic activity causing what can be considered a global problem, namely antibiotic resistance or AMR.

The purpose of this paper is to bring attention to the development of alternative therapies to the use of antibiotics and to raise awareness of the moderate and conscious use of drugs.

The first chapter will discuss antibiotics, their discovery, and their development. It will focus on to the main classes of antibiotics including β-lactams, which are the most widely used products. the most important antibiotic discovered and used is penicillin, which is, indeed, part of the β-lactam antibiotics. Further antibiotic class is the macrolides which were discovered in 1952 and have a broader spectrum of action than the penicillins; of this class the most widely used molecule is azithromycin. The third and final class is represented by the tetracyclines

In the second chapter, the problem of antibiotic resistance (AMR) will be addressed, highlighting what are the main causes that lead to the occurrence of this problem, among which overuse, inadequate prescription, and livestock use (by consuming the meat of these animals, people can acquire microorganisms that may or may not be pathogenic but still exhibit antibiotic resistance) are recognized. AMR is certainly a process that occurs naturally by bacteria, but the main source of the problem of uncontrollable AMR dispersal is overuse. Attention has been paid to the development of biofilms by bacteria that result in antibiotic resistance; of a certain interest is the abuse of antibiotics during the COVID-19 pandemic which further contributed to the dispersion of AMR . One of the ways to fight AMR is definitely that given by the "One Health" perspective where all sanitary disciplines (human and veterinary medicine) must coordinate to solve the problem.

The third and final chapter will examine various possible alternative therapies including the use of bacteriophages, antimicrobial peptides, and nanoparticles (silver, zinc, titanium, and iron).

1. WHAT IS AN ANTIBIOTIC?

The introduction of antibiotics into clinical (Figure 1) use was arguably the greatest medical breakthrough of the 20th century. [1, 2]

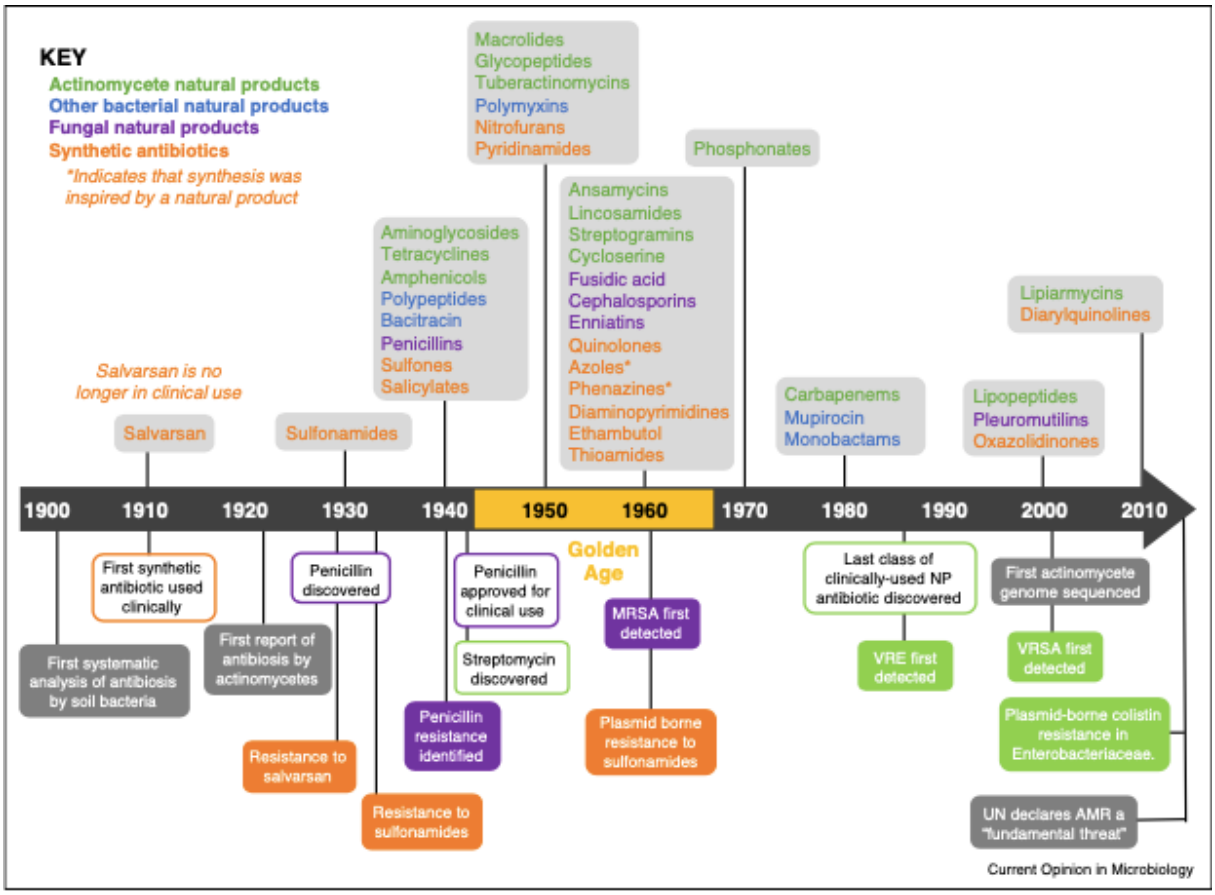

Figure 1 - Timeline showing the decade new classes of antibiotics reached the clinic. The antibiotics are colored per their source: green = actinomycetes, blue = other bacteria, purple = fungi, and orange = synthetic. At the bottom of the timeline are key dates relating to antibiotic discovery and antimicrobial resistance, including the first reports of drug-resistant strains methicillin-resistant S. aureus (MRSA), vancomycin-resistant enterococci (VRE), vancomycin-resistant S. aureus (VRSA) and plasmid-borne colistin resistance in Enterobacteriaceae. [1]

The term antibiotic was coined from the word „antibiosis" which means, literally, „against life". In the past, antibiotics were considered to be organic compounds produced by one microorganism which are toxic to other microorganisms. [3, 4]

An antibiotic is a substance produced by a microorganism, capable of killing others; they are often used to treat or prevent bacterial diffusion and next infections. They can be considered as life-saving treatments that transformed medicine; it all started with the discovery

of penicillin by Alexander Fleming in 1928. Indeed, it has been estimated that the widespread availability of antibiotics has added 30 years to human life expectancy in developed countries. [5, 6]

The use of antibiotic-producing bacteria to prevent disease stretches back millennia, with traditional poultices of moldy bread being used to treat open wounds in Serbia, China, Greece, and Egypt more than 2000 years ago. An Anglo-Saxon recipe from 1000 years ago was also recently shown to kill MRSA (methicillin-resistant Staphylococcus aureus). [1, 7]

Many antibiotics show a broad spectrum of activity which means that they can be effective against both Gram-positive and Gram-negative bacteria. Since the discovery of these drugs, there has been a question asked: “What is an ideal antibiotic?”. While specific circumstances may vary, philosophically, an ideal antibiotic is an antibacterial agent that kills or inhibits the growth of harmful bacteria in a host regardless of site of infection without affecting beneficial microbes (gut/skin flora) or causing undue toxicity to the host with low potential for resistance. [5]

Whatever the scenario, an antibiotic would eventually lose its effectiveness due to the development of resistance, whether it was optimal or not. Unlike drugs used to treat non-infectious diseases, antibiotics have a short effective life due to the resistance developed. As a result, an ideal antibiotic will never last forever and will eventually need to be replaced with a newer one when the old one stops working.

Between 2000 and 2010, worldwide consumption of antibiotics by humans increased by 36%, with Brazil, Russia, India, China, and South Africa (BRICS) accounting for three-quarters of this increase despite collectively representing only 40% of the world’s population. [8]

Antibiotic consumption in hospitals is increasing rapidly in China, which accounted for 57% of the increase in hospital sales of antibiotics in the BRICS countries. The pattern of antibiotic consumption has shifted towards newer broad-spectrum antibiotics, including cephalosporins, broad-spectrum penicillin, and fluoroquinolones. [6]

Since their clinical implementation eight decades ago, antibiotics have become the foundation of modern medicine. However, their continued efficacy is threatened by the global dissemination of antibiotic-resistance determinants, driven in large part by improper use of antibiotics in clinical, community, and agricultural settings. [9] To develop effective next-generation antibacterial therapies, it is imperative that we gain a more thorough understanding of how bacteria respond to antibiotics and leverage this understanding toward the development of treatments that expand drug efficacy beyond the current state of the art. [10]

Three hypotheses can be used to describe antibiotic effectiveness in bacterial metabolism:

1. Antibiotics alter the metabolic state of bacteria, which contributes to the resulting death or stasis.
2. The metabolic state of bacteria influences their susceptibility to antibiotics.
3. Antibiotic efficacy can be enhanced by altering the metabolic state of bacteria.

It is believed that these postulates unify decades of independent observations into a mechanistically coherent framework, which will allow for the more rational development of antibiotics and synergistic therapeutic combinations going forward. [10]

There is an immediate requirement for new antibiotics due to the threat given by the rise of highly resistant microorganisms, but the discovery of new antibiotics is becoming increasingly difficult. Natural product discovery is now plagued by the dereplication problem, wherein the same molecules are being repeatedly discovered. [11] Novel approaches to antibiotic discovery are needed to increase the rate at which new antibiotics are identified and simultaneously decrease the associated cost of early lead discovery. [12]

Three different approaches have been applied for effective drug discovery programs:

1. Historically, substances, crude extracts, or purified chemicals were screened for biological activity mostly in whole cell assays without knowing the drug target. Only after an active substance has been identified, serious efforts have been made to analyze the target and the mode of action of the compound.

2. Another approach in identifying new drug substances is denominated as *chemical screening*, which aims to identify novel, chemically diverse molecules without considering their biological activity. The substances used in this approach can originate from biological sources (such as metabolites produced by microorganisms) or chemical libraries.
3. In contrast to chemical screening, the *target-oriented screening* aims to identify compounds that hit a known and validated molecular target. Thereby, the target represents a cellular or molecular structure involved in the pathology of interest that the drug-in-development is meant to act on. Different attributes of the target have to be taken into account. [13]

The primary drawback of these approaches was the high number of metabolites that were continually found. It is assumed that most of the molecules have been already discovered and only a few new metabolites are left to be identified. The inability of many bacteria to be cultivated under standard laboratory settings is a major barrier to the identification of new species. A wide variety of previously uncultivable bacteria can now be grown, thanks to several fresh and ground-breaking cultivations techniques that have emerged in recent years.

In addition to the progress in finding new secondary metabolites, also new ideas came up, and old strategies were revived, which may help in the search for novel compounds or in their more effective application.

- In order to overcome the resistance problem, compounds that do not kill the pathogen but only prevent its pathogenic action may be used. This will reduce the pressure on the pathogen to acquire mutations, which will allow it to survive in the presence of the drug. This approach that we could define as the creation of an "anti-virulent" state can be used in combination with "classical" antibiotics.

- Another strategy to reduce the occurrence of resistance is the development of new drug combinations. This has been proven to be successful in the treatment of tuberculosis and HIV but is not a routine procedure in other medicating infections.
- Many of the most effective drugs used in human therapy have more than one target in the bacterial cell. A criterion for the future selection of drug candidates may be, therefore, the interaction with more than one target. The newly emerging research field of 'cell biology of antibiotic action' will deliver a deeper understanding on the mode of action of many antibiotics. This should enable the development of new approaches for the search for novel antibiotics.
- According to recent predictions, Gram-negative pathogens will constitute a major threat in the future. Although Gram-positives presently cause the majority of deaths in clinics, a greater arsenal is required to combat Gram-negatives. A critical step which prevents the application of many compounds in Gram-negatives is the passage across the outer membrane. Here, the combination of known antibiotics with substances, which permeabilize the outer membranes, may help. Alternatively, antibiotics can be combined with siderophore structures that promote the uptake. This 'trojan horse' strategy has successfully been applied in model systems. [13]

Undoubtedly, we will require new antibiotics in the future, and the best source is likely to be natural products o alternative therapies (such as bacteriophages, active peptides). Worldwide, scientists will eventually develop new technologies, and this will result in the discovery of new metabolites in the next years. While academics have largely been the source of developing these new tools and technologies, academia and industry will need to collaborate to produce the vast quantities of chemicals necessary to create an antibiotic that is effective enough to be used in hospitals.

The Gram-positive bacteria consists of a cytoplasmic membrane surrounded by a tough and rigid mesh called a cell wall. In contrast, Gram-negative bacteria consist of a thin cell wall

that is surrounded by a second lipid membrane called outer membrane (OM). The space between the OM and cytoplasmic membrane is referred as periplasm (Figure 2). The OM is an additional protective layer in Gram-negative bacteria and prevents many substances from entering the bacterium. The cell wall is a tough layer that gives bacterium a characteristic shape and prevents it from osmotic and mechanical stresses. The cytoplasmatic membrane prevents ions from flowing into or out of the cell and maintains the cytoplasmatic and bacterial components in a defined space. [14]

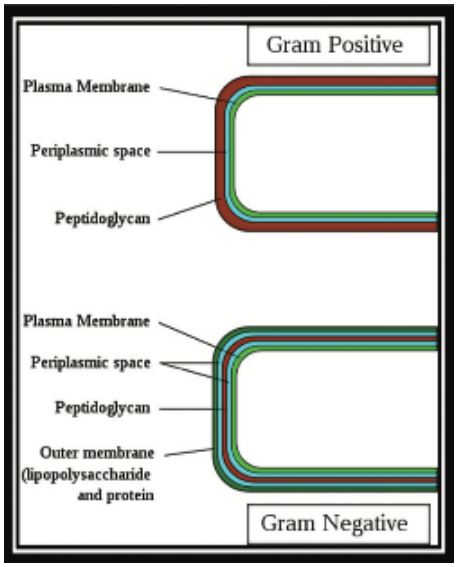

Figure 2 – Structure of bacterial cell envelope [14]

The different cell walls allow identifying Gram-negative and Gram-positive bacteria through Gram staining. Compared to Gram-negative bacteria, Gram-positive bacteria have thicker and more robust cell walls because they contain more peptidoglycan (PNG). The PNG is located near the cell exterior. The decolorizing agent, typically ethanol, used for washing after the dye has been employed is crucial. The PNG on the wall condenses and dehydrates when the excess dye is removed with ethanol, preserving the complex that produces the color. As a result, Gram-positive bacteria acquire their distinctive purple color, but Gram-negative bacteria, whose PNG content is lower, are unable to hold onto the dye complex.

There are various classifications and categories for antibiotics, but the most popular ones are based on their chemical structures, modes of action, and spectrum of activity. There are other classification schemes as well.

1.1 BETA-LACTAMS

Members of this class of antibiotics contain a 3-carbon and 1-nitrogen ring that is highly reactive (Figures 3 and 4). They interfere with proteins essential for synthesis of bacterial cell wall, and in the process either kills or inhibits their growth. More succinctly, certain bacterial enzymes termed penicillin-binding protein (PBP) are responsible for cross-linking peptide units during synthesis of peptidoglycan. Members of beta-lactam antibiotics are able to bind themselves to these PBP enzymes, and in the process, they interfere with the synthesis of peptidoglycan resulting to lysis and cell death. [3, 15]

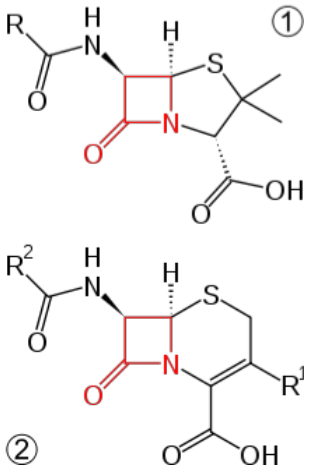

Figure 3 – Chemical structure of beta-lactam structure. Core structure of penicillin (top) and cephalosporins (bottom). [16]

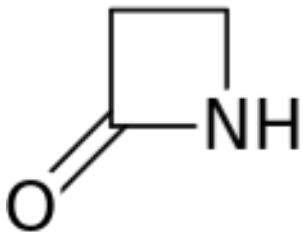

Figure 4 – Chemical structure of a beta-lactam ring. [17]

1.1.1 Penicillin

Penicillins are the first antibiotic that has ever been discovered in 1928 by Alexander Fleming. Particularly Penicillin G was the first ever produced. Although Penicillin G was discovered by Alexander Fleming in the 1920s, it took the efforts of several other workers such as Ernst Chain, Edward Abraham, Norman Heatley, and Howard Florey in 1945 to understand the cultural requirements of the fungus and its clinical effectiveness. [3]

Although Penicillin G was originally discovered and isolated from the fungus *Penicillum notatum* by Fleming, a close relative *Penicillun chrysogenum* is the preferred choice of source. [3] Penicillin G has a narrow antimicrobial spectrum. It is active with respect to Gram-positive bacteria (*Staphylococcus, Streptococcus,* and *Pneumococci*) and *Bacillus anthracis*, while Gram-negative bacteria are resistant to it. [18] Another Gram-negative bacteria sensitive to Penicillin G is *Treponema pallidum* which causes syphilis.

A great concern is the *Staphylococcus aureus* which is Penicillin G resistant. The basis of *S. aureus* resistance to Penicillin G was elucidated by Kirby in 1944, [19] who demonstrated the capacity of bacterial enzymes to hydrolyze the β-lactam ring of Penicillin G, inactivating it. These enzymes were denominated β-lactamases. [19] The first semi-synthetic penicillin with clinical efficacy against Penicillin G-resistant *S. aureus* was methicillin. [3] Methicillin cannot be taken orally by men because it is unstable in acidic conditions, and it is not absorbed by them. Gram-negative bacteria's outer membrane resists its easy diffusion. *S. aureus* methicillin-resistant (MRSA) should be regarded as potentially resistant to additional penicillin and cephalosporins.

1.1.2 Cephalosporins

In terms of both structure (Figure 5) and method of action, cephalosporins, and penicillin are comparable; they are among the most often recommended and used antibiotics. The first known member of this group of antibiotics was first isolated by Guiseppe Brotzu in 1945 from the fungus *Cephalosporium acremonium*. Although the drug was first isolated by Guiseppe Brotzu, it was Edward Abraham who got the credit to patent it having been able to extract the compound. [3]

Cephalosporins are alternative β-lactam antibiotics, which are highly effective and commonly used for mild to severe infectious diseases. [20]

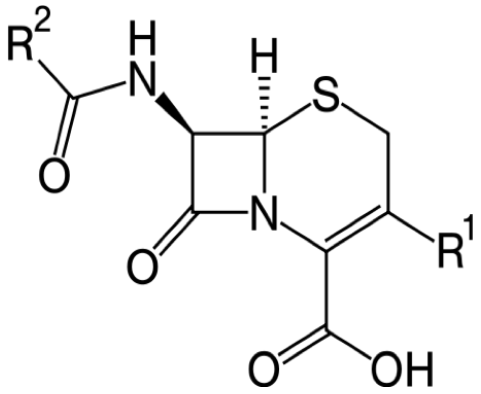

Figure 5 – Structure of Cephalosporins [21]

Semi-synthetic cephalosporins are broadly active against both Gram-positive and Gram-negative bacteria, and they are synthesized based on the bicyclic nucleus consisting of a six-membered dihydrothiazine ring attached to a beta-lactam ring. Two carbons of the cephalosporin scaffold, C3, and C7, confer a huge possibility for introducing variable side chains that significantly extend antibacterial activities as well as enhance the structural stability against beta-lactamases.

Cephalosporin C (CPC) was the first cephalosporin antibiotic compound isolated. [20] CPCs are crucial for preventing and treating infectious disorders that affect skin, ears, and bones, as well as urinary and upper respiratory infections.

