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REVIEW

The war against bacteria, from the past to present and beyond

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ABSTRACT

Introduction: The human defense against microorganisms dates back to the ancient civilizations, with attempts to use substances from vegetal, animal, or inorganic origin to fight infections. Today, the emerging threat of multidrug-resistant bacteria highlights the consequences of antibiotics inappropriate use, and the urgent need for novel effective molecules.

Methods and Materials: We extensively researched on more recent data within PubMed, Medline, Web of Science, Elsevier's EMBASE, Cochrane Review for the modern pharmacology in between 1987 - 2021. The historical evolution included a detailed analysis of past studies on the significance of medical applications in the ancient therapeutic field.

Areas covered: We examined the history of antibiotics development and discovery, the most relevant biochemical aspects of their mode of action, and the biomolecular mechanisms conferring bacterial resistance to antibiotics.

Expert opinion: The list of pathogens showing low sensitivity or full resistance to most currently available antibiotics is growing worldwide. Long after the 'golden age' of antibiotic discovery, the most novel molecules should be carefully reserved to treat serious bacterial infections of susceptible bacteria. A correct diagnostic and therapeutic procedure can slow down the spreading of nosocomial and community infections sustained by multidrug-resistant bacterial strains.

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Microbiology; history of medicine; history of pharmacology; antibiotics; antibiotic resistance

1. Introduction

The term 'antibiotic' (Greek, αντὶ = against, and βιωτικό = useful in life) originally refers to substances produced by microorganisms that could inhibit the growth of bacteria, although nowadays any natural or synthetic drug used to fight bacterial infections is often defined as 'antibiotic' [1]. Instead, natural substances that kill bacteria but are not produced by microorganisms (such as gastric fluid and hydrogen peroxide) are excluded from the original definition. Several natural antibiotics (as, for example, substances produced by actinomycetes and fungi, soil samples, plants, marine organisms) have been subsequently used as templates for chemical synthesis, with the aim to improve some properties of natural derivatives in the attempt to kill or inhibit the growth of harmful microorganisms [2,3]. Many antibiotics are relatively small molecules, with a molecular weight of less than 2,000 Da. Although some antibiotic agents are still produced and isolated from living organisms, such as aminoglycosides, the majority of currently used antibiotics are semi-synthetic derivatives of the original compounds, or molecules synthetically produced, such as quinolones and oxazolidinones [4,5].

The wider definition of antimicrobial agent, on the other hand, is reserved for any chemical substance – natural or synthetic – that can inhibit the growth of both bacteria and other microorganisms [4,6]. Although the terms antibiotics and antimicrobial agents are sometimes utilized interchangeably in a common language, the difference is relevant and must be underlined: while antibiotics specifically target bacteria, antimicrobials encompass a broader range of products able to act on bacteria, fungi, protozoa, and viruses. Antimicrobial chemotherapy is a strategy to counteract infections intended to selectively destroy or inhibit pathological microbial development, without altering the function or damaging the structure of host cells (selective cell toxicity) (Figure 1). Ideally, the appropriate antimicrobial agent should: a) show selective toxicity (enhanced activity toward target microorganisms, not harmful to humans), b) not induce hypersensitivity reactions in the host, c) not extensively alter the host microbiota's eubiosis, d) display appropriate pharmacokinetic properties (absorption, distribution, metabolism, and excretion) when administered systemically, and g) have affordable costs [7–11].

Antimicrobial therapy should be performed, whenever possible, with molecules that target pathogenic microorganisms showing sensitivity to the antimicrobial agent administered; however, for infections sustained by unknown microorganisms of undetermined sensitivity, treatment is generally initiated on an empirical basis with molecules able to interfere with a wide

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Article highlights

- Antibiotics include natural substances (mainly produced in actinomycetes and fungi, soil samples, plants, marine organisms), semi-synthetic derivatives modified from the original compounds, or molecules synthetically produced.
- Antimicrobial therapy is a strategy to counteract infections intended to selectively destroy or inhibit pathological microbial development, without altering the function or damaging the structure of host cells (selective cell toxicity).
- Over the years, biomolecular mechanisms underlying the anti-infective activity of antibiotics have been identified. The correct choice of an antibiotic must take into account multiple factors that aim for a better and lasting efficacy of antimicrobial therapy.
- Misuse of antibiotics represents a major cause for selection of mutations responsible for the antibiotic resistance. It includes (but it is not limited to) empirical use (treatment of a disease from unknown etiological agents), prophylaxis in surgery, inappropriate use of molecules with a broad spectrum of action, auxinic use in farm animals, administration in pediatric patients with viral infections and improper patient compliance.
- One of the biggest and urgent challenges today is the development of effective novel molecules to counteract antibiotic resistance.
- The antibiogram remains an extremely useful method to guide the correct treatment in single patients, to help identify antibiotic-resistant hospital infections, and for epidemiological purposes.

spectrum of pathogens; moreover, under specific circumstances, antimicrobial agents might be administered for preventing infections in vulnerable subjects. The most desirable features of an antibiotic substance to be useful for therapy are as follows: (a) a selective toxicity (higher antimicrobial activity and lower toxicity to human tissues), (b) a minimum risk of hypersensitivity reactions in the host, (c) a reduced interference with the human microbiota, (d) the appropriate pharmacokinetic characteristics (absorption, distribution, metabolism, and excretion), and (e) a low economic cost. Thus, the correct choice of the appropriate antibiotic/antimicrobial agent depends on multiple factors, recapitulated in Figure 2 [12].

More and more often, especially considering the broad use of antimicrobial agents in farm animals, the inappropriate administration of antimicrobial therapies in humans, and the rapid evolving pace of microorganisms, germs may build up defensive mechanisms that confer resistance to antimicrobial agents (AMR). To cope with these resilient strains and develop novel drugs that may overcome the microbial resistance, it is essential to understand the mechanisms underlying the AMR, including the nature of substances secreted by bacteria, the specific enzymes synthesized, or the alternative metabolic routes adopted by germs to survive [2].

The historical course of the review was carried out through international scientific databases, historical and medical books, translations of ancient Greek manuscripts from texts of the National Library of Greece (Stavros Niarchos Foundation), and the School of Health Sciences of the National and Kapodistrian University of Athens (Greece). The research languages were English, Italian, and Greek for this selection of historical works. We have selected documents and texts focused on a historical/medical point of view on the infections' therapy and its applications in the past. For the modern antibiotic therapy, we made an extensive research on the more recent data using the keywords 'antibiotics,' 'antibiotic,' 'antimicrobial resistance' and 'infections' on the PubMed, Medline, Web of Science, Elsevier's EMBASE, Cochrane Review databases. Additionally, we have considered some statements from National and International Government Agencies on Antibiotic Safety and Drug Research.

The following chapters will recapitulate our knowledge on the use of antibiotics, starting from ancient times up to the current scenario.

2. The timeline history of the antimicrobial therapy

2.1. *The ancient medicine's era against infectious diseases*

The human defense against microorganisms dates back to the earliest witness of ancient civilizations, with attempts to use substances from vegetal, animal, or inorganic origin for inhibition of microorganism spreading and healing purposes. The search and development of substances with antimicrobial

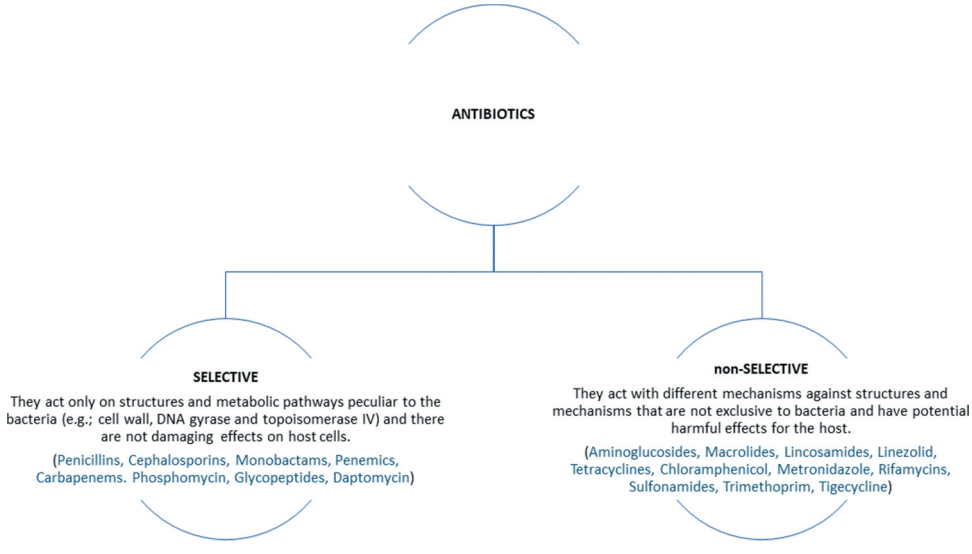

Figure 1. Selective or nonselective antimicrobial activity/toxicity of antibiotics.