Their subdivision is based on the target organisms, and they go from 1st to 5th generation according to their effectiveness against Gram-negative bacteria.

1.1.3 Monobactams

The discovery of this antibiotic was found in *Chromobacterium violaceum*. In this case, as opposed to regular β-lactams, the β-lactam ring is positioned alone and not bonded to another ring (Figure 6).

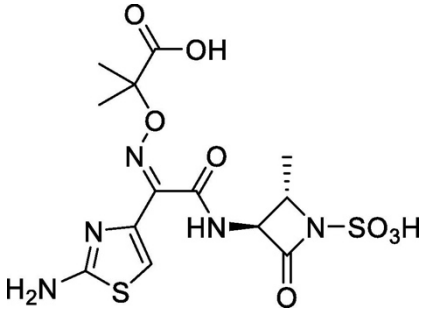

Figure 6 – Structure of Monobactams. [22]

Aztreonam is the only monobactam that is marketed and only works against aerobic Gram-negative bacteria (e.g., *Pseudomonas*). Aztreonam has been used alone or in combination with other antibiotics to treat a wide variety of infections, which include urinary tract infections, lower respiratory infections, intra-abdominal and gynecological infections, and skin/ soft tissue infections. [23]

1.1.4 Carbapenems

Carbapenems represent one of the most important classes of β-lactam antibiotics. They have a penicillin-like five-membered ring, but the sulfur at C-1 in the five-membered ring is replaced with a carbon atom, and a double bond between C-2 and C-3 is introduced. [24]

(Figure 7) They are relatively resistant to hydrolysis by most β-lactamases, being able in some cases to act as slow substrates or inhibitors of β-lactamases. They penetrate the outer membranes of Gram-negative bacteria through porin channels and target penicillin-binding proteins. They have broad-spectrum antibacterial activity against a variety of Gram-negative and Gram-positive pathogens. [18, 25]

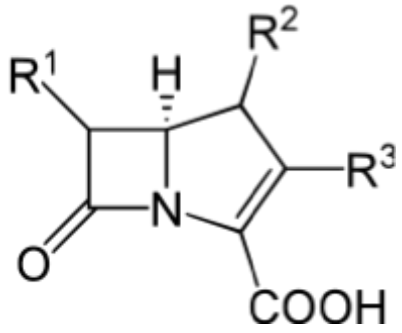

Figure 7 – Structure of Carbapenem [25]

This group of antibiotics was discovered in 1976. Due to the advent of β-lactamase in bacteria, before this, in the late 1960s, penicillin's efficacy was seriously endangered. Resistance to penicillin was given to bacteria via bacterial β-lactamases. This circumstance prompted researchers to start a broad search for β-lactamase inhibitors. The breakthrough came precisely in 1976 when olivanic acid (produced by Gram-negative *Streptomyces claveligerus*) was found to be a β-lactamase inhibitor. Sadly, olivanic acid was not chemically stable, so researchers had to keep working until they found two more beta-lactamase inhibitors: clavulanic acid and thienamycin (according to reports, is regarded as the original "carbapenem" and sets the pattern for all subsequent carbapenems).

Carbapenems occupy a very important place in our fight against bacterial infections. This is because they can resist the hydrolytic action of the β-lactamase enzyme. Among the several hundreds of known β-lactams, carbapenems possess the broadest spectrum of activity and greatest potency against Gram-positive and Gram-negative bacteria. As a result, they are often

called “antibiotics of last resort” and are administered when patients with infections become gravely ill or are suspected of harboring resistant bacteria. [26]

Examples classes of carbapenem are:

1. Imipenem: a broad spectrum effective against aerobic and anaerobic pathogens, usually taken orally and active in low concentrations, with minimal allergy side effects.
2. Meropenem: a broad spectrum effective against non-fermentative Gram-negative bacilli particularly against acquired infections.
3. Ertapenem: a broad spectrum with limited activity against non-fermentative Gram-negative bacilli. [3, 27]

1.2 MACROLIDES

1952 saw the discovery and isolation of the first antibiotic in this class isolated from *Streptomyces*. They have a wider spectrum of antibiotic activity than penicillins and are often administrated to patients allergic to penicillin. [28] Macrolide antibiotics are classified according to the size of the macrocyclic lactone ring as being either 12-, 14-, 15-, or 16-membered ring macrolides. (Figure 8) Most macrolides contain amino sugar and/or neutral sugar moieties connected to the lactone ring via a glycosylic bond. [29]

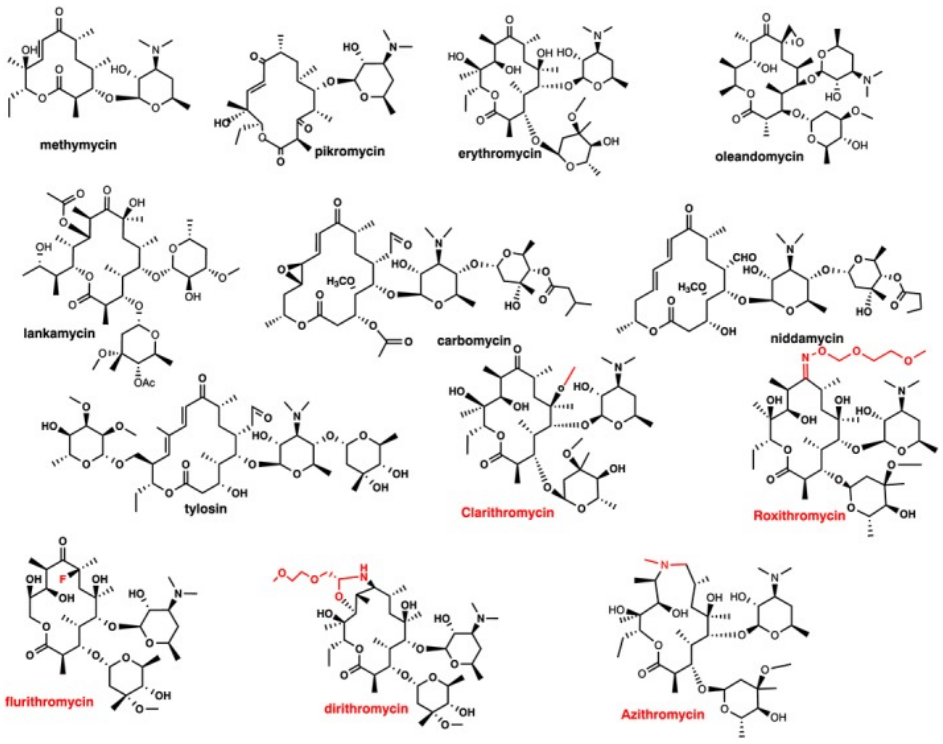

Figure 8 - Macrolide structures. First generation: 12-membered (methymycin), 14-membered (pikromycin, erythromycin, oleandomycin, and lankamycin), and 16-membered (carbomycin, niddamycin, and tylosin), all-natural products. Second generation: 14-membered (clarithromycin, roxithromycin, flurithromycin dirithromycin) and 15-membered (azithromycin). The red color indicates modifications inserted in the erythromycin molecule to generate the second generation of 14- and 15-membered macrolides. [29]

Generally speaking, Gram-positive bacteria are the target of macrolide antibiotic action, with Gram-negative bacteria showing relatively sporadic activity. This group of antibiotics is quite effective against *Staphylococcus* and *Streptococcus.* Although macrolides display excellent antibacterial activity, their generally poor bioavailability, unpredictable pharmacokinetics, and low stability in the acidic pH of the stomach prompted early searches for new derivatives with improved properties. [29]

Five erythromycin byproducts have been created and sold:

- Dirithromycin.
- Clarithromycin.
- Flurithtromicyn.
- Roxithromycin.
- Azithromycin.

With azithromycin being the most used in this antibiotic class.

1.2.1 Azithromycin

Azithromycin (AZM) is a second-generation, broad-spectrum, synthetic macrolide antibiotic used to treat a wide range of bacterial and mycobacterial infections of respiratory and skin infections. [30]

Since its discovery, it has been FDA-approved for respiratory tract infections such as pneumonia, genitourinary infections such as chlamydia, and enteric infections such as typhoid, and has also been extensively studied with malaria. [31] The World Health Organization (WHO) lists azithromycin as one of the safest drugs for any national health system. [32, 33]

Indeed, over the last several decades, its administration for respiratory diseases [34, 35] has resulted in few short-term side effects relative to other antibiotics, even in pregnant women and children. [36] Results from a recent trial for COVID-19 further indicate that azithromycin is not only safe, but a more clinically effective drug candidate against the disease compared to hydroxychloroquine, with all azithromycin monotherapy patients displaying signs of recovery. [37]

It has an intriguing range of anti-viral and anti-inflammatory properties, and it has been used as a treatment in previous coronavirus diseases during the epidemics of severe acute respiratory syndrome (SARS) in 2003 and Middle East respiratory syndrome (MERS) in 2012. [30] Has therefore been used to treat SARS-CoV-2, which causes coronavirus-19 disease (COVID-19).

1.3 TETRACYCLINES

Tetracycline antibiotics are well known for their broad spectrum of activity, spanning a wide range of Gram-positive and -negative bacteria, spirochetes, obligate intracellular bacteria, as well as protozoan parasites. The first tetracyclines were natural products derived from the fermentations of actinomycetes. Chlortetracycline, produced by *Streptomyces aureofaciens*, and marketed as Aureomycin, was first reported by Benjamin Duggar at Lederle Laboratories in 1948 and approved for clinical use that same year. [38, 39]

According to how they were created, the members of this class of antibiotics, are divided into various generations (Figure 9):

1. First generation: obtained by biosynthesis.
    a. Tetracycline;
    b. Oxytetracycline;
    c. Chlortetracycline;
    d. Demeclocycline;
2. Second generation: obtained by semi-synthesis.
    a. Doxycycline;
    b. Meclocycline;
    c. Lymecycline;
    d. Minocycline;
    e. Methacycline;
    f. Rolitetracycline.
3. Third generation: obtained from total synthesis.
    a. Tigecycline.

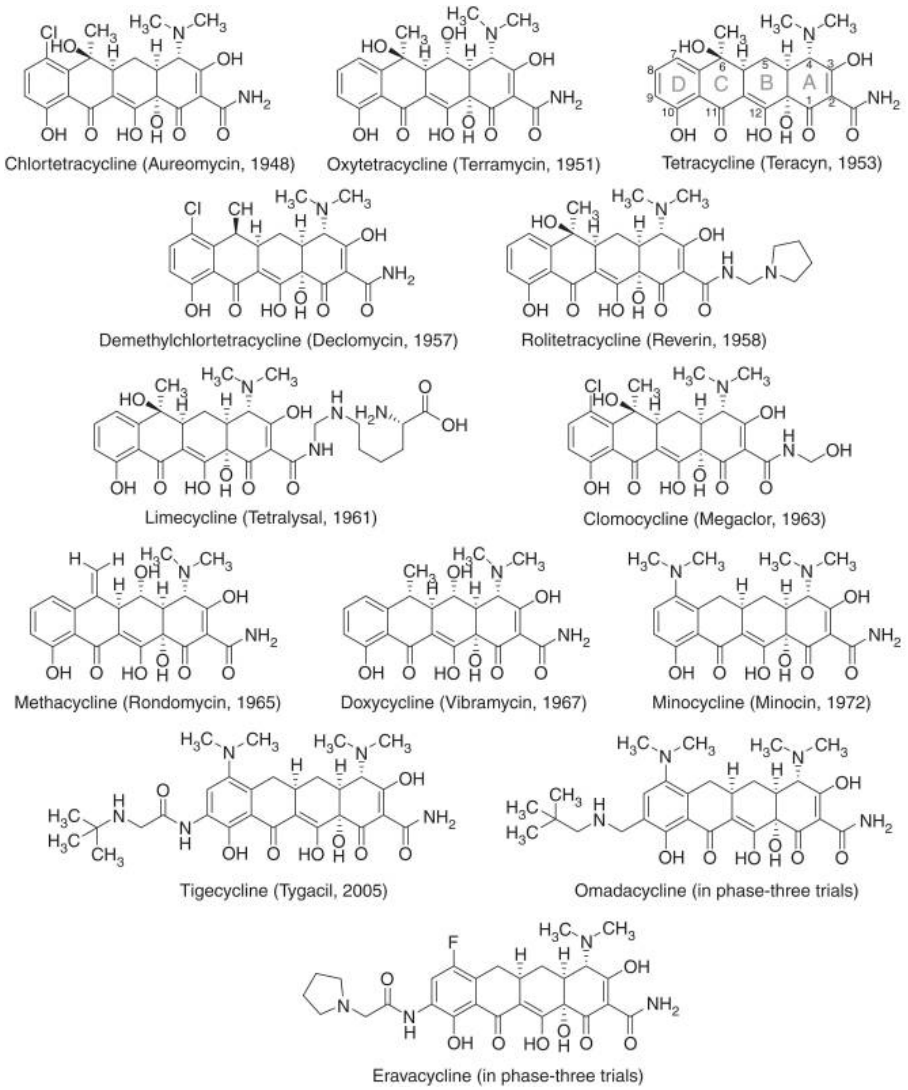

Figure 9 - Chemical structures of clinically used tetracyclines and development candidates. Tetracycline structures are labeled with generic names; trade names and year of discovery are indicated within parentheses. The core structure rings (A–D) and carbons (1–12) are labeled in the chemical structure of tetracycline using the convention for tetracycline carbon numbering and ring letter assignments. [38]

Their target of antimicrobial activity in bacteria is the ribosome. Patients are advised to take tetracyclines at least two hours before or after meals for better absorption. All tetracyclines are recommended for patients above eight years because the drugs have been shown to cause teeth discoloration among patients below this age and can be used in treating malaria, elephantiasis, amoebic parasites, and rickettsia. [3, 40]

2 ANTIBIOTIC RESISTANCE (AMR: ANTIMICROBIAL RESISTANCE)

Antibiotic resistance has been described as one of the greatest global threats of the 21st century. [41, 42] In the last two decades, antimicrobial resistance is considered a global health and development threat. WHO (World Health Organization) has declared that AMR (Antimicrobial Resistance) is one of the top 10 global public health threats facing humanity. [43]

Infectious diseases are rising again, especially those that can't be treated employing already-known antibiotics. Infectious pathogens can evolve and therefore over time, many have developed resistance to the currently prescribed and newly developed antibiotics. [44] Antibiotic resistance has become a critical threat to the global population, and being increased drastically over the past years, reaching a sort of "pre-antibiotic era" again. There is concern that antibiotic may lose their effectiveness over the next years due to a combination of both self-medication and irrational prescription and use of these therapeutic agents, which has led to the development of multi-resistant bacterial strains, and in fact, some of them are resistant to all available antibiotics. [45]

However, saying that we might go back to a pre-antibiotic era is a hyperbolic forcing. History never comes back again, and we will never be back to the dark ages of deadly infections. Not only antibiotics are responsible for the decline in infectious diseases, in fact, infectious diseases started declining much before the discovery of antimicrobial agents, because of the progress of hygiene-ecology and social welfare. [46] Progresses in medicine can control in our days most of these host processes. In fact, many severe infectious diseases can now be treated without the need of antibiotics**.** In modern Western countries, in our developed world, a world of wonders, there is a dominant "culture of fear" which takes the form of the Alexis de Tocqueville (1805–1859) paradox: "plus tout va bien, plus on a peur" or "the better things are, the more fear we have." [47]

Fear unavoidably has some undesirable side effects. As has been demonstrated, fear is a societal construct that tends to fix the worst expectations; it creates a misleading analogy by differentiating between dangerous situations and those that are only scary. The perpetuation of dread in the absence of clear remedies reflects poorly on scientific efforts, the issue of antibiotic resistance has been discussed for many decades without a possible solution. Fear doesn't have only negative aspects, for example, it promotes human quick analysis of microbial threats, as with AIDS or the COVID-19 pandemics, but it also undoubtedly helped to stimulate antibiotic development. Fear improves social and organizational cooperation, and certainly fear of antibiotic resistance has significantly contributed to the interaction of scientists all around the world. Fear raises our awareness not to stop progress in any sense for the profit of a single humanity. [47] The European Centre for Disease Prevention and Control (ECDC) reported that, each year, 25.000 people die from infections caused by multi-resistant bacteria and also added that these microorganisms' cost about 1.5 billion euros in extra healthcare services and productivity losses per year to Europe. [45, 48]

But there's more in a perspective view: some reviews estimated that antimicrobial resistance (AMR) could cause 10 million deaths a year by 2050 (Figure 10).

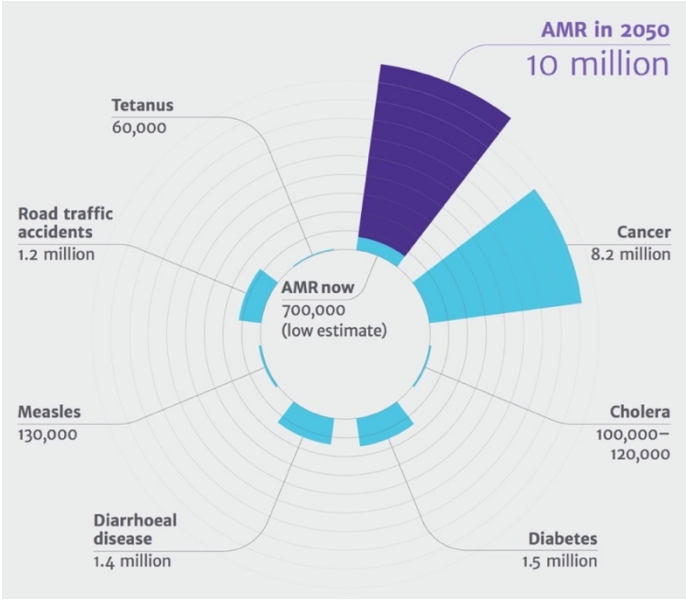

Figure 10 – Deaths attributable to AMR every year compared to other major causes of death. [49]

In recent decades, an increase in the use of antibiotics has been observed. This is mainly due to a growth in the number of people requiring medical care; because of the elderly population's rising, there is an increase in chronic diseases and healthcare-associated infections, also called nosocomial infections, which is a planetary health problem.

Self-medication represents an important factor that contributes to an intensification of the problem, and this leads to an overuse of drugs; another issue is represented by the incorrect use by the population. Additionally, the misuse of antibiotics in livestock, whether for therapeutic, preventative, or even growth-promoting objectives, makes the situation even direr since it encourages the selection of commensal and pathogenic bacteria resistant to any antibiotic molecule.

Summarizing, these are the predominant causes of AMR:

1. Overuse. Lack of information and diagnostic criteria are major causes of the abuse of these drugs. Recent data show that at least 58% of the children with diagnostic of flu syndromes (of viral origin) received antibiotics.
2. Inadequate prescription. Studies show errors in the choice of drug and treatment time of about 30–50% of the employment of antibiotics. [50]
3. Use in livestock. The use of antibiotics in meat production (animal products chain) has been regarded as largely responsible for the increase in bacterial resistance indicators. [51] By eating the meat of those animals, the human being acquires these microorganisms, pathogenic or not, but which carry resistance genes, exposing humans to infections untreatable with antibiotics. [45, 50]

Although antimicrobial resistance (AMR) is a natural process, the public health emergency due to the uncontrolled spread of this phenomenon depends primarily on the overuse of antibiotics. [52, 53]. Fleming himself winning the Nobel Prize for medicine in 1945, stated that anyone who plays with penicillin without thinking about the consequences is morally responsible for the death of anyone who dies from an infection caused by a penicillin-resistant microorganism.

But the overuse can't be considered the only cause of AMR; in addition to this aspect, there is a variety of factors involved such as poor infection control in clinics and hospitals, poor community hygiene, accumulations of antibiotics in the environment (especially in waters), and their heavy use in the animal industry.

Both developing countries and industrialized countries are equally affected by this issue, therefore it is fundamental analyzing the aspects with a Planetary Health perspective. Even though it is difficult to get accurate numbers regarding antibiotic resistance it is predicted that resistant infections will cause by 2050 approximately 10 million deaths per year (compared to a hypothetical 8 million deaths attributable to neoplastic problems).

One of the issues regarding developing countries is that there is almost no surveillance and few biosecurity tools; several nations lack effective quality control procedures to guarantee that the given antibiotics are of the highest caliber. It has been demonstrated that using pediatric antibiotics that had beyond their expiration date resulted in increased rates of resistance compared to using new medications. Utilizing expired drugs increases resistance rates by a factor ranging from 2 to 6. Toward the end of the product's shelf life, many of them are rebranded and gifted rather than sold.

Another important matter regards the clinical misuse of antibiotics. It has been shown that proper diagnostic methods are often not utilized when treating infections and therefore antibiotics are prescribed when not necessary. [44] Microbiological bacterial culture and drug sensitivity tests are rarely done in many developing countries (susceptibility test according to Kirby Bauer, E-test or MIC). Other diagnostic tests used to confirm infection are uncommon and unreliable, and therefore, many clinicians only rely on clinical signs and symptoms rather than the recommended laboratory tests. Consequently, there is an increased use of broad-spectrum antibiotics, which in turn breeds resistance because it selects for resistant organisms within the normal intestinal flora which may be harmless at index antibiotic administration but can transfer resistance to future pathogenic infections. [44, 54]

On the other hand, in developed nations for example, for routine operations such as surgery or organ transplants, prophylactic antibiotics are frequently used. Moreover, patients facing chemotherapy are given antibiotics as a preventative measure which weakens the person's immunity. Hospital-acquired infections (nosocomial infections) remain one of the major sources of antibiotic-resistant infections in developed countries. Antibiotics are used extensively in hospitals to not just treat patients with bacterial infectious diseases but also prophylactically to reduce the risk of infections during procedures and surgeries. [44] It has been discovered that switching up the used antibiotics from one category to another can reduce the likelihood of resistance developing by relieving the organisms' dependence on a single antibacterial agent.

Drug resistance is bred for example in the United States by similar variables to those in developing countries, such as inappropriate prescribing by physicians and low adherence from patients. Many people without insurance and those who are below the poverty level obtain antibiotics from unreliable sources. Self-medication is considered another issue in developed countries, furthermore circa 17% of patients have taken "left-over" drugs when experiencing a sore throat.

The aforementioned problem referred to the use of antibiotics in food-producing animals (production chain) can also be handled in terms of developed countries. Antibiotics are not just used to treat illnesses, but also as a preventive measure against infections and as non-therapeutic means of promoting animal growth (fortunately European legislation has prevented this use). Nearly 7–9 million medically important drugs and 4–6 million nonmedically important drugs were sold annually for use in food-producing animals since 2009, in which medically important drugs refer to the FDA's guidelines for industry use of medications important for human medical therapy. [44, 55]

The large use of antibiotics in animals employed for food production as well as the transmission to humans of antibiotic-resistant species is very concerning.

The first use of antibiotics in food animals was during the final phases of the Second World War, when accessibility to lyophilized preparations of penicillin was allowed for veterinarians owing to its abundant production at the time to cater for war casualties. [56, 57] Prior diagnosis of infection before treatment with antibiotics is recommended in food animals [58, 59], and the use of these drugs responsibly in animals for medical reasons is essential for the general well-being of animals. [56] This must be done to stop the spread of zoonotic diseases.

Although the occurrence of ARMs is possibly naturally happening in some cases, certain farm management practices can help control the load of ARMs acquired by animals. For example, the correlation between ARMs prevalence in cattle and animal management, including different farm characteristics, feeding practices, and farm hygiene were the most investigated factors.

The size of the livestock farm seems to be directly related to the prevalence of antibiotic resistance (AMRs).

Another critical farm management practice is its hygienic-sanitary level. Isolating sick animals, burying deceased cattle, and cleaning drinking water troughs more than once a month are practices that are associated with a lower prevalence of CRB (cefotaxime-resistant bacteria). [60]

Another issue can be denoted for wildlife species and not only for food animals. Migratory birds, serve as potential reservoirs of ARMs and have a critical role in spreading ARMs globally. [61, 62]

Even though antibiotics are not administrated, wild animals can pick up resistance mechanisms through interactions with the environment, which may contain medications and is home to many species. For example, bacteria can develop a resistance to antibiotics since they are created naturally by microbes. Another problem is given by the fact that the irrigation water used for forage, sometimes, comes directly from hospitals. The antibiotics and their metabolites continue their transmission dynamically among surface water, groundwater, and soil, which

contributes to the promotion of ARMs in the environment resulting in the increasing exposure of ARMs to wildlife. [61]

A method to prevent possible antibiotic resistance transmission between wildlife and livestock is through the regulation of wildlife population growth, as this can increase the number of AMR carriers in both wildlife and livestock populations.

2.1 BIOFILM

One of the strategies employed by bacteria to exert their antimicrobial activity corresponds to their ability to form biofilms. Biofilms are surface-attached bacteria encased in a self-produced extracellular polymeric matrix. The hallmark of the biofilm lifestyle is increased resistance to a wide range of stressors including the immune system, disinfectants, and antibiotics. The mechanisms underlying this resistance are extremely complex. [63, 64]

To develop a relationship with the host, to show resistance towards hostile external conditions, and to cope with the known antibiotics and other environmental cues, the microorganisms have evolved to form a protective cover around themselves. [65] Biofilm formation contributes towards the development of antibiotic resistance and the formation of persistent cells which are responsible for the unmanageable persistence of microbial infections. [66, 67]

In biofilms, extracellular polymeric substances (EPS) play a vital role in the formation of physical and social interactions, an enhanced rate of gene exchange, and antimicrobials tolerance. [68] EPS consist of cellulose, alginates, poly-N-acetyl glucosamine, extracellular teichoic acid, proteins, lipids, nucleic acids, phospholipids, polysaccharides, extracellular DNA, and other organic compounds. [69] About 90% of biofilms biomass is comprised of EPS that contribute to the resemblance of the “mushroom-like” structure. [70] Besides, the

mechanical firmness of biofilms is attributed to the viscoelastic features of the EPS matrix. [71, 72]

Biofilm is a heterogeneous structure comprising mainly of microbial cells (10–25%) and a self-produced EPS matrix (75–90%). [67, 73]

EPS forms a scaffold that holds the biofilm together and, thus, helps in cell-to-cell communication and provides adhesion and cohesion forces required for biofilm formation. EPS helps in nutrient cycling, maintaining the availability of deoxyribonucleic acid (DNA) for horizontal gene transfer (HGT), and acts as a protective barrier against oxidizing biocides, antibiotics, ultraviolet radiations, desiccation, and host immune defense system. [67, 74]

The fundamental components of EPS could be considered polysaccharides, extracellular proteins, extracellular DNA, surfactants, lipids, and water.

A multi-layer defense system is constituted in biofilm by the formation of persistent cells, development of adaptive stress responses, very less antibiotic penetration, limited nutrition, less growth, and metabolic activity [75], and inactivation of antimicrobials within the components of the EPS matrix. [67, 64]

Structural and physiological change takes place after cells have been attached to conditioned surfaces. Structural polymeric substances produced are acting as a sort of barrier [76] and prevent the entrance of antibiotics and sanitizer agents. [77] Within the biofilm, the bacterial cell's growth is very slow, and it generates cells that may withstand harsh circumstances such as exposure to antibiotics and other biocidal molecules.

As per the reports of the National Institutes of Health (NIH), about 65% and 80% of microbial and chronic infections, respectively, are caused by microbial biofilms, infecting both tissues and medically implanted devices. [67] Breast implants, ventricular shunts, tissue fillers, ventricular-assisted devices, contact lenses, catheters, joint prostheses, urinary catheters, orthopedic implants, pacemakers, mechanical heart valves, defibrillator, vascular grafts,

endotracheal tubes, voice prostheses, etc. are some examples of medically implanted devices often infected by microbial biofilms. [67, 78]

Furthermore, different sectors of the food industry, viz. poultry, dairy, ready-to-eat, aquaculture, etc., are severely affected by biofilm-producing microorganisms resulting in food spoilage, disease outbreaks, and deaths. [67, 79]

Biofilm production can be influenced by a variable number of factors such as: surface conditions, chemical and physical growth factors, cellular structures, and any other challenges. [77]

Biofilm formation (Figure 11) requires four stages:

1. Attachment. Planktonic bacteria adhering to surfaces initiate the process that leads to biofilm formation, which is thought to be a crucial stage in the development of the freely moving microbes into a built-up community composition. During the early stages of formation, microorganisms are loose and reversibly connected to surfaces. Following that, bacteria tend to adopt a flattened orientation on surfaces and pursue permanent adhesion, creating resistance to a variety of physical conditions that prevent the formation of biofilms.
2. Growth. Soon after the successful adhesion of microorganisms to the surfaces, the adhered microorganisms start multiplication and aggregation within self-produced EPS leading to the microcolony formation in presence of a high concentration of c-di-GMP (Bis-(3′-5′)-cyclic dimeric guanosine monophosphate).
3. Maturation. EPS plays a crucial role in biofilm maturation as it helps in microbial attachment to surfaces, stabilizing the 3-D structure of the biofilm, grouping cells together, protecting from various stresses like host immune system response, antimicrobials, oxidative damage, and metallic cations, but also encapsulating signaling molecules required for quorum sensing, metabolic products, and enzymes. [80] A mature biofilm may acquire a “mushroom” or “tower” shape structure in which

microorganisms are arranged as per aero-tolerance and metabolism rate. [81] A mature biofilm is a three-layered structure: inner regulating layer, middle microbial basement layer, and outer layer inhabited by the planktonic form of microorganisms that are ready to exit the biofilm. [82]

4. Dispersion. Finally, matured biofilm ruptures actively (motility and EPS degradation–dependent dispersion) or passively (physical factors like liquid flow-dependent dispersion) to disperse the microorganisms to start a new cycle of biofilm formation.

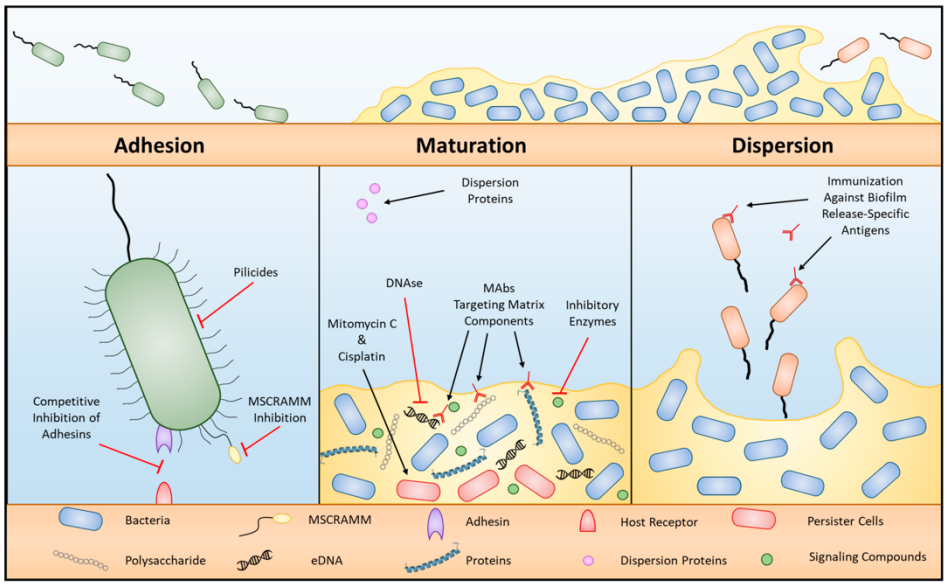

Figure 11 – Biofilm formation in bacteria. [83]

Biofilm is highly resistant to conventional antibiotics, so there is an urgent requirement to develop alternative potent therapeutic solutions to overcome the problem and improve healthcare, food safety, and other industrial sectors. [67, 79]

The study of various biofilm model systems enhances the knowledge regarding the biofilm biology and physiology. The biofilms are studied using both in-vivo and in-vitro model systems. In-vitro biofilm model systems are broadly classified into 3 major types including closed or static models, open or dynamic models and microcosms. The most frequently used closed model systems are microtiter plate-based model systems which uses static and batch growth conditions. [84] In this model, there is no flow of media, product, or waste materials into or out of the reactor, so the experimental conditions change gradually in the wells like

accumulation of signaling components, increase of bacterial population, and depletion of nutrients in media. [85] Many tests can be run simultaneously because it is needed a little number of reagents.

Among the open and dynamic models, flow displacement biofilm model is most commonly used to study biofilms. [85] This model allows the addition of nutrients and the discharge of waste products in contrast to the prior method.

Both in-vitro and in-vivo systems can be turned into a microcosm by using the same medium and creating an artificial environment to assess cell metabolism and behavior. Apart from this, there exists an ex-vivo model system, which deals with the tissues and organs extracted from organisms for further analysis and experimentation in artificial environment. This model can be useful to monitor the bacterial colonization and progression in the given tissue or organ. To validate the simplified results provided by the in-vitro model studies, certain in-vivo model system studies should be performed. To address various therapeutic and diagnostic challenges the studies of mammalian models closer to the humans is necessary. [85]

Since biofilm formation contributes to bacterial pathogenicity and resistance toward antibiotics, there must be certain strategies to deal with this problem. Recently, Wu and his staff have reported the use of foreign bodies as a major cause of increase in biofilm infections. [86] So, to treat such biofilm-associated infections it is imperative to remove the indwelling medical equipment followed by replacement with new uninfected ones along with sensitive and aggressive administration of antibiotics. Moreover, the implant removal should be timed properly so that the new or replaced implant does not get infected when inserted into the patient's body. In cases where removal is not possible, a long-term administration of antibiotics is recommended so as to avert the biofilm from growing. According to the previous reports, the premature biofilms can be treated more effectively with antibiotics than that of the mature biofilms. [85]

The antibiotic used for the treatment of biofilms should be legitimately selected on the basis of sensitivity as well as the capacity to penetrate properly through the biofilm matrix. [85, 86]

AMR is higher in biofilm-associated bacteria than in planktonic microorganisms. Consequently, using a combination of therapies is better than using only one antibiotic. Using multiple therapies can be an advantage since the various agents can function differently. Every anti-biofilm molecules have their specific modes of action, but a single molecule may follow more than one mechanism. [85]

Information regarding the mechanism of action provides better understanding about the nature of biofilms, which can be further used to develop new and successful drug molecules with the previously known target of action. This can bring about improvement in the efficacy of the previously known drugs. It can be achieved either by making suitable modifications or by using combinatorial therapy which includes the previously reported less effective drug against bacterial infections along with the potent anti-biofilm agents thereby raising the activity of the antibiotics. [85]

2.2 ANTIBIOTIC RESISTANCE AND COVID-19

On 11 March 2020, the WHO announced the COVID-19 pandemic. [87] The disease known as COVID-19 or SARS-2 spread rapidly from Wuhan City, China, to the rest of the globe. [88] As of early July 2022, roughly 547,901,157 COVID-19 cases and 6,339,899 deaths have been officially reported; while, actually (as of May 16, 2023) the confirmed cases are 765.903.278 (in Europe 276.136.217) and the death are 6.927.378. [89]

COVID-19 infection is a viral disease -untreatable by antibiotics, but the viral respiratory infections clinically progressed to bacterial pneumonia- the bacterial superinfection and other nosocomial infections- requiring antibiotic administration. Initial reports of Wuhan Pulmonary

Hospital, China, published in '*The Lancet*,' show an approximate 50% secondary bacterial infection occurrence rate in COVID-19 casualties. [90, 91, 92]

During the COVID-19 pandemic, there were improper uses of antibiotics either in healthcare institutions or in communities, which in turn played a role in the increase in AR. [93, 94, 95]

It has been documented that about 72% of COVID-19-admitted patients were treated with antimicrobials, whereas solely 8% of these patients had bacterial or fungal co-infection. [93] Additionally, different antibiotics have been explored or suggested to cure COVID-19 patients, e.g., azithromycin. [93, 94] Both the worry and the improper use of antibiotics directly impact access to antibiotics without a prescription, particularly in low- and middle-income countries that have a weak system of antibiotic control. In this correlation, Zavala-Flores and his research team, reported that nearly 69% of COVID-19 patients stated that they had used antibiotics (namely, ceftriaxone and azithromycin) before being admitted to the hospital. [95, 96]

Overall, more than one-half of COVID-19 patients may receive an i.v. antibiotic and this number can be higher in patients with severe disease.

This high rate of antibiotic utilization for patients infected with SARS-CoV-2, particularly in severe COVID-19 cases, could be due to the following:

1. As the prevalent presentations of SARS-CoV-2 infection (cough, fever, and radiological infiltrates) are also hallmarks of community-acquired bacterial pneumonia, clinicians empirically add a broad-spectrum antibiotic despite the suspicion of a viral origin.
2. The anxiety and uncertainty regarding the COVID-19 outbreak as well as the absence of effective anti-SARS-CoV-2 treatments are potential drivers of widespread and excessive prescription of antibiotics.
3. Co-bacterial, fungal, or secondary infection with COVID-19 is possible; however, it is difficult to differentiate between sole SARS-CoV-2 infection and co- or secondary infections. [97]

Clinicians are challenged with competing priorities: prescribing a broad enough spectrum antimicrobial to ensure the organism is sensitive, while at the same time avoiding the unnecessary use of antimicrobials, particularly those of last resort, when a more commonly used or narrower-spectrum antimicrobial would suffice. Inappropriate treatment in either direction has been associated with increased risk of mortality. [98, 99]

The evolution of AMR in a population is determined by three components: emergence-, transmission-, and population-level infection burden. COVID-19 has the potential to affect all three of these components through the direct or indirect consequences of pandemic responses. Because a patient with COVID-19 can present with non-specific symptoms (e.g. fever and/or persistent cough), these could be mistaken for other diseases such as malaria [100] or tuberculosis (TB) [101], and vice versa. These overlapping symptoms may result in inappropriate prescribing or a lack of prescribing and misdiagnosis, depending on COVID-19 prevalence. This could impact future drug resistance levels of other pathogens, as mentioned above.

The importance of such bystander selection has already been seen following the widespread use of azithromycin for WHO-recommended mass drug administration campaigns for trachoma. [102] This is particularly relevant for COVID-19 as azithromycin has been proposed as a potential therapy. [103]

In places where SARS-CoV-2 infections are prevalent, particularly considering the atypical symptoms, low-cost and quick diagnostics may help detect infections early and hence reduce the need for antibiotics. This could be crucial in low- and middle-income countries (LMIC) where antibiotics could be obtained without a prescription and where testing is prioritized over vaccinations, which may take longer to become more utilized. The question is whether the accessibility and cost of these tests will result in a significant reduction in the use of antibiotics. Furthermore, some of the current testing done on asymptomatic patients may result in a “false negative” result, leading to an inaccurate sense of security.

COVID-19 has marked an important change in the interactions of people, who have become more aware of various hygiene practices such as putting on a mask and washing hands frequently. These hygiene practices can prevent the spread of infectious diseases, including those that are resistant to antibiotics.