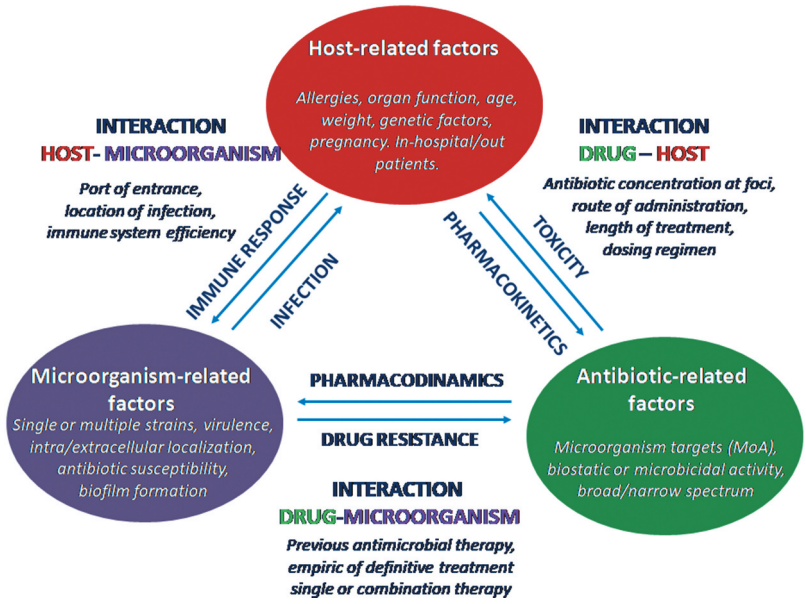

Figure 2. The antibiotic make-decision factors for the most effective antimicrobial therapy.

potential began probably about 2,500 years ago, when Chinese doctors discovered the healing properties of moldy soybean meal and used this substance to treat wounds and infections [13,14]. The adoption of therapeutic remedies has been reported in several other ancient cultures, including Mesopotamians, Ancient Egyptians, Ancient Greeks, and Romans. Among the Sumerian population, clay tablets written around 3000 B.C. report medical and healing content: one of these tablets (dating around 1700 B.C., Nippur, near Babylon) describes the preparation of soap, likely for disinfection and body care, by adding natural fats to the ashes of a plant with a high sodium content [13,15,16].

The Egyptian mythology includes some deities who ruled over human health, such as the deity of medicine Isis. She was supposed to know the properties of all drugs and possess a wide therapeutic knowledge, which was transmitted to Thoth (god of wisdom corresponding to Hermes for the ancient Greeks), who authored 42 hermetic books, 6 of which reporting medical content [1,17,18]. Indeed, the medical text of the Ebers papyrus (around 1550 B.C.) is believed to correspond to the *Hermetic's* books '*Περί φαρμάκων*' (About Medicines). The ancient Greek writer, geographer, and historian Herodotus (around 484–425 B.C.) in his *'Ιστορίαι'* (The Histories) reports that in Egypt the practice of healing was classified into branches, and parasitology was particularly developed due to the subtropical climate of the region, with parasitic diseases often depicted on the murals. Several herbal and other remedies, such as the ointments from *Ricinus communis* oil mixed with beer, were commonly used to heal septic wounds and burn lesions [16,19]. During four centuries of contact with Egyptian culture, Jewish medicine was greatly influenced. With roots in religion and public hygiene laws, Jews physicians paid particular attention to infectious diseases: they knew the transmission route by insects, mice, and other carriers, or by direct contact with the infected subjects and kept them isolated outside the community until recovery. All purification ceremonies (cleaning baths and others) of patients suffering with leprosy or gonorrhea had a religious perspective [16,20].

Similarly, in the archaic ages, both the Minoan and Mycenaean civilizations were acquainted with the use of roots, wood, bark, flowers, shoots, fruits, seeds, oils, and resins from medicinal plants and herbs growing on the Greek peninsula, as well as on the Islands of Crete and Santorini for accelerating the healing of infections [21].

Concerning Indian medicine, two main texts refer to health and healing: the Characa's Ayur-Veda (dealing with the science of life) and Susruta's Ayur-Veda (including chapters on pathology, anatomy, toxicology, and therapy). Approximately eight hundred herbal products are enlisted here and grouped in 38 sections according to their therapeutic potential as anti-gonorrhea, anti-helminthic, anti-pyretic agents, poison antidotes, and so forth. Interestingly, the ancient Greeks were aware of Indian medicine [22,23]. Indeed, the Greek physician Hippocrates (Kos, 460–370 B.C.) mentions the Indian remedies, and the historian Megasthenes (350–290 B.C.) wrote a text referring to the flora, fauna, social classes, and healing activities of Indian doctors. The physician Ktisias of Knidos (4th century B.C.) describes the flora and fauna of India in his book '*Ινδικά*' (Indica). Although the original text has been lost, the content of this opera is available from one epitome synthesis by the Imperial Patriarch of Nova Roma (Constantinople), Saint Photius I (9th century) [16,21].

The oldest Chinese medicine's book '*Inner Canon of the Yellow Emperor*' (黄帝内经 = Huangdi Neijing) is allegedly dated during Han dynasty, between 206 B.C. and 220 A.D. For treatments for infectious diseases, Chinese physicians adopted mercury for syphilis, and pomegranate root and garlic based on their anti-helminthic properties. For the prevention and treatment of smallpox, their method required to

pulverize the patient blisters, and make the subject inhale the obtained powder as a prototypical type of vaccination. The fruit juice of *Punica granatum* was known for its anti-helminthic properties and *Allium sativum* was considered a powerful