The pandemic has brought to light the need for improved infection prevention, which, together with protocols, help minimize the risk of hospitalization. COVID-19 has facilitated wider awareness and reinforced the role of a global One Health approach. The application of the One Health approach has the potential to effectively combat COVID-19, [104] and this approach can be leveraged to tackle the rise of antimicrobial resistance. [105]

2.3 ONE HEALTH AND ANTIBIOTIC RESISTANCE

The issue regarding AMR can be faced under various aspects, one of these is what is the One Health perspective. One Health is defined as "the collaborative effort of multiple health science professions, together with their related disciplines and institutions working locally, nationally, and globally—to attain optimal health for people, domestic animals, wildlife, plants, and our environment". [106]

The origins of One Health are centuries old (the concept echoes the writings of ancient philosophers, in particular, as early as c.460-c.377 B.C., Hippocrates wrote that human health depends on the environment in his book "On Airs, Waters and Places") and are based on the mutual dependency of humans and animals and the recognition that they share not only the same environment but also many infectious diseases. [107] It has been estimated that as many as 75% of human infectious diseases that have emerged or re-emerged in recent decades are zoonotic; that is, they originated in animals. [108, 109]

Calvin Schwabe, a veterinarian, coined a new term: “One Medicine” and it means that there are many similarities in both animal and human medicine. Moreover, many procedures done in veterinary can affect or benefit human health. More specifically, One Health promotes the idea that to ensure the long-term health and well-being of people, animals, and the environment, health professions must cooperate.

Certain antibiotics or antimicrobials are only used for veterinary purposes because they may be hazardous to people, while others are only used in humans for conditions for which animals are not treated. Nonetheless, both humans and animals are exposed to most antimicrobials.

In veterinary medicine, there are notable differences in the ways that antimicrobials are used in companion animals (e.g., dogs, cats, pet birds, horses) compared to food-producing animals. Antimicrobial use practices in companion animals are broadly like those in humans; that is, the drugs are mostly administered on an individual animal basis for the treatment of clinical infection, with some use for prophylaxis in individual animals, such as post-surgery. [110, 111]

In the case of food animals, however, when some animals in a group are clinically infected and in need of antimicrobial therapy, for reasons of practicality and efficiency, the drugs are frequently administered through feed or water to the entire group (e.g., pens of pigs, flocks of broilers), even when most of the animals are not displaying signs of infection (in effect, prophylaxis). This is, however, now defined inappropriately by many in the animal health sector as “therapeutic” use. In addition, there is use in food animals similar to what happens in people, when antimicrobials are used to treat individual clinically sick animals (e.g., dairy cows with mastitis). [108, 112]

The term “metaphylaxis” is used to describe a treatment done to a group, typically when administrated to a large group of animals at high risk of infection. On the other hand, is very uncommon to do a prophylactic administration of antimicrobials on people unless there is a case of very serious infections.

Many in the industry justify group-level treatments as therapeutic when clinical infections are observed in at least some of the animals in the pen or flock, or prophylactic when there are no sick animals present, but they are at high risk of clinical bacterial infection due to exposure to infectious agents (e.g., mixing of animals from different sources), unsanitary or crowded conditions, or other factors (e.g., age, stress of transport). [108, 113] The antibiotic administration is recommended when the risk of prophylactic bacterial infection is high due to mixing of new animals, crowded or unsanitary conditions, the stress of transport, and age-related factors. [114, 113]

There are many discussions regarding the use of medications to promote the growth of food-producing animals because they are administrated to a group of animals for a long period, contributing to antibiotic or antimicrobial resistance. Sometimes these growth promoters are used to hide a condition of poor health and hygiene; because of this the WHO (World Health Organization) supports the interruption of the use of antibiotics as growth promoters; Europe has already outlawed this practice.

Recently, the World Organization for Animal Health (OIE) reported that 41% of 146 countries reporting on antimicrobial use in animals allow the use of antimicrobial growth promoters. This represents a reduction from 51% of 151 reporting countries in 2012. [108, 115] According to estimates, the global use of antibiotics was 131,000 tons in animal production during 2013, which is projected to increase to 200,000 tons in 2030. [114, 116]

In 2014 an alliance was formed between OIE (Office International des Epizooties – World Organization for Animal Health), FAO (Food and Agriculture Organization of the United Nations), and WHO to improve global coordination and collaboration between disciplines: animal and public health, and food safety. It was then established that this issue must be resolved with the One Health approach. AMR was identified as a pressing danger that needed to be managed globally.

The usage of antibiotics, persistence of antibiotic residues, and presence of resistant bacteria in the human-animal-environment niches are associated with the One Health triad due to the interdependence of these pillars in the food chain and environment. [114]

At the most basic level, everyone should understand the principles of basic hygiene to prevent the spread of infections, understand the need to follow the antimicrobial prescriber's instructions for treatment and have a basic appreciation of the risks to themselves and others associated with antimicrobial use, in addition to the benefits [117, 118]. This applies to antimicrobial use in humans as well as animals. [108]

3 ALTERNATIVE STRATEGIES TO ANTIBIOTICS

Finding new antibiotics is crucial in the ongoing battle against AMR. Even though antibiotics have been helpful for humanity, the capability of bacteria to rapidly evolve has made it urgent to find other options for the use of antibiotics. Some new strategies may be considered to be bacteriophages, antimicrobial peptides, and nanoparticles.

3.1 BACTERIOPHAGES

Bacteriophages were discovered by Frederick Twort and Félix d'Hérelle in 1915 and 1917, respectively. Since then, it has been suggested that the administration of these viruses could be used to treat bacterial infections (these phages kill bacteria specifically but cannot infect other kinds of organisms). [119]

Bacteriophages, or phages, are viruses that only infect bacterial cells. [45] They are biological entities completely devoid of any metabolic machinery and thus are obligate intercellular parasites that require a bacterium to replicate themselves, through their genetic material, by taking over the biochemical machinery of the bacterial cells. [120, 121, 122, 45] Phage therapy is characterized by its specificity to single bacterial species and usually to a subset of strains within that species. [123] To be active against >90% of strains within a bacterial resistance to a single phage, mixtures (cocktails) of different phages, often more than ten phages, are used for therapy. [124]

Bacteriophages can be used for the treatment of infections by various bacteria, ranging from *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Shigella*, and *Salmonella* (Table 1). [125]

Table 1 – Bacteriophages that are approved or are undergoing clinical trials for human use. *[125]*

Product	Company	Condition	Phase (status)
ListShield	Intralytix	Food industry (*Listeria monocytogenes*) Approved Food industry (*E. coli*)	Approved
EcoShield	Intralytix	Food industry (*Salmonella enterica*)	Approved
SalmoShield	Intralytix	Burn infections (*Pseudomonas aeruginosa* and *E. coli*)	Approved
ABPA01	AmpliPhi	Chronic rhinosinusitis and cystic fibrosis (*P. aeruginosa*)	Preclinical
PHOSA	Multiple centres	Treatment and prophylaxis of gastrointestinal infections (multiple organisms)	Preclinical
Phagesti	Biochimpharm	Treatment and prophylaxis of bacterial purulent–inflammatory infections (multiple microorganisms)	Approved
Phagyo	Biochimpharm	Treatment and prophylaxis of bacterial purulent–inflammatory infections (multiple microorganisms)	Approved
Phagedys	Biochimpharm	Treatment and prophylaxis of dysentery (*Shigella*)	Approved
Phagetyph and Phagesal	Biochimpharm	Treatment and prophylaxis of enteric fever and salmonellosis (*Salmonella*)	Approved
Phagestaph	Biochimpharm	Treatment and prophylaxis of bacterial purulent–inflammatory infections (*Staphylococcus aureus*)	Approved
Phagepy	Biochimpharm	Treatment and prophylaxis of bacterial purulent–inflammatory infections (*P. aeruginosa*)	Approved
Pyo-Phage	Eliava Institute	Urogenital infections; pyo-inflammatory gynecologic diseases; enteric infections; dysbacteriosis, surgical infections	Approved

Intesti-Phage	Micro-gen	Bacterial dysentery; salmonellosis; dyspepsia; dysbacteriosis; enterocolitis, colitis	Approved
SES-Phage	Eliava	Pyo-inflammatory and enteric infections caused by staphylococci, streptococci, and enteropathogenic E. coli	Approved
ENKO-Phage	Eliava	Pyo-inflammatory and enteric infections caused by *Shigella*, *Salmonella*, different types of *E. coli*, species of pathogenic staphylococci	Approved
Fersisi-Phage	Eliava	Pyo-inflammatory and enteric infections caused by staphylococci and streptococci	Approved
ABSA01	AmpliPhi	Chronic rhinosinusitis, bacteremia, endocarditis, prosthetic joint infections, osteomyelitis, diabetic foot ulcers (*S. aureus*)	Phase I
PhagoBurn	Multiple centres	*E. colior, P. aeruginosa* burn wound infections	PhaseI/II

There are several challenges to the clinical use of phage treatment. These include the following ones:

1. The prerequisite of selecting appropriate phages to achieve an significant range of activity and prevent the development of bacterial resistance.
2. Manufacturing phages under good manufacturing practices (GMP) and chemistry, manufacturing, and control (CMC) guidance. Some progress has been achieved when tackling the insurmountable challenge regarding CMC, especially production, stability, purity, and quality control.
3. Considering phage biology in the design of phage treatment is a prerequisite of any successful approach. [126] Unique pharmacokinetics (PKs) and pharmacodynamics (PDs) of phages mean that dose-finding processes are challenging. The immense size

of phages when compared to small-molecule antibiotics results in a wide range of PK challenges and is the reason why many sites of infection are not accessible by phages. The concept of phage therapy is based on localized amplification in the presence of specific susceptible bacteria. High bacterial loads are necessary for amplification, and their localization is a complex pathophysiological issue. [127]

4. Showing efficacy in clinical trials and thus gaining regulatory approval.
5. Developing and implementing appropriate diagnostics are essential to support use in patients. [124]

Phage treatment will likely not take over the role antibiotics play in more typical bacterial infections. However, synergy with antibiotics has been seen *in vitro* and animal models. [128] Therefore, phage therapy may be a promising adjunctive treatment in specific indications or salvage therapy for patients with infections not responding to any other treatment. [124]

There are thought to be ten distinct bacteriophages for each bacterial cell, some of which are extremely specialized for their host and only recognize one type of receptor (monophage); others, instead, have a wider host range and recognize multiple types of receptors (polyphage). In several nations where the bacteriophage research and development centers were constructed specifically with phage therapy in mind, this therapy has been used during the past few decades to treat bacterial infections. The research carried out in these facilities led to outstanding clinical outcomes.

Phages have different infection processes and following bacterial infection, they may respond either in a lytic or lysogenic way, depending on their type (intrinsic biological characteristics).

During a lytic replication cycle, a phage attaches to a susceptible host bacterium, introduces its genome into the host cell cytoplasm, and utilizes the ribosomes of the host for manufacturing its proteins. The host cell resources are rapidly converted to viral genomes and capsid proteins, which assemble into multiple copies of the original phage. As the host cell dies, it is either

actively or passively lysed, releasing the new bacteriophage to infect another available and susceptible host cell. [129]

A promoter sequence in the phage genome initially attracts RNA polymerase to the site, and the transcription process results in the production of viral mRNA, which will be translated, leading to viral proteins' synthesis; this step inhibits the transcription process of the host's genome and stimulates the replication of the exogenous genetic material and enables the taking over and control of the bacterial cell mechanism, known as phagocytosis. Phage therapy seeks and employs particles of this kind.

In the lysogenic replication cycle, the phage also attaches to a susceptible host bacterium and introduces its genome into the host cell cytoplasm. However, the phage genome is instead integrated into the bacterial cell chromosome or maintained as an episomal element where, in both cases, it is replicated and passed on to daughter bacterial cells without killing them. [129] In this instance, phage reproduction takes place at a later stage; the phage replicates without lysing the host, and the bacterium develops a resistance to attacks from other phages of the same strain, turning it into a lysogenic bacterium. This bacterium is distinguished by the presence of a prophage (inactive phage) integrated into its genome that persists in a latent state for numerous bacterial cell divisions. The prophage is activated following *stress* processes or cellular damage of the host, inducing its replication via a lytic pathway, after it exists in the bacterial genome. [45]

The discovery and development of new antibiotics have decreased because of the large amount of time and money required, resulting in increasingly difficult clinical management of infections and, in some cases, infections that are impossible to treat. [130, 131, 132, 119] For this reason, phage therapy is considered a valid alternative to antibiotics because its mechanisms of action differ from that of antibiotics.

Moreover, lytic bacteriophages have had an impact on the clinical treatment of multidrug-resistant bacteria because of their capacity to naturally control bacterial populations. [133, 134]

These viruses provide novel advantages, such as the safe treatment of infections, as they are harmless to eukaryotic cells, do not cause harmful side effects, and demonstrate high host specificity. This is because they only replicate in the presence of bacteria causing the infection, thus reducing damage to the natural microflora. [119]

However, bacteriophage therapy has both advantages and disadvantages. In the first case, it can be said that this kind of therapy is an attractive antibacterial strategy in which a specific type of virus is used to inhibit or kill harmful bacteria. Bacteriophages exhibit a significant bactericidal effect by increasing the number of self-reproductions but only minimally disrupting the normal flora. [135]

- Lytic bacteriophages render bacteria infected with them incapable of regaining vitality. As opposed to being bactericidal, some antibiotics, are bacteriostatic. Antibiotics may thereby encourage the emergence of bacterial resistance (susceptible bacterial populations do not perish instantaneously in the presence of bactericidal or above all bacteriostatic antibiotics).
- Bacteriophage-based treatments come in a variety of shapes, including liquids, and tiny needles. It is possible to combine various bacteriophages to increase the antibiogram and produce a variety of antibacterial effects.
- They can multiply while eradicating bacteria, this ability to reproduce benefits the treatment of pathogens.
- Non-toxic nucleic acids and proteins make up the majority of bacteriophages. They can only infect a small number of pathogenic bacteria due to their host specificity, while the majority of normal tissues are unaffected.
- They are ubiquitous in the environment and one of the most abundant entities on the planet and are very easy to isolate. [45]
- Patients with allergies to antibiotics can safely be treated with phage therapy.

Phage therapy also carries a series of disadvantages such as:

- At present, a potential problem with phage therapy is that specific phages can modify host bacteria to make them pathogenic. [136] Lysogenic phages can integrate their genome into the target bacteria so this becomes a symbiotic relationship because the phage doesn't immediately kill the bacteria. Phages exist as bacterial components that form lysogens. Lysogen-infected bacteria do not die from infection. Bacterial lysogens tend to be resistant to the same lysogen infection and phage type. Even if the same type of phage later infects bacteria, it does not lead to bacterial death. [137, 135] It is therefore inferred that only lytic phages can be used for therapies.
- Phages are unstable, which makes it difficult to produce, store, and manage them. The main difficulties may occur due to the structural instability of virions as well as an accelerated genetic variability in phage genomes both in vivo and in vitro. But not only, they may be subjected to harsh solvents, pressures, and temperatures during the current manufacturing process. To maintain their effectiveness, most phage products are kept in cold chains for storage stability; they must be kept at cryogenic temperatures in certain circumstances.
- In the course of production or use, phages can develop and replicate themselves. Self-renewal phages have the potential to produce significant long-term therapeutic effects by administering only one dose. [138] Although this ability might be key to the development of vaccine-like treatments for chronic diseases, self-renewal phages significantly complicate pharmacokinetics. [135]
- It has to be defined which is the best route of administration, the optimal dose, the frequency of administration, and the average duration of treatment with these biological entities.

- Bacteria developed many different types of mechanisms that confer resistance to phages (in fact, the rapid appearance of phage-resistant bacterial variants is conceivable, which could hinder the favorable outcomes of the treatment itself). [45]

The use of phages in food products for human consumption has been studied, more specifically in live animals. The number of naturally occurring bacteria and bacteriophages are likely to fluctuate during passage through animal bodies, and be influenced by diet, contact with other animals, and incidents of disease. This might suggest that a dynamic predator-prey relationship exists within animal populations and that animals could provide a valuable resource for the discovery of new bacteriophages against a range of pathogenic bacteria. Bacteriophages with broad host specificity are able to be isolated from the environment and be cultivated on a laboratory scale to demonstrable effect in agricultural animals. Application of this technology could mean a decreased disease load and animal death through infection might allow for reduced disruption to milk or meat production, and thus reduce the economic burden on farmers through the loss that would be associated with bacterial disease. However, it must be noted that repeat application of bacteriophages could lead to a specific host immune response. [139] An essential problem is the efficient transfer of bacteriophages into animal bodies. The phage needs to reach the required location, stay alive during delivery, and be present in large enough numbers to have an impact on the host microorganism population. Phages can be introduced in a live animal, but the most commonly used way is the oral one, either with food or water. This process can be used both to make the phage get to one single animal or as a metaphylaxis approach.

Moreover, regarding meat products, phages have been used to reduce living bacterial cells. Key to the procedure is to ensure that the bacteriophages remain viable when applied post-slaughter, and perhaps post-processing. The bacteriophages must remain viable during storage conditions in order to reduce the bacterial load. However, once this has been achieved, the delivery of viable bacteriophages to the human consumer is not necessarily required. [139]

Studies have found that higher temperatures facilitate lytic activity against bacteria leading to a reduction of cell numbers (using equivalent combinations of time and temperature); so by increasing the temperature, and reducing time, the treatment had the same result in terms of logarithmic decimal reduction of bacterial cells; but still it was found that at 4°C phages were able to induce the lysis of the cell. Research indicates that bacteriophage infection and reproduction require the presence of metabolically active host cells. To this end, the refrigeration of foods would appear to allow for bacteriophage infection of host cells, but at sub-optimal rates. Also of critical importance is the ratio of bacteriophage to host cell needed for the reduction of bacterial cell numbers. Studies agree that the use of a higher concentration of bacteriophages is optimal, but it must be noted that the studies published only usually refer to one to two species of bacteria. If such techniques are to be effective, then the use of bacteriophage cocktails is perhaps an avenue for further research, given the potential for foods to contain a range of bacterial pathogens from multiple sources. However, the selection of appropriate bacteriophages would be critical to the success of such an endeavor, requiring a full investigation of their host range. [139] Finally, it is important, to remember that the use of bacteriophages as biocontrol agents might mean that viable bacteriophages enter the human food chain post-cooking/processing, which presents several consumer health-related issues. [140] It is important to assess how cooking affects phage survival for each food item because research has long shown that environmental factors like temperature are essential to bacteriophage survival in food. DiGirolamo [141] and colleagues demonstrated that the temperatures used for cooking experimentally contaminated crabs were insufficient to inactivate the bacteriophage therein.

Recent studies have demonstrated that bacteriophages can be administrated by either intraperitoneal or intravenous way; another way can be considered the topical one, where bacterial skin conditions can be treated with just a topical (local and external) application.

3.2 ANTIMICROBIAL PEPTIDES (AMP)

Antimicrobial peptides are small peptides crucial for the innate immune response. Sometimes referred to as “host-defense peptides”, AMPs are ubiquitous in the epithelial barriers and systemic induced defenses of multicellular eukaryotes. [142] They are highly diverse within and across species, with most plant and animal genomes encoding 5 to 10 distinct AMP gene families that range in size from one to more than 15 paralogous genes. [143] At the moment there are more than 1700 AMPs known, they are made up of under 100 amino acids, and just one gene encodes them. They are synthesized during inflammation or in the presence of molecules produced by pathogenic agents. [144]

Per their secondary structure and amino acid makeup, AMPs can be divided into four classes:

1. Linear peptides.
2. With α-helical structure.
3. With a β-stranded structure.
4. With a loop structure.

The type of structure of AMPs is directly correlated to their size, mechanism of action, hydrophobicity, net charge, and polar angle. [145, 45]

The galloping increase of bacterial resistance to chemical antibiotics and the possibility of these pharmaceutical compounds losing their effectiveness in the treatment of bacterial infections in the next five years exponentially increased the interest of both researchers and pharmaceutical industries in the application of AMPs as therapeutic antimicrobial agents. [146, 147, 148, 45]

AMPs are considered potential drug candidates for the treatment of infections caused by otherwise untreatable microorganisms. [149, 150] The first AMP, gramicidin, was discovered in 1939 from the soil bacteria *Bacillus brevis* and showed in vitro and in vivo antibacterial activity against many Gram-positive bacteria. [151, 152, 153]

AMPs have a variety of reported modes of action, most of which involve direct pathogen killing, however, some AMPs also have the potential to kill indirectly by altering host immune responses. An important feature that sets AMPs apart from conventional antibiotics is their attack on multiple low-affinity targets such as bacterial membranes, which is thought to mitigate the development of antimicrobial resistance. [154, 153]

The cell wall is a potential target for AMPs to recognize microbial cells. AMPs are acting on the cell wall and exert antibacterial effects mainly by affecting the synthesis of cell wall components and destroying the cell wall structure. (Figure 12) [155]

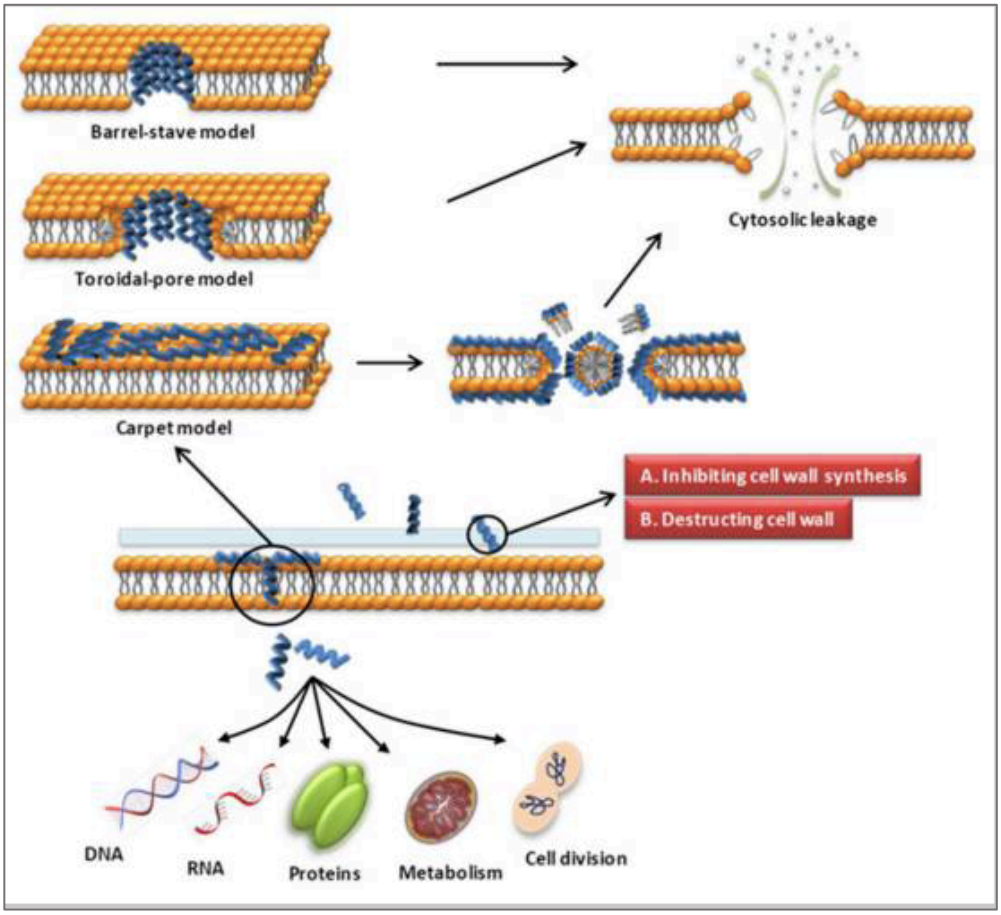

Figure 12 - Schematic presentation of the antibacterial mechanism of AMPs. Some AMPs act on cell membranes through different modes of action, increasing membrane permeability, leading to leakage of cell contents and cell death. Modes of action include barrel-stave, toroidal-pore, carpet models. Some AMPs act on the cell wall and exert antibacterial effects by affecting the synthesis of cell wall components and destroying the cell wall structure. Some AMPs enter the cell through direct penetration or endocytosis and exert anti-microbial effects by targeting the nucleus, organelles, present in fungi, or intracellular proteins. [155]

AMPs biological function can be divided into the following aspects:

- Direct bactericidal effect: AMPs directly kill microorganisms by acting on the microbial membrane or intercellular targets.
- Antibiofilm activity: several studies have shown that AMPs have antibiofilm activity, whether the concentration is equal to or higher than the minimum inhibitory concentration (MIC) for corresponding planktonic cells or the concentration is lower than the MIC for corresponding planktonic cells. [156, 157]

- Immune system regulation: this mechanism includes the regulation of proinflammatory and anti-inflammatory responses, cell differentiation, wound healing, autophagy, and apoptosis.
- When used in combination with traditional antibiotics, AMPs also show a synergic effect by promoting the absorption of antibiotics. [155] For example, a recent study on multidrug-resistant Gram-negative bacteria showed strong synergism between the antibiotic azithromycin – which showed no activity against Gram-negative bacteria in standard MIC tests – and the AMPs colistin and LL-37 [158, 143] (the only AMPs in the cathelicidin family that exists in the human body, produced by mainly epithelial cells and neutrophils). [155]

The pharmacodynamics of AMPs reduces the probability of resistance evolution. [159] Contrary to many antibiotics, which can increase the rate of bacterial mutations by inducing bacterial stress responses, the majority of AMPs interact with the bacterial cell's surface and do not directly cause bacterial mutations. AMPs kill faster than antibiotics - within minutes instead of hours [160] - allowing many fewer bacterial generations in which resistance could evolve. Because resistance to AMPs tends to be by nonspecific mechanisms, there may be fewer mutational routes by which resistance to AMPs can evolve [161] and lower likelihood of horizontal gene transfer that confers resistance. [162, 143]

The wise use of synergic and potent AMP combinations that eradicate bacteria before resistant forms can evolve is most likely the most efficient strategy to prevent widespread, generic resistance to AMPs. These cocktails could occasionally be expertly crafted using information about the properties of the constituent ingredients known in advance. Of greater concern, evolved resistance to therapeutically applied AMPs could result in undesirable cross-resistance to endogenous host AMPs. [163] The only explicit test of this hypothesis has been carried out in the model insect *Tenebrio* [164, 165], in which some AMP-resistant *S. aureus* survived better in the host, albeit without showing increased virulence. However, similar experiments in a

mammalian system or with human AMPs are lacking. This is particularly concerning given that at least 11 of the AMPs that are currently under clinical trial are of human origin. [143]

Peptides exert their effect either by compromising the integrity of the bacterial cell wall or through interferences with intracellular metabolic processes. [166, 167] Also, peptides' exact mechanisms of action against all pathogens are not the same. While the initiation of interaction depends solely on electrostatic forces, [168, 169] the effects AMPs exert on the membrane are dependent on the properties of the peptides. [170]

In terms of how peptides work, there are typically two main processes to take into account: direct cell death by rupture of the cell membrane and immunomodulatory effect.

In bacteria species, AMPs initiate cell disruption in three major steps:

1. The attraction phase involves the interaction of the cationic peptides with the bacterial surface. The cationic peptide binds to the bacterial outer membrane surfaces using lipopolysaccharide as a receptor. Since the AMPs are positively charged, the attachment makes it displace cationic ions such as Ca^{2+} and Mg^{2+}, causing a disruption of the outer membrane, allowing the AMPs to reach the intracellular target (negatively charged phospholipids). [171, 172]
2. Attachment: it involves the penetration of the polysaccharides and their attachment and interaction with cellular targets such as the protective capsular coats. [171]
3. Once successful in crossing the cell membrane, AMPs interact with the lipids in the cytoplasmatic membrane such as phosphatidylglycerol and cardiolipin, destabilizing the bonds of the phospholipids, resulting in disintegration or permeability. Furthermore, there is the insertion stage, where peptides position themselves within the membrane bilayer, leading to membrane thinning, curvature, and disruption of the membrane barrier. [173, 170]

AMPs surely carry many advantages for example, they are more efficient than traditional antibiotics. They display their advantages over conventional antibiotics with the

broad-spectrum antibacterial, antifungal, and antivirus activities. [150, 174] They are also potent with rapid germ-killing ability and low bactericidal concentration, even effective on traditional antibiotic-resistant strains, and even have synergistic effects with typical antibiotics to neutralize endotoxin. [175, 150] Furthermore, these AMPs are safe with no toxic side effects or less, and hard to induce bacterial drug resistance compared to conventional antibiotics. [176, 177]

They are small synthetic molecules with low cost, simple structure-activity relationship, and weak or low sensitization. [178, 179] They can be widely used in medical development. For instance, daptomycin, one of the AMPs, has been approved and marketed in 2003 as an anionic antibacterial peptide employed to treat skin infections caused by Gram-positive bacteria (Table 2). This peptide even showed inhibitory effects on highly drug-resistant Typhoid bacillus and *Staphylococcus aureus*. Moreover, these peptides displayed their inhibitory ability to cancer cells. [150, 180] Many studies have demonstrated that cancer cells are more sensitive to AMPs than normal cells. Cancer cells may have changes to their membrane, cytoskeleton, or extracellular matrix due to their high metabolism. The lipid membranes are easily penetrated by these peptides, which then create ion channels or pores that eventually kill cancer cells or cause cell contents to seep out. These membrane-permeabilizing AMPs represent a potential new therapy against drug-resistant microbes that result in more morbidity and mortality and may be clinically applied as a strategy to overcome the frequent resistance of many common microbes to conventional antibiotics. [177]

Table 2 - The antimicrobial peptide drugs approved by Food and Drug Administration (FDA) [177]

Drugs	Trade names	Antimicrobial activities	Administrations	In use
Bacitracin	Baciim	Gram-positive bacteria	Topical	Localized skin and eye infections, wound infections.
Dalbavancin	Dalvance, Xydalba	Gram-positive bacteria	Intravenous	Acute bacterial skin infections.
Daptomycin	Cubicin	Gram-positive bacteria	Intravenous	Bacterial skin infections.
Enfuvirtide	Fuzeon	Virus	Subcutaneous	HIV-1 infection.
Oritavancin	Orbactiv	Gram-positive bacteria	Intravenous	Bacterial skin infection.
Teicoplanin	Targocid	Gram-positive bacteria	Intravenous & intramuscular	Bacterial infections.
Telaprevir	Incivo, Incivek	Virus	Oral	Hepatitis C.
Telavancin	Vibativ	Gram-positive bacteria	Intravenous	Bacterial skin infection.
Vancomycin	Vancocin	Gram-positive bacteria	Oral & intravenous	Bacterial infections.

On the other hand, AMPs are easily deactivated by proteases and are therefore vulnerable to their action, which may provide a barrier to the systemic administration of AMPs. When given in high doses, AMPs can cause cytotoxicity in many host cells and may disrupt cell membranes. PH variations can also influence AMPs' activities resulting in a loose of their activity. After several treatments, they could cause sensitivities and allergies.

AMPs appear to be promising therapeutic drugs for different skin and soft tissue infections. They present a broad spectrum of antimicrobial activity, wound-healing promoting activities, such as angiogenesis, and induction of cell migration and proliferation, in addition to immune-modulatory activity. [181] As reported in Table 2, daptomycin (approved in 2003), telavancin (approved in 2014), and dalbavancin (approved in 2009) are used for injections against complicated skin, and skin structure infections caused by different Gram-positive bacterial infections. [182] Furthermore, AMPs with anti-tumor activity called anticancer peptides (ACPs), are new drugs that may overcome the problems associated with tumor resistance to conventional chemotherapy. In recent years, the number of natural AMPs that have antitumor activity has increased. [183, 184]

3.3 NANOPARTICLES

The field of nanotechnology has advanced exponentially in the last decade and many products containing nanoparticles are now used in various applications such as in food science, cosmetics, and pharmaceuticals. [185]

Nanoparticles (NPs) are defined as particles with one dimension ranging between 1 and 100 nm. NPs exhibit different properties depending on their size and surface functionalities. [186] The small size and large surface area account for the extensive use of NPs in various areas such as cosmetics, electronics, and both diagnostic and therapeutic medical applications. [187] NPs are used as pharmaceutical drug carriers with applications in both diagnostics and therapy field. These NPs, including polymeric NPs, nanoemulsions, liposomes, and solid NPs, are suggested to have potential clinical applications. Their clinical applicability depends on different parameters such as their physical and chemical properties, drug loading efficiency, drug release, and most importantly low or no toxicity of the carrier itself. [188, 189]

Advancement in the nanotechnology area offers synthesis of various nanosized inorganic/organic molecules having potential applicability in the fields of medicine, therapeutics, and diagnostics sectors [190] having a profound impact on human health improvement while nanoparticles are considered as innovative tools due to their inimitable physicochemical properties such as strength, durability, performance, and flexibility. These nanosized particles can be used as diagnostic agents, targeted drug delivery vehicles, noninvasive imaging technologies, oncology, dermatology, cardiovascular medications, and other chronic disease care. [191, 192] Development of these nanoparticles as antimicrobial compounds proves as a substitute approach to solve the problem related to antimicrobial resistance [191] as nanoparticles rely on entirely different mechanisms compared with traditional antibiotics. [193]

When discussing nanomaterials fabrication, two main approaches can be distinguished: top-down and bottom-up. The top-down approach implies starting from larger structures and reducing their size by means of mechanical force and the aid of finer and finer tools until reaching dimensions in the nano range. [194, 195] These methods are preferred in industrial settings, as they can be easily scaled up and produce fine particles with fine particle-producing capacity and reproducibility. In opposition to top-down techniques, bottom-up processes assume the fabrication of nanoparticles through the growth and self-assembly of smaller components of atomic or molecular dimensions, conforming to a natural physical principle or an externally applied driving force. [195] Such methods are simple, rapid, energy-efficient, and cost-effective, being ideal options for laboratory-scale production of amorphous particles with reduced dimensions, narrow particle size distribution, increased solubility, and enhanced bioavailability. However, NPs obtained in this manner tend to agglomerate and might also present stability issues, while there are also several drawbacks associated with the fabrication processes. [194, 196]

A variety of physical, chemical, and biological fabrication methods are available for the synthesis of nanostructures (Table 3). [197]

Table 3 – Classification of NP synthesis methods. [197]

Synthesis approach	Nature of involved process	Examples of techniques
Top-down approach	Physical methods	Ball milling, Laser ablation, Electron beam deposition, Sputtering, Aerosol spray.
Bottom-up approach	Chemical methods	Co-precipitation, Thermal decomposition, Sol-gel, Microemulsion Sonochemical, Hydrothermal, Microwave-assisted, Chemical reduction, Electrochemical, Solvothermal.
	Biological methods	Bacteria-based, Plant-based.

3.3.1 Silver nanoparticles (AgNPs)

Silver nanoparticles have been imposed as an excellent antimicrobial agent being able to combat bacteria in vitro and in vivo causing infections. Gram-positive and Gram-negative bacteria, including multidrug-resistant strains, are all covered by AgNPs' antibacterial ability. AgNPs exhibit multiple and simultaneous mechanisms of action and in combination with antibacterial agents such as organic compounds or antibiotics, it has shown synergistic effect against pathogens bacteria. The characteristics of silver nanoparticles make them suitable for their application in medical and healthcare products where they may treat infections or prevent them efficiently. [198]

Throughout history, silver in all of its forms has been used either alone or in conjunction with other technologies as an antimicrobial agent; due to its low cytotoxicity, it is known to be effective against a variety of both Gram-positive and Gram-negative bacteria. Because of the knowledge and evidence existing of the antibacterial activity of silver [199], with the emergence of nanotechnology, the exploration of the antibacterial capacity of AgNPs was an evident path. [198]

Although AgNP is integrated into many areas, the exact mechanism explaining the particle formation is not fully uncovered yet. The traditional method for the synthesis of AgNP is to use physical and chemical approaches to produce nanoparticles with controlled and well-defined sizes and shapes. [200] Recently, the synthesis of AgNP through biological methods has been studied intensely. The biological method offers nanoparticles with high yield and stability compared to the conventional physical and chemical approach. [201] AgNP can be biosynthesized by bacteria, fungi, yeast, actinomycetes, and plant, thus avoiding the use of toxic substances and enabling further application in medical and pharmaceutical field. [202] The application of plants for the synthesis of AgNP has gained significant attention. [203] AgNPs' mechanism of action is still not fully understood, but several theories could account for their

antibacterial, anti-inflammatory, and anti-cancer properties. It is known that nanoparticles have a large surface area that either penetrates the cell or attaches itself to the cell wall, [202] causing a disturbance in the membrane permeability making it porous, [204] and this action leads to a further leakage of cell content. Moreover, the appearance of pores on the membrane result in the diffusion of nanoparticles into the cell where it binds with sulfur and phosphorus-containing proteins, thus leading to the inactivation of proteins and DNA. [205, 203]

Extremely interesting, in this regard is the work carried out by Professor Elviri of the University of Parma in collaboration with the Department of Veterinary Medicine Sciences. They created a formulation of silver nanoparticles (AgNP) and two natural polymers such as alginate (ALG) and nanocrystalline cellulose (CNC) which have been developed for 3D printing of scaffold. The antimicrobial activity of 3D-printed developed scaffolds has been determined as shown in Table 4. These exhibited a large interface surface area, improved mechanical strength, and the ability to promote antimicrobial actions and cytotoxic effects.

Table 4 – Determination of antimicrobial activity of 3D-printed developed scaffolds. [206]

SCAFFOLD	***Staphylococcus aureus***		***Pseudomonas aeruginosa***	
	Ø Inhibition Diameter (mm)			
ALG	0	0	0	0
ALG/CNC	0	0	0	0
ALG/CNC + AgNP (100 µg/mL)	6	6	6	6
ALG/CNC + AgNP (10 µg/mL)	6	6	6	6
ALG/CNC + AgNP (5 µg/mL)	0	0	0	0
ALG/CNC + AgNP (1 µg/mL)	0	0	0	0

Calcium alginate (ALG) is worldwide considered a highly absorbent, biocompatible and biodegradable hydrogel suitable for dressing purposes [207], it derives from seaweed, is generally regarded as safe and its known strength relies on the capacity to keep the wound

environment moist, favoring healing as well as the formation of granulation tissue and being easily removable with painless dressing changes. [208, 209]

Crystalline nanocellulose (CNC) is a promising biomaterial derived from different cellulose sources by means of various processing methodologies. [210] The cellulose nanoparticles are characterized by elevated surface area and crystallinity, they have a very large elasticity modulus, and they have high strength, low toxicity and high biocompatibility.

As for morphology, SEM (Scanning Electron Microscopy) images of ALG and ALG/CNC scaffolds exhibited rough homogeneous surfaces with optimal macro pore size, ranging from 174 to 201 µm. (Figure 13 A,C) [206]

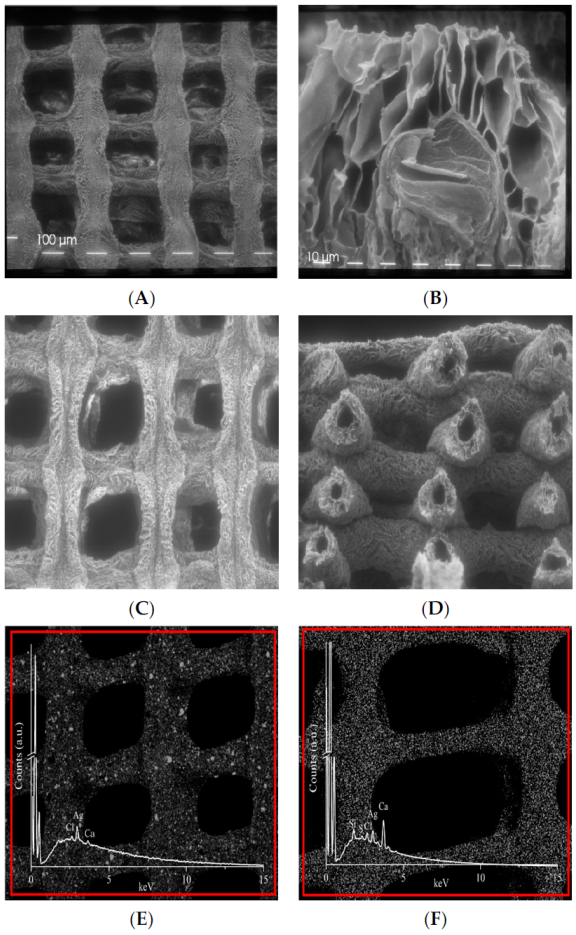

*Figure 13 - Scanning electron microscope images at different magnification of: (**A**) ALG 5% (w/v) scaffold (80×); (**B**) ALG 5% (w/v) scaffold (360×); (**C**) ALG/CNC (5% and 3% w/v) scaffold (80×); (**D**) ALG/CNC (5% and 3% w/v) scaffold cross section (80×). Energy-dispersive X-ray spectroscopy (EDS) map distribution and EDS spectrum of Ag in: (**E**) ALG 5% (w/v) scaffold (80×); (**F**) ALG/CNC (5% and 3% w/v) scaffold (80×). [206]*

3.3.2 Zinc nanoparticles

Zinc nanoparticles (ZnONPs) are an eco-friendly and biosafe material having a small size and high surface area. These NPs possess an attractive antimicrobial property due to the generation of ROS [H_2O_2 (hydrogen peroxide), OH^- (hydroxyl radicals), and O_2^{-2} (peroxide)] which behave as a major factor in cellular damage, enhancing the permeability of cell membrane, nanoparticles internalization due to proton motive force loss and toxic dissolved zinc ions uptake that lead to decrease in the activity of mitochondria and produce ROS compounds that cause cell death. [193] ZnONP has gained considerable attention out of all other nanoparticles because of its unique electronic, optical, and medicinal properties. [211] Zinc oxide nanoparticle is highly biocompatible and its electron transport kinetics rate is fast so, it's suitable to use as a biological membrane or for other biological applications. [212] Review literature clearly illustrates the antimicrobial activity of ZnONPs against a broad spectrum of pathogenic bacteria like *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Mycobacterium tuberculosis,* and *Bacillus subtilis*. [213] The functional relevance of the antibacterial efficacy of ZnO was discussed in the food packaging sector where they are used as antibacterial agents against food-borne pathogens. [214, 193]

3.3.3 Titanium nanoparticles

Titanium dioxide (TiO_2) nanoparticles (NPs) – also called titania [215] -, were first developed in the 1990s, and have been applied in numerous biomedical fields such as tissue engineering and therapeutic drug development. In recent years, TiO_2-based drug delivery systems have demonstrated the ability to decrease the risk of tumorigenesis and improve cancer therapy. the detailed molecular mechanisms by which drug delivery to cancer cells alters sensing of gene mutations, protein degradation, and metabolite changes as well as its associated cumulative

effects that determine the microenvironmental mechanosensitive metabolism have not yet been clearly elucidated.

Titanium dioxide (TiO_2) is abundantly available in nature and possesses specific properties such as biocompatibility, lightness, high corrosion resistance, high thermal stability, low ion release, and non-magnetic properties. [216, 217, 218] The current bulk manufacturing of TiO_2 further allows its application in sunscreens, implants, and as a pigment in paint additives. Furthermore, titanium-based nickel alloys are widely used in surgical implants. [219, 220] Nanoscale titanium, with a size of less than 100nm, has recently been the subject of research aimed at the creation of biocompatible and non-toxic instruments. Nanoscale TiO2 particles offer special therapeutic qualities and have been used in numerous biomedical applications as a result. These NPs have been approved by the Food and Drug Administration (FDA) as safe, biocompatible, highly reactive, and chemically stable substances. It is estimated that the global annual production of TiO_2NPs was 1,175,176 tons in 2012 [221] and it will reach up to 2.5 million metric tons in 2050, which will convert nearly 100% of the total TiO_2 market into nano. [222] Nanostructures of TiO_2 have profound applications in the agriculture and food industry, food packaging, textile, energy, ceramics, cosmetics, medical devices, pharmacy, and theranostics of various diseases. [223, 224, 215] Thin film transparency, biological and chemical awareness, low toxicity, and excellent chemical stability are only a few of the traits of TiO_2NPs that have led to their considerable research. The medical uses of $TiNO_2$NPs, among others, are noteworthy and could significantly contribute to the expansion of the healthcare industry. For instance, the photocatalytic capability of photoexcited TiO_2NPs illustrates the ability to significantly destroy cancer cells. TiO_2NPs have also been regarded as an appealing antimicrobial agent. [225] Moreover, TiO_2NPs are one of the increasingly used metal oxide NPs in biomedicine because of their antimicrobial activity. [226]

The literature reports that the antibacterial activity of TiO_2NPs against MRSA was investigated. A study was designed to evaluate the effect of TiO_2NPs against biofilm formation by MRSA

using the tissue culture plate method. A total of 30 isolates were taken and out of them, 22 were involved in strong biofilm formation and 2 were weak in the formation of biofilm. The TiO_2NPs (500µg/ml) inhibited the growth of both strong and weak MRSA, thus showing that the TiO_2NPs are promising antibacterial candidates. [227, 215]

3.3.4 Iron nanoparticles

Iron nanoparticles (FeNPs) represent an additional class of antimicrobial materials that are usually inert when present in bulk form and did not possess antimicrobial properties. Several studies reported that by modifying their surfaces their anti-adherent as well as anti-bacterial properties get activated resulting in the abolition of both Gram-negative and Gram-positive bacterial biofilms. [228, 193]

Superparamagnetic iron oxide nanoparticles (SPIONs) are also used. They are a special class of metal-oxide NPs with magnetic properties and excellent biocompatibility. Their shape, size, and magnetic nature enable them to kill microorganisms through the application of an external magnetic field, resulting in an increase of the therapeutic antimicrobial properties, especially when compared to conventional antimicrobial compounds. [229] The superparamagnetism is generated due to the reduced size of these nanoparticles which allow for a higher surface-to-volume ratio, increasing the surface of the atoms. [230, 231]

CONCLUSION

The purpose of this paper has been to bring to light the serious problem of antibiotic resistance which is continuing to spread globally both in developed and developing countries.

The discovery and development of new alternative therapies to replace the use of antibiotics, which, however important and useful they have been, are becoming a problem for humans, animals, and the environment, is urgent.

The prospect is to reduce or, at least, contain the problem of antibiotic resistance even with the use of the "One Health" policy and therefore pay attention not only to antibiotics in humans but also in livestock as once the animals are consumed, there is a possibility that antibiotic-resistant pathogens are also assimilated by humans.

Deaths from antibiotic resistance are expected to be 10 million people by 2050, for this reason, it is imperative to find a solution to this problem as soon as possible.

In addition to using the alternative therapies that have been discovered, one option for solving the problem of antibiotic resistance could be to educate and make people understand how to use antibiotics correctly while avoiding overuse and misuse.

The hope is surely that the situation will improve and that with the cooperation of more areas, people, and medical staff the problem can become controllable again.

BIBLIOGRAPHY

[1] M. I. Hutchings, A. W. Truman and B. Wilkinson, "Antibiotic: past, present and future," *Current Opinion in Microbiology,* 2019.

[2] L. Katz and R. H. Baltz, "Natural product discovery: past, present and future," *Industrial Microbiology and Biotechnology,* 2016.

[3] E. Etebu and I. Arikekpar, "Antibiotics: Classification and mechanisms of action with emphasis on molecular perspectives," *International Journal of Applied Microbiology and Biotechnology Research,* 2016.

[4] D. A. Russel, "Types of antibiotics and synthetic antimicrobial agents," in *Hugo and Russel's pharmaceutical microbiology*, Blackwell Science, 2004.

[5] S. B. Singh, K. Young and L. L. Silver, "What is an "ideal" antibiotic? Discovery challenges and path forward.," *Biochemical Pharmacology,* 2017.

[6] R. Laxminarayan, P. Matoso, S. Pant, C. Bower, J.-A. Røttingen, K. Klugman and S. Davies, "Access to effective antimicrobials: a worldwide challenge.," *The Lancet,* 2016.

[7] F. Harrison, A. E. L. Roberts, R. Gabrilska, K. P. Rumbaugh, C. Lee and S. . P. Diggle, "A 1,000-Year-Old Antimicrobial Remedy with Antistaphylococcal Activity," *mBio,* 2015.

[8] T. P. Van Boeckel, S. Gandra, A. Ashok, Q. Cuadron, B. T. Grenfell, S. A. Levin and R. Laxminarayan, "Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data.," *The Lancet Infectious Diseases,* 2014.

[9] K. Bush, P. Courvalin, G. Dantas, J. Davies, B. Eisenstein, P. Huovinen, G. A. Jacoby, R. Kishony, B. N. Kreiswirth, E. Kutter, S. A. Lerner, S. Levy, K. Lewis, O. Lomovskaya and J. Miller, "Tackling Antibiotic Resistance," *Nature Reviews Microbiology,* 2011.

[10] J. M. Stokes, A. J. Lopatkin, M. A. Lobritz and J. J. Collins, "Bacterial Metabolism and Antibiotic Efficacy," *Cell Metabolism,* 2019.

[11] G. Cox, A. Sieron, A. M. King, G. De Pascale, A. C. Pawlowski, K. Koteva and G. D. Wright, "A Common Platform for Antibiotic Dereplication and Adjuvant Discovery," *Cell Chemical Biology,* 2017.

[12] J. M. Stokes, K. Yang, K. Swanson, W. Jin, A. Cubillos-Ruiz, N. M. Donghia, C. R. MacNair, S. French, L. A. Carfrae, Z. Bloom-Ackerman, V. M. Tran, A. Chiappino-Pepe, A. H. Badran and I. Andrews, "A deep learning approach to antibiotic discovery," *Cell,* 2020.

[13] W. Wohlleben, Y. Mast, E. Stegmann and N. Ziemert, "Antibiotic drug discovery," *Microbial Biotechnology,* 2016.

[14] G. Kapoor, S. Saigal and A. Elongavan, "Action and resistance mechanisms of antibiotics: a guide for clinicians," *Journal of Anaesthesiology Clinical Pharmacology,* 2017.

[15] J. Heesemann, "Mechanisms of resistance to beta-lactam antibiotics.," *Infection,* 1993.

[16] K. B. Holten and E. M. Onusko, "Appropriate Prescribing of Oral Beta-Lactam Antibiotics," *American Family Physicians,* 2000.

[17] T. T. Tidwell, "Hugo (Ugo) Schiff, Schiff Bases, and a Century of β-Lactam Synthesis," *Angewandte Chemie International Edition,* 2008.

[18] L. M. Lima, B. N. M. da Silva, G. Barbosa and E. J. Barreiro, "β-lactam antibiotics: An overview from a medicinal chemistry perspective," *European Journal of Medicinal Chemistry,* 2020.

[19] W. Kirby, "Extraction of a highly potent penicillin inactivator from resistant Staphylococci," *Science,* 1944.

[20] X. Lin and U. Kück, "Cephalosporins as key lead generation beta-lactam antibiotics," *Applied Microbiology and Biotechnology,* 2022.

[21] S. Pegler and B. Healy, "In patients allergic to penicillin, consider second and third generation cephalosporins for life threatening infections," *BMJ,* 2007.

[22] Z. M. Thu, J. Sun, J. Ji, L. He, Z. Iqbal, K. K. Myo, Y. Gao, L. Zhai, Y. Mu, D. Tang, G. Vidari, H. Yang and Z. Yang, "Synthesis and antibacterial evaluation of new monobactams," *Bioorganic & Medicinal Chemistry Letters,* 2021.

[23] L. E. Asbel and M. E. Levison, "CEPHALOSPORINS, CARBAPENEMS, AND MONOBACTAMS," *Infectious Disease Clinics of North America,* 2000.

[24] R. C. Moellering Jr, G. M. Eliopoulos and D. E. Sentochnik, "The carbapenems: new broad spectrum beta-lactam antibiotics," *The Journal of Antimicrobial Chemotherapy,* 1989.

[25] K. M. Papp-Wallace, A. Endimiani, M. A. Taracila and R. A. Bonomo, "Carbapenems: Past, Present, and Future," *Antimicrobial Agents and Chemotherapy,* 2011.

[26] J. A. Torres, M. V. Villegas and J. P. Quinn, "Current concepts in antibiotic-resistant gram-negative bacteria," *Expert Review of Anti-infective Therapy,* 2007.

[27] A. J. Brink, C. Feldman, D. C. Grolman, D. Muckart, J. Pretorius, G. A. RIchards, M. Senekal and W. Sieling, "Appropriate use of the carbapenems," *South African Medical Journal,* 2004.

[28] D. Moore, 2015. [Online]. Available: http://www.orthobullets.com/basic-science/9059/antibiotic- classification-and-mechanism.. [Accessed 1 September 2016].

[29] G. P. Dinos, "The macrolide antibiotic renaissance," *British Journal of Pharmacology,* 2017.

[30] M. E. Oliver and T. S. Hinks, "Azithromycin in viral infections," *Reviews in Medical Virology,* 2020.

[31] W. R. Taylor, T. L. Richie, D. J. Fryauff, C. Ohrt, H. Picarima, D. Tang, G. S. Murphy, H. Widjaja, D. Braitman, E. Tjitra, A. Ganjar, T. R. Jones, H. Basri and J. Berman, "Tolerability of Azithromycin as Malaria Prophylaxis in Adults in Northeast Papua, Indonesia," *Antimicrobial Agents and Chemotherapy,* 2003.

[32] A. Firth and P. Prathapan, "Azithromycin: The First Broad-spectrum Therapeutic," *European Journal of Medicinal Chemistry,* 2020.

[33] World Health Organization, "World Health Organization Model List of Essential Medicines: 21st List.," Geneva, 2019.

[34] M. Socan, "Treatment of atypical pneumonia with azithromycin: comparison of a 5-day and a 3-day course," *Journal of Chemotherapy,* 1998.

[35] P. Zarogoulidis, N. Papanas, I. Kioumis, E. Chatzaki, E. Maltezos and K. Zarogoulidis, "Macrolides: from in vitro anti-inflammatory and immunomodulatory properties to clinical practice in respiratory diseases," *European Journal of Clinical Pharmacology,* 2012.

[36] H. Li, D.-H. Liu, L.-L. Chen, Q. Zhao, Y.-Z. Yu, J.-J. DIng, L.-Y. Miao, Y.-L. Xiao, H.-R. Cai, D.-P. Zhang, Y.-B. Guo and C.-M. Xie, "Meta-analysis of the adverse effects of long-term azithromycin use in patients with chronic lung diseases," *Antimicrobial Agents and Chemotherapy,* 2014.

[37] P. Gautret, J.-C. Lagier, P. Parola, V. T. Hoang, L. Meddeb, M. Mailhe, B. Doudier, J. Courjon, V. GIordanengo, V. E. Vieira, H. T. Dupont, S. Honoré, P. Colson and Chabrièr, "Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial," *International Journal of Antimicrobial Agents,* 2020.

[38] T. H. Grossman, "Tetracycline Antibiotics and Resistance," *Cold Spring Harbor Perspective in Medicine,* 2016.

[39] B. Duggar, "Aureomycin; a product of the continuing search for new antibiotics," *Annals of the New York Academy of Sciences,* 1948.

[40] A. R. Sánchez, R. S. Rogers 3rd and P. J. Sheridan, "Tetracycline and other tetracycline-derivative staining of the teeth and oral cavity," *International Journal of Dermatology,* 2004.

[41] N. Sabtu, D. Enoch and M. Brown, "Antibiotic resistance: what, why, where, when and how?," *British Medical Bulletin,* 2015.

[42] J. Conly and B. Johnston, "Where are all the new antibiotics? The new antibiotic paradox.," *Canadian Journal of Infectious Diseases and Medical Microbiology,* 2005.

[43] S. Ghosh, C. Bornman and M. Z. Zafer, "Antimicrobial Resistance Threats in the emerging COVID-19 pandemic: Where do we stand?," *Journal of Infection and Public Health,* 2021.

[44] A. Chokshi, Z. Sifri, D. Cennimo and H. Horng, "Global contributors to antibiotic resistance," *J. Glob Infect Dis.,* 2019.

[45] A. C. Rios, C. G. Moutinho, F. C. Pinto, F. S. Del Fiol, A. Jozala, M. V. Chaud, M. M. Vila and J. A. Teixeira, "Alternatives to overcoming bacterial resistances: State-of-the-art," *Microbiological Research,* 2016.

[46] T. McKeown, The role of medicine: dream, mirage, or nemesis?, Princeton Legacy Library: Princeton University Press, 2016.

[47] F. Baquero, "Threats of antibiotic resistance: an obliged reappraisal," *International Microbiology,* 2021.

[48] J. Carlet and J. L. Mainardi, "Antibacterial agents: back to the future? Can we live with only colistin, co-trimoxazole and fosfomycin?," *Clinical Microbiology and Infection,* 2011.

[49] J. O. Neill, "Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations," 2014.

[50] C. L. Ventola, "The Antibiotic Resistance Crisis," *Pharmacy and Therapeutics,* 2015.

[51] P. Boerlin and R. J. Reid-Smith, "Antimicrobial resistance: its emergence and transmission.," *Animal Health Research Review,* 2008.

[52] G. Mancuso, A. Midiri, E. Gerace and C. Biondo, "Bacterial antibiotic resistance: the most critical pathogens," *New Insights into Bacterial Pathogenesis,* 2021.

[53] B. Coculescu, "Antimicrobial resistance induced by genetic changes.," *J Med Life,* 2009.

[54] S. Dancer, "The dangers of broad spectrum antibiotics.," *BMJ,* 2003.

[55] FDA, "SUMMARY REPORT on Antimicrobials Sold or Distributed for Use in Food-Producing Animals.," 2016.

[56] T. T. H. Van, Z. Yidana, P. M. Smooker and P. J. Coloe, "Antibiotic use in Food Animals Worldwide, with a focus on Africa: Plus and Minuses," *Journal of Global Antimicrobial Resistance,* 2019.

[57] R. Gustafson and R. Bowen, "Antibiotic use in animal agriculture," *Journal of Applied Microbiology,* 1997.

[58] N. De Briyne , J. Atkinson, L. Pokludová and S. P. Borriello, "Antibiotic used most commonly to treat animals in Europe," *Veterinary Record,* 2014.

[59] M. L. Gilchrist, C. Greko, D. B. Wallinga, G. W. Beran, D. G. Riley and P. S. Thorne, "The Potential Role of Concentrated Animal Feeding Operations in Infectious Disease Epidemics and Antibiotic Resistance," *Environmental Health Perspective,* 2007.

[60] S. Markland, T. A. Weppelmann, Z. Ma, S. Lee, R. A. Mir, L. Teng, A. Ginn, C. Lee, M. Ukhanova, S. Galindo, C. Carr, N. DiLorenzo, S. Ahn, J.-H. Mah, H.-Y. Kim, V. Mai and M, "High Prevalence of Cefotaxime Resistant Bacteria in Grazing Beef Cattle: A Cross-Sectional Study," *Frontiers in Microbiology,* 2019.

[61] Z. Ma, S. Lee and K. C. Jeong, "Mitigation Antibiotic Resistance at the Livestock Environment Interfaces: A Review," *Journal of Microbiology and Biotechnology,* 2019.

[62] J. Greig, A. Rajić, M. Mascarenhas, L. Waddell and J. LeJeune, "A scoping review of the role of wildlife in the transmission of bacterial pathogens and antimicrobial resistance to the Food Chain," *Zoonoses and Public Health,* 2015.

[63] E. Banin, D. Hughes and O. P. Kuipers, "Editorial: bacterial pathogens, antibiotics and antibiotic resistance," *FEMS Microbiology Reviews,* 2017.

[64] C. Hall and T. F. Mah, "Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria.," *FEMS Microbial Reviews,* 2017.

[65] L. F. Castiblanco and G. W. Sundin, "New insights on molecular regulation of biofilm formation in plant-associated bacteria," *Journal of Integrative Plant Biology,* 2016.

[66] Z. Pang, R. Raudonis, B. R. Glick, T.-J. Lin and Z. Cheng, "Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and alternative therapeutic strategies.," *Biotechnology advances,* 2019.

[67] M. A. Rather, K. Gupta and M. Mandal, "Microbial biofilm: formation, architecture, antibiotic resistance, and control strategies," *Brazilian Journal of Microbiology,* 2021.

[68] H.-C. Flemming, J. Wingender, U. Szewzyk, P. Steinberg, S. A. Rice and S. Kjelleberg, "Biofilms: an emergent form of bacterial life.," *Nature reviews microbiology,* 2016.

[69] M. Simões, L. C. Simões and M. J. Vieira, "A review of current and emergent biofilm control strategies," *LWT - Food Science and Technology,* 2010.

[70] P. S. Stewart and M. J. Franklin, "Physiological heterogeneity in biofilms.," *Nature Reviews Microbiology,* 2008.

[71] G. Gebreyohannes, A. Nyerere, C. Bii and D. Berhe Sbhatu, "Challenges of intervention, treatment, and antibiotic resistance of biofilm-forming microorganisms," *Helion,* 2019.

[72] M. Jamal, W. Ahmad, S. Andleeb, F. Jalil, M. Imran, M. A. Nawaz, T. Hussain, M. Ali, M. Rafiq and M. A. Kamil, "Bacterial biofilm and associated infections.," *Journal of the Chinese Medical Association ,* 2018.

[73] T. K. Lu and J. J. Collins, "Dispersing biofilms with engineered enzymatic bacteriophage.," *Proceedings of the National Academy of Science,* 2007.

[74] H.-C. Flemming, J. Wingender, T. Griebe and C. Mayer, "Physico-chemical Properties of Biofilms," in *Biofilms: Recent Advances in their Study and Control*, Harwood Academic Publishers, 2000, pp. 19-34.

[75] P. S. Stewart, "Mechanisms of antibiotic resistance in bacterial biofilms.," *International Journal of Medical Microbiology,* 2002.

[76] W. Yiu, Y. Wang, L. Liu and J. He, "Antibiofilm agents: a new perspective for antimicrobial strategy.," *Journal of Microbiology,* 2017.

[77] C. Chavez Lopez, "The role of bacterial biofilm in antibiotic resistance and food contamination," *International Journal of Microbiology,* 2020.

[78] M. Jamal, U. Tasneem, T. Hussain and S. Andleeb, "Bacterial biofilm: its composition, formation and role in human infections.," *Research & Reviews: Journal of Microbiology and Biotechnology,* 2015.

[79] E. E. Giaouris and M. V. Simões, "Pathogenic Biofilms Formation in the Food Industry and Alternative Control Strategies," *Foodborne Diseases,* 2018.

[80] M. Toyofuku, T. Inaba, T. Kiyokawa, N. Obana, Y. Yawata and N. Nomura, "Environmental factors that shape biofilm formation," *Bioscience, Biotechnology & Biochemistry,* 2016.

[81] N. Rabin, Y. Zheng, C. Opoku-Temeng, Y. Du, E. Bonsu and H. O. Sintim, "Biofilm formation mechanisms and targets for developing antibiofilm agents," *Future Medical Chemistry,* 2015.

[82] X. Zhao, F. Zhao, J. Wang and N. Zhong, "Biofilm formation and control strategies of foodborne pathogens: food safety perspectives.," *Royal Society of Chemistry,* 2017.

[83] M. Beitelshees, A. Hill, C. H. Jones and B. A. Pfeifer, "Phenotypic Variation during Biofilm Formation: Implications for Anti-Biofilm Therapeutic Design," Materials, 2018.

[84] M. Hamilton, J. Heersink, K. Buckingham-Meyer and D. Goeres, The Biofilm Laboratory: step-by-step protocols for experimental design, analysis, and data interpretation., Cytergy Publishing, 2003.

[85] R. Roy, M. Tiwari, G. Donelli and V. Tiwari, "Strategies for combating bacterial biofilms: A focus on anti-biofilms agents and their mechanisms of action.," *Virulence,* 2018.

[86] H. Wu, C. Moser, H.-Z. Wang, N. Høiby and Z.-J. Song, "Strategies for combating bacterial biofilm infections.," *International Journal of Oral Science,* 2015.

[87] World Health Organization, "WHO Director-General's Remarks at the Media Briefing on 2019.," 2020. [Online]. Available: https://www.who.int/director-general/speeches/detail/who-director-general-s-remarks-at-the-media-briefing-on-2019-ncov-on-11-february-2020.

[88] C. Wang, P. W. Horby, F. G. Hayden and G. F. Gao, "A novel coronavirus outbreak of global health concern," *The Lancet,* 2020.

[89] World Health Organization, "Coronavirus Disease (COVID-19) Pandemics," 2021. [Online]. Available: https://www.who.int/emergencies/diseases/novel-coronavirus-2019.

[90] S. G. Rizvi and S. Z. Ahammad, "COVID-19 and antimicrobial resistance: A cross-study," *Science of the Total Environment,* 2022.

[91] F. Zhou, T. Yu, R. Du, G. Fan, Y. Lin, Z. Liu, J. Xiang, Y. Wang, B. Song, X. Gu, L. Guan, Y. Wei, H. Li, X. Wu, J. Xu, S. Tu, Y. Zhang, H. Chen and B. Cao, "Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study," *The Lancet,* 2020.

[92] H. Zhou, Z. Cao, M. Zhang, Z. Ying and L. Ma, "Zero-valent iron enhanced in-situ advanced anaerobic digestion for the removal of antibiotics and antibiotic resistance genes in sewage sludge," *Science of the Total Environment,* 2021.

[93] H. Getahun, I. Smith, K. Trivedi, S. Paulin and H. H. Balkhy, "Tackling antimicrobial resistance in the COVID-19 pandemic," *Bulletin of the World Health Organization,* 2020.

[94] C. Lynch, N. Mahida and J. Gray, "Antimicrobial stewardship: a COVID casualty?," *The Journal of Hospital Infection,* 2020.

[95] E. Zavala-Flores and J. Salcedo-Matienzo, "Medicación prehospitalaria en pacientes hospitalizados por COVID-19 en un hospital público de Lima-Perú," *Acta Médica Peruana,* 2020.

[96] H. J. A. Sulayyim, R. Ismail, A. A. Hamid and N. A. Ghafar, "Antibiotic Resistance during COVID-19: A Systemic Review," *International Journal of Environmental Research and Public Health,* 2022.

[97] C.-C. Lai, S.-Y. Chen, W.-C. Ko and P.-R. Hsueh, "Increased Antimicrobial Resistance during the COVID-19 pandemic," *International Journal of Antimicrobial Agents,* 2021.

[98] B. Gutiérrez-Gutiérrez, E. Salamanca, M. de Cueto, P.-R. Hsueh, P. Viale, J.-R. Paño-Pardo, M. Venditti, M. Tumbarello, G. Daikos, R. Cantón, Y. Doi, F. F. Tuon, I. Karaiskos and Pérez-Nadale, "Effect of appropriate combination therapy on mortality of patients with bloodstream infections due to carbapenemase-producing Enterobacteriaceae (INCREMENT): a retrospective cohort study," *The Lancet Infectious Diseases,* 2017.

[99] M. Paul, V. Shani, E. Muchtar, G. Kariv, E. Robenshtok and L. Leibovici, "Systematic review and meta-analysis of the efficacy of appropriate empiric antibiotic therapy for sepsis," *Antimicrobial Agents and Chemotherapy,* 2010.

[100] P. Chanda-Kapata, N. Kapata and A. Zumala, "COVID-19 and malaria: A symptom screening challenge for malaria endemic countries," *International Journal of Infectious Diseases,* 2020.

[101] The Lancet HIV, "When pandemics collide," *The Lancet HIV,* 2020.

[102] K. S. O'Brien, P. Emerson, P. Hooper, A. L. Reingold, E. G. Dennis, J. D. Keenan, T. M. Lietman and C. E. Oldenburg, "Antimicrobial resistance following mass azithromycin distribution for trachoma: a systematic review," *The Lancet Infectious Diseases,* 2019.

[103] G. M. Knight, R. E. Glover, C. F. McQuaid, I. D. Olaru, K. Gallandat, Q. J. Leclerc, N. M. Fuller, S. J. Willcocks, R. Hasan, E. van Kleef and C. I. Chandler, "Antimicrobial resistance and COVID-19: Intersections and implications," *Elife,* 2021.

[104] A. Ruckert, K. Zinszer, C. Zarowsky, R. Labonté and H. Carabin, "What role for One Health in the COVID-19 pandemic?," *Canadian Journal of Public Health,* 2020.

[105] S. Choudhury, A. Medina-Lara and R. Smith, "Antimicrobial resistance and the COVID-19 pandemic," *Bulletin of the World Health Organization,* 2022.

[106] One Health Commission, "What is One Health?," 2018. [Online]. Available: https://www.onehealthcommission.org/en/why_one_health/what_is_one_health/.

[107] J. Zinsstag, A. Meisser, E. Schelling, B. Bonfoh and M. Tanner, "From 'two medicines' to 'One Health' and beyond," *Onderstepoort Journal of Veterinary Research,* 2012.

[108] S. A. McEwen and P. J. Collignon, "Antimicrobial Resistance: a One Health Perspective," *Microbiology Spectrum,* 2018.

[109] M. E. J. Woolhouse and S. Gowtage-Sequeria, "Host Range and Emerging and Reemerging Pathogens," *Emerging Infectious Diseases,* 2005.

[110] J. E. Sykes, "Antimicrobial Drug Use in Dogs and Cats," *Antimicrobial Therapy in Veterinary Medicine,* 2013.

[111] S. Giguère, A. C. Abrams-Ogg and S. A. Kruth, "Prophylactic Use of Antimicrobial Agents, and Antimicrobial Chemotherapy for the Neutropenic Patient," *Antimicrobial Therapy in Veterinary Medicine,* 2013.

[112] S. A. McEwen and P. J. Fedorka-Cray, "Antimicrobial Use and Resistance in Animals," *Clinical Infectious Diseases ,* 2002.

[113] National Research Council (US) Committee on Drug Use in Food Animals, The Use of Drugs in Food Animals: Benefits and Risks, Washington, DC: National Research Council, 1999.

[114] B. Aslam, M. Khurshid, M. I. Arshad, S. Muzammil, M. Rasool, N. Yasmeen, T. Shah, T. H. Chaudhry, M. H. Rasool, A. Shahid, X. Xueshan and Z. Baloch, "Antibiotic Resistance: One Health One World Outlook," *Frontiers in Cellular and Infection Microbiology,* 2021.

[115] World Organisation for Animal Health, "OIE annual report on the use of antimicrobial agents in animals. Second report.," 2017.

[116] T. P. Van Boeckel, E. E. Glennon, D. Chen, M. Gilbert, T. P. Robinson, B. T. Grenfell, S. A. Levin, S. Bonhoeffer and R. Laxminarayan, "Reducing Antimicrobial Use in Food Animals," *Science,* 2017.

[117] J. O'Neill, "Tackling drug-resistant infections globally: final report and recommendations.," *The review on antimicrobial resistance,* 2016.

[118] World Health Organization, "Global Action Plan on Antimicrobial Resistance," Geneva, Switzerland, 2015.

[119] C. Aranaga, L. D. Pentoja, E. A. Martínez and A. Falco, "Phage Therapy in the Era of Multidrug Resistance in Bacteria: A Systematic Review," *Iternational Journal of Molecular Science,* 2022.

[120] M. Skurnik and E. Strauch, "Phage therapy: Facts and fiction," *International Journal of Medical Microbiology,* 2006.

[121] J. A. Hermoso, J. L. García and P. García, "Taking aim on bacterial pathogens: from phage therapy to enzybiotics," *Current Opinion on Microbiology,* 2007.

[122] P. Hyman and S. T. Abedon, "Bacteriophage Host Range and Bacterial Resistance," *Advances in Applied Microbiology,* 2010.

[123] K. E. Kortright, B. K. Chan, J. L. Koff and P. E. Turner, "Phage Therapy: A Renewed Approach to Combat Antibiotic-Resistant Bacteria," *Cell Host & Microbe,* 2019.

[124] U. Theuretzbacher and L. J. Piddock, "Non-traditional Antibacterial Therapeutic Options and Challenges," *Cell Host & Microbe,* 2019.

[125] C. Ghosh, P. Sarkar, R. Issa and J. Haldar, "Alternatives to Conventional Antibiotics in the Era of Antimicrobial Resistance," *Trends in Microbiology,* 2019.

[126] J. J. Bull and J. J. Gill, "The habits of highly effective phages: population dynamics as a framework for identifying therapeutic phages," *Frontiers in Microbiology,* 2014.

[127] C. Rohde, J. Wittmann and E. Kutter, "Bacteriophages: A Therapy Concept against Multi-Drug-Resistant Bacteria," *Surgical Infections,* 2018.

[128] F. Oechslin, P. Piccardi, S. Mancini, J. Gabard, P. Moreillon, J. M. Entenza, G. Resch and Y.-A. Que, "Synergistic Interaction Between Phage Therapy and Antibiotics Clears Pseudomonas Aeruginosa Infection in Endocarditis and Reduces Virulence," *The Journal of Infectious Diseases,* 2017.

[129] L. M. Kasman and L. D. Porter, "Bacteriophages," in *StatPearls [Internet]*, Treasure Island, FL: StatPearls Publishing, 2022.

[130] E. Tacconelli, E. Carrara, A. Savoldi, S. Harbarth, M. Mendelson, D. L. Monnet, C. Pulcini, G. Kahlmeter, J. Kluytmans, Y. Carmeli, M. Ouellette, K. Outterson, J. Patel, M. Cavaleri and E. Cox, "Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis," *The Lancet Infectious Diseases,* 2018.

[131] Y. Suzuki, T. Ishimoto, S. Fujita, S. Kiryu, M. Wada, T. Akatsuka, M. Saito and M. Kawano, "Antimicrobial antisense RNA delivery to F-pili producing multidrug-resistant bacteria via a

genetically engineered bacteriophage," *Biochemical and Biophysical Research Communications,* 2020.

[132] J. M. Tinoco, N. Liss, H. Zhang, R. Nissan, W. Gordon, E. Tinoco, L. Sassone and R. Stevens, "Antibacterial effect of genetically-engineered bacteriophage ϕEf11/ϕFL1C(Δ36)PnisA on dentin infected with antibiotic-resistant Enterococcus faecalis," *Archives of Oral Biology,* 2017.

[133] A. Sinha, K. Eniyan, P. Manohar, N. Ramesh and U. Bajpai, "Characterization and genome analysis of B1 sub-cluster mycobacteriophage PDRPxv," *Virus Research,* 2020.

[134] J. Romero, C. G. Feijoo and P. Navarrete, "Antibiotics in Aquaculture – Use, Abuse and Alternatives," *Health and Environment in Aquaculture,* 2012.

[135] H. Ling, X. Lou, Q. Luo, Z. He, M. Sun and J. Sun, "Recent advances in bacteriophage-based therapeutics: Insight into the post-antibiotic era," *Acta Pharmaceutica Sinica B,* 2022.

[136] V. N. Krylov, "Phage Therapy in Terms of Bacteriophage Genetics: Hopes, Prospects, Safety, Limitations," *Russian Journal of Genetics,* 2001.

[137] I. Y. Sengun and S. Karabiyikli, "Importance of acetic acid bacteria in food industry," *Food Control,* 2011.

[138] C. B. Kurtz, Y. A. Millet, M. K. Puurunen, M. Perrault, M. R. Charbonneau, V. M. Isabella, J. W. Kotula, E. Antipov, Y. Dagon, W. S. Denney, D. A. Wagner, K. A. West, A. J. Degar and A. Brennan, "An engineered E. coli Nissle improves hyperammonemia and survival in mice and shows dose-dependent exposure in healthy humans," *Science Translational Medicine,* 2019.

[139] I. R. Cooper, "A review of current methods using bacteriophages in live animals, food and animal products intended for human consumption," *Journal of Microbiological Methods,* 2016.

[140] S. T. Abedon, S. J. Kuhl, B. G. Blasdel and E. M. Kutter, "Phage treatment of human infections," *Bacteriophage,* 2011.

[141] R. DiGirolamo, L. Wiczynski, M. Daley, F. Miranda and C. Viehweger, "Uptake of bacteriophage and their subsequent survival in edible West Coast crabs after processing.," *Applied Microbiology,* 1972.

[142] M. Zasloff, "Antimicrobial peptides of multicellular organisms," *Nature,* 2002.

[143] B. P. Lazzaro, M. Zasloff and J. Rolff, "Antimicrobial peptides: application informed by evolution," *Science,* 2020.

[144] O. Bruhn, J. Grörzinger, I. Cascorbi and S. Jung, "Antimicrobial peptides and proteins of the horse - insights into a well-armed organism," *Veterinary Research,* 2011.

[145] J. Cho, I.-S. Hwang, H. Choi, J. H. Hwang, J.-S. Hwang and D. G. Lee, "The Novel Biological Action of Antimicrobial Peptides via Apoptosis Induction," *Bioactive Compounds and Chemical Biology,* 2012.

[146] M.-D. Seo, H.-S. Won, J.-H. Kim, T. Mishig-Ochir and B.-J. Lee, "Antimicrobial Peptides for Therapeutic Applications: A Review," *Molecules,* 2012.

[147] C. Björn, J. Håkansson, E. Myhrman, V. Sjöstrand, T. Haug, K. Lindgren, H.-M. Blencke, K. Stensvåg and M. Mahlapuu, "Anti-infectious and anti-inflammatory effects of peptide fragments sequentially derived from the antimicrobial peptide centrocin 1 isolated from the green sea urchin, Strongylocentrotus droebachiensis," *AMB Express,* 2012.

[148] J. Carlet, C. Rambaud and C. Pulcini, "WAAR (World Alliance against Antibiotic Resistance): Safeguarding antibiotics," *Antimicrobial Resistance & Infection Control,* 2012.

[149] M. Upton, P. Cotter and J. Tagg, "Antimicrobial peptides as therapeutic agents," *International Journal of Microbiology,* 2012.

[150] M. Mahlapuu, J. Håkansson, L. Ringstad and C. Björn, "Antimicrobial Peptides: An Emerging Category of Therapeutic Agents," *Frontiers in Cellular and Infection Microbiology,* 2016.

[151] R. J. Dubos, "Studies on a bactericidal agent extracted from a soil bacillus: I. preparation of the agent. Its activity in vitro.," *The Journal of Experimental Medicine,* 1939.

[152] R. J. Dubos, "Studies on a bactericidal agent extracted from a soil bacillus: II. protective effect of the bactericidal agent against experimental Pneumococcus infections in mice," *The Journal of Experimental Medicine,* 1939.

[153] A. Bin Hafeez, X. Jiang, P. J. Bergen and Y. Zhu, "Antimicrobial Peptides: An Update on Classifications and Databases," *International Journal of Molecular Science,* 2021.

[154] M. Mahlapuu, C. Björn and J. Ekblom, "Antimicrobial peptides as therapeutic agents: opportunities and challenges," *Critical Reviews in Biotechnology,* 2020.

[155] X. Li, S. Zuo, B. Wang, K. Zhang and Y. Wang, "Antimicrobial Mechanisms and Clinical Application Prospects of Antimicrobial Peptides," *Molecules,* 2022.

[156] K. Zhang, X. Li, C. Yu and Y. Wang, "Promising Therapeutic Strategies Against Microbial Biofilm Challenges," *Frontiers in Cellular and Infection Microbiology,* 2020.

[157] G. Batoni, G. Maisetta and S. Esin, "Antimicrobial peptides and their interaction with biofilms of medically relevant bacteriaAntimicrobial peptides and their interaction with biofilms of medically relevant bacteria," *Biochimica et biophysica acta,* 2016.

[158] L. Lin, P. Nonejuie, J. Munguia, A. Hollands, J. Olson, Q. Dam, M. Kumaraswamy, H. Rivera Jr, R. Corriden, M. Rohde, M. E. Hensler, M. D. Burkart, J. Pogliano, G. Sakoulas and V. Nizet, "Azithromycin Synergizes with Cationic Antimicrobial Peptides to Exert Bactericidal and Therapeutic Activity Against Highly Multidrug-Resistant Gram-Negative Bacterial Pathogens," *EBioMedicine,* 2015.

[159] G. Yu, D. Y. Baeder, R. R. Regoes and J. Rolff, "Predicting drug resistance evolution: insights from antimicrobial peptides and antibiotics," *Proceedings Biological Sciences,* 2018.

[160] G. E. Fantner, R. J. Barbero, D. S. Gray and A. M. Belcher, "Kinetics of antimicrobial peptide activity measured on individual bacterial cells using high-speed atomic force microscopy," *Nature Nanotechnology,* 2010.

[161] N. Jochumsen, R. L. Marvig, S. Damkiær, R. L. Jensen, W. Paulander, S. Molin, L. Jelsbak and A. Folkesson, "The evolution of antimicrobial peptide resistance in Pseudomonas aeruginosa is shaped by strong epistatic interactions," *Nature Communications,* 2016.

[162] B. Kintses, O. Méhi, E. Ari, M. Számel, Á. Györkei, P. K. Jangir, I. Nagy, F. Pál, G. Fekete, R. Tengölics, Á. Nyerges, I. Likó, A. Bálint, T. Molnár, B. Bálint and B. M. Vásárhelyi, "Phylogenetic barriers to horizontal transfer of antimicrobial peptide resistance genes in the human gut microbiota," *Nature Microbiology,* 2019.

[163] G. Bell and P.-H. Gouyon, "Arming the enemy: the evolution of resistance to self-proteins," *Microbiology (Reading),* 2003.

[164] A. J. Dobson, J. Purves and J. Rolff, "Increased survival of experimentally evolved antimicrobial peptide-resistant Staphylococcus aureus in an animal host," *Evolutionary Applications,* 2014.

[165] B. El Shazely, A. Urbański, P. R. Johnston and J. Rolff, "In vivo exposure of insect AMP resistant Staphylococcus aureus to an insect immune system," *Insect Biochemistry and Molecular Biology,* 2019.

[166] B. Mishra, S. Reiling, D. Zarena and G. Wang, "Host defense antimicrobial peptides as antibiotics: design and application strategies," *Current Opinion in Chemical Microbiology,* 2017.

[167] M. S. Zharkova, D. S. Orlov, O. Y. Golubeva, O. B. Chakchir, I. E. Eliseev, T. M. Grinchuk and O. V. Shamova, "Application of Antimicrobial Peptides of the Innate Immune System in Combination With Conventional Antibiotics-A Novel Way to Combat Antibiotic Resistance?," *Frontiers in Cellular and Infection Microbiology,* 2019.

[168] H. Jenssen, P. Hamill and R. E. W. Hancock, "Peptide antimicrobial agents," *Clinical Microbiology Reviews,* 2006.

[169] A. Pfalzgraff , K. Brandenburg and G. Weindl , "Antimicrobial Peptides and Their Therapeutic Potential for Bacterial Skin Infections and Wounds," *Frontiers in Pharmacology,* 2018.

[170] I. E. Mba and E. I. Nweze, "Antimicrobial Peptides Therapy: An Emerging Alternative for Treating Drug-Resistant Bacteria," *The Yale Journal of Biology and Medicine,* 2022.

[171] S. Mukhopadhyay, A. S. Bharath Prasad, C. H. Mehta and U. Y. Nayak, "Antimicrobial peptide polymers: no escape to ESKAPE pathogens-a review," *World Journal of Microbiology & Biotechnology,* 2020.

[172] Y. Wu, N. Yang, R. Mao, Y. Hao, D. Teng and J. Wang, "In Vitro Pharmacodynamics and Bactericidal Mechanism of Fungal Defensin-Derived Peptides NZX and P2 against Streptococcus agalactiae," *Microorganisms,* 2022.

[173] N. Raheem and S. K. Straus, "Mechanisms of Action for Antimicrobial Peptides With Antibacterial and Antibiofilm Functions," *Frontiers in Microbiology,* 2019.

[174] Z. Amso and Z. Hayouka, "Antimicrobial random peptide cocktails: a new approach to fight pathogenic bacteria," *Chemical Communications (Cambridge, England),* 2019.

[175] A. A. Bahar and D. Ren, "Antimicrobial peptides," *Pharmaceuticals,* 2013.

[176] R. Rathinakumar, W. F. Walkenhorst and W. C. Wimley, "Broad-spectrum antimicrobial peptides by rational combinatorial design and high-throughput screening: the importance of interfacial activity," *Journal of the American Chemical Society,* 2009.

[177] J. Lei, L. Sun, S. Huang, C. Zhu, P. Li, J. He, V. Mackey, D. H. Coy and Q. He, "The antimicrobial peptides and their potential clinical applications," *American Journal of Translational Research,* 2019.

[178] T. L. Raguse, E. A. Porter, B. Weisblum and S. H. Gellman, "Structure-activity studies of 14-helical antimicrobial beta-peptides: probing the relationship between conformational stability and antimicrobial potency," *Journal of the American Chemical Society,* 2002.

[179] D. L. Lee and R. S. Hodges, "Structure-activity relationships of de novo designed cyclic antimicrobial peptides based on gramicidin S," *Bipolymers,* 2003.

[180] F. Schweizer, "Cationic amphiphilic peptides with cancer-selective toxicity," *European Journal of Pharmacology,* 2009.

[181] A. L. Hilchie, D. W. Hoskin and M. R. Power Coombs, "Anticancer Activities of Natural and Synthetic Peptides," *Advances in Experimental Medicine and Biology,* 2019.

[182] C. H. Chen and T. K. Lu, "Development and Challenges of Antimicrobial Peptides for Therapeutic Applications," *Antibiotics,* 2020.

[183] A. L. Tornasello, A. Borrelli, L. Buonaguro, F. M. Buonaguro and M. L. Tornasello, "Antimicrobial Peptides as Anticancer Agents: Functional Properties and Biological Activities," *Molecules: a journal of synthetic chemistry and natural product chemistry,* 2020.

[184] D. M. Mabrouk, "Antimicrobial peptides: features, applications and the potential use against covid-19," *Molecular Biology Reports,* 2022.

[185] V. Kumar, A. Kumari, P. Guleria and S. K. Yadav, "Evaluating the toxicity of selected types of nanochemicals," *Reviews of environmental contamination and toxicology,* 2012.

[186] M. R. Gwinn and V. Vallyathan, "Nanoparticles: health effects--pros and cons," *Environmental Health Perspectives,* 2006.

[187] W. N. Missaoui, R. D. Arnold and B. S. Cummings, "Toxicological status of nanoparticles: What we know and what we don't know," *Chemico-biological interactions,* 2018.

[188] A. Puri, K. Loomis, B. Smith, J.-H. Lee, A. Yavlovich, E. Heldman and R. Blumenthal, "Lipid-based nanoparticles as pharmaceutical drug carriers: from concepts to clinic," *Critical reviews in therapeutic drug carrier systems,* 2009.

[189] W. Najahi-Missaoui, R. D. Arnold and B. S. Cummings, "Safe Nanoparticles: Are We There Yet?," *International journal of molecular sciences,* 2020.

[190] A. K. Thabit , J. L. Crandon and D. P. Nicolau, "Antimicrobial resistance: impact on clinical and economic outcomes and the need for new antimicrobials," *Expert opinion on pharmacotherapy,* 2015.

[191] M. S. Akhtar, M. K. Swamy, A. Umar and A. A. Al Sahli, "Biosynthesis and Characterization of Silver Nanoparticles from Methanol Leaf Extract of Cassia didymobotyra and Assessment of Their Antioxidant and Antibacterial Activities," *Journal of Nanoscience adn Nanotechnology,* 2015.

[192] I. Y. Wong, S. N. Bhatia and M. Toner, "Nanotechnology: emerging tools for biology and medicine," *Genes & Development,* 2013.

[193] F. Fatima, S. Siddiqui and W. A. Khan, "Nanoparticles as Novel Emerging Therapeutic Antibacterial Agents in the Antibiotics Resistant Era," *Biological Trace Element Research,* 2021.

[194] A.-G. Niculescu, C. Chircov, A. C. Bîrcă and A. M. Grumezescu, "Nanomaterials Synthesis through Microfluidic Methods: An Updated Overview," *Nanomaterials,* 2021.

[195] V. M. Arole and S. V. Munde, "Fabrication of nanomaterials by top-down and bottom-up approaches-an overview," *Journal of Material Science,* 2014.

[196] P. Shrimal, G. Jadeja and S. Patel, "A review on novel methodologies for drug nanoparticle preparation: Microfluidic approach," *Chemical Engineering Research and Design,* 2020.

[197] D.-A. Mercan, A.-G. Niculescu and A. M. Grumezescu, "Nanoparticles for Antimicrobial Agents Delivery—An Up-to-Date Review," *International Journal of Molecular Sciences,* 2022.

[198] T. Bruna, F. Maldonado-Bravo, P. Jara and N. Caro, "Silver Nanoparticles and Their Antibacterial Applications," *International Journal of Molecular Sciences,* 2021.

[199] A. Kędziora, M. Speruda, E. Krzyżewska, J. Rybka, A. Łukowiak and G. Bugla-Płoskońska, "Similarities and Differences between Silver Ions and Silver in Nanoforms as Antibacterial Agents," *International Journal of Molecular Sciences,* 2018.

[200] K. Mounika, J. Venakatesh, K. Manogna and A. Paul, "Biosynthesis and characterization of silver nanoparticles from tuber extract of Cyperus rotundus and study of its antibacterial activity.," *Research Journal of Pharmaceutical, Biological and Chemical Sciences,* 2017.

[201] S. H. Lee and B.-H. Jun, "Silver Nanoparticles: Synthesis and Application for Nanomedicine," *International Journal of Molecular Sciences,* 2019.

[202] K. S. Siddiqi, A. Husen and R. A. K. Rao, "A review on biosynthesis of silver nanoparticles and their biocidal properties," *Journal of Nanobiotechnology,* 2018.

[203] E. Urnukhsaikhan, B.-E. Bold, A. Gunbileg, N. Sukhbaatar and T. Mishig-Ochir, "Antibacterial activity and characteristics of silver nanoparticles biosynthesized from Carduus crispus," *Scientific Reports,* 2021.

[204] E. K. Kambale , C. I. Nkanga, B.-P. I. Mutonkole, A. M. Bapolisi , D. O. Tassa, J.-M. I. Liesse, R. W. Krause and P. B. Memvanga, "Green synthesis of antimicrobial silver nanoparticles using aqueous leaf extracts from three Congolese plant species (Brillantaisia patula, Crossopteryx febrifuga and Senna siamea)," *Heliyon,* 2020.

[205] R. Vijayan, S. Joseph and B. Mathew, "Green Synthesis, Characterization and Applications of Noble Metal Nanoparticles Using Myxopyrum serratulum A. W. Hill Leaf Extract," *BioNanoScience,* 2017.

[206] C. Bergonzi, G. Remaggi, C. Graiff, L. Bergamonti, M. Potenza, M. C. Ossiprandi, I. Zanotti, F. Bernini, R. Bettini and L. Elviri, "Three-Dimensional (3D) Printed Silver Nanoparticles/Alginate/Nanocrystalline Cellulose Hydrogels: Study of the Antimicrobial and Cytotoxicity Efficacy," *Nanomaterials (Basel),* 2020.

[207] S. Dhivya, V. V. Padma and E. Santhini, "Wound dressings - a review," *BioMedicine,* 2015.

[208] B. A. Aderibigbe and B. Buyana, "Alginate in Wound Dressings," *Pharmaceutics,* 2018.

[209] C. Knill, J. Kennedy, J. Mistry, M. Miraftab, G. Smart, M. Groocock and H. Williams, "Alginate fibres modified with unhydrolysed and hydrolysed chitosans for wound dressings," *Carbohydrate Polymers,* 2004.

[210] S. Mondal, "Preparation, properties and applications of nanocellulosic materials," *Carbohydrate Polymers,* 2017.

[211] L. S. Reddi, M. M. Nisha, M. Joice and P. N. Shilpa, "Antimicrobial activity of zinc oxide (ZnO) nanoparticle against Klebsiella pneumoniae," *Pharmaceutical Biology,* 2014.

[212] Y.-W. Huan, C.-h. Wu and R. S. Aronstam, "Toxicity of Transition Metal Oxide Nanoparticles: Recent Insights from in vitro Studies," *Materials,* 2010.

[213] H. Agarwal, S. Menon, S. Venkat Kumar and S. Rajeshkumar, "Mechanistic study on antibacterial action of zinc oxide nanoparticles synthesized using green route," *Chemico-Biological Interactions,* 2018.

[214] P. Chaudhary, F. Fatima and A. Kumar, "Relevance of Nanomaterials in Food Packaging and its Advanced Future Prospects," *Journal of Inorganic and Organometallic Polymers and Materials,* 2020.

[215] R. Javed, N. u. Ain, A. Gul, M. Arslan Ahmad, W. Guo, Q. Ao and S. Tian, "Diverse biotechnological applications of multifunctional titanium dioxide nanoparticles: An up-to-date review," *IET Nanobiotechnology,* 2022.

[216] A. Weir, P. Westerhoff, L. Fabricius, K. Hristovski and N. von Goetz, "Titanium dioxide nanoparticles in food and personal care products," *Environmental Science & Technology,* 2012.

[217] T. Maier and H. C. Korting, "Sunscreens - which and what for?," *Skin Pharmacology and Physiology,* 2005.

[218] P. Tucci, G. Porta, M. Agostini, D. Dinsdale, I. Iavicoli, K. Cain, A. Finazzi-Agró, G. Melino and A. Willis, "Metabolic effects of TiO2 nanoparticles, a common component of sunscreens and cosmetics, on human keratinocytes," *Cell death & disease,* 2013.

[219] D. Buser, N. Broggini, M. Wieland, R. K. Schenk, A. J. Denzer, D. L. Cochran, B. Hoffmann, A. Lussi and S. G. Steinemann, "Enhanced bone apposition to a chemically modified SLA titanium surface," *Journal of Dental Research,* 2004.

[220] G. Raja, S. Cao, D.-H. Kim and T.-J. Kim, "Mechanoregulation of titanium dioxide nanoparticles in cancer therapy," *Material Sicence & Engineering. C, Materials for Biological Applications,* 2019.

[221] F. Piccinno, F. Gottschalk, S. Seeger and B. Nowack, "Industrial production quantities and uses of ten engineered nanomaterials in Europe and the world," *Journal of Nanoparticle Research,* 2012.

[222] C. O. Robichaud, A. E. Uyar, M. R. Darby, L. G. Zucker and M. R. Wiesner, "Estimates of upper bounds and trends in nano-TiO2 production as a basis for exposure assessment," *Environmental Science & Technology,* 2009.

[223] M. Alizadeh Sani, M. Maleki, H. Eghbaljoo-Gharehgheshlaghi , A. Khezerlou, E. Mohammadian, Q. Liu and S. M. Jafari, "Titanium dioxide nanoparticles as multifunctional surface-active materials for smart/active nanocomposite packaging films," *Advances in colloid and Interface Science,* 2021.

[224] V. Rodríguez-González, C. Terashima and A. Fujishima, "Applications of photocatalytic titanium dioxide-based nanomaterials in sustainable agriculture," *Journal of Photochemistry and Photobiology C: Photochemistry Reviews,* 2019.

[225] C. L. de Castillo, "Antimicrobial effect of titanium dioxide nanoparticles," *Antimicrobial Resistance - A One Health Perspective,* 2020.

[226] A. Sobha, K. Surendranath, V. Meena, T. Keerthi Jwala, N. Swetha and K. S. M. Latha, "Emerging trends in nanobiotechnology," *Biotechnology and Molecular Biology Reviews,* 2010.

[227] A. Jesline, N. P. John, P. M. Narayanan, C. Vani and S. Murugan, "Antimicrobial activity of zinc and titanium dioxide nanoparticles against biofilm-producing methicillin-resistant Staphylococcus aureus," *Applied Nanoscience,* 2014.

[228] N. Beyth, Y. Houri-Haddad, A. Domb, W. Khan and R. Hazan, "Alternative antimicrobial approach: nano-antimicrobial materials," *Evidence-based complementary and alternative medicine: eCAM,* 2015.

[229] D. Stanicki, L. Vander Elst, R. N. Muller and S. Laurent, "Synthesis and processing of magnetic nanoparticles," *Current Opinion in Chemical Engineering,* 2016.

[230] A. B. Seabra, M. T. Pelegrino and P. S. Haddad, "Antimicrobial Applications of Superparamagnetic Iron Oxide Nanoparticles: Perspectives and Challenges," in *Micro and Nanotechnologies*, vol. Nanostructures for Antimicrobial Therapy, Amsterdam.

[231] M. Guerrero Correa, F. B. Martínez, C. P. Vidal, C. Streitt, J. Escrig and C. L. de Castillo, "Antimicrobial metal-based nanoparticles: a review on their synthesis, types and antimicrobial action," *Beilstein Journal of Nanotechnology,* 2020.

[232] J. O. Neill, "Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations," 2014.

[233] D. M. Camacho, K. M. Collins, R. K. Powers, J. C. Costello and J. J. Collins, "Next-Generations Machine Learning for Biological Networks," *Cell,* 2018.

[234] A. Bala and G. Rani, "A review on phytosynthesis, affecting factors and characterization techniques of silver nanoparticles designed by green approach," *International Nano Letters,* 2020.

[235] R. Rajendran, N. Ganesan, S. K. Balu, S. Alagar, P. Thandavamoorthy and D. Thiruvengadam, "Green synthesis, characterization, antimicrobial and cytotoxic effects of silver nanoparticles using Origanum heracleoticum L. leaf extract.," *International Journal of Pharmacy and Pharmaceutical Sciences,* 2015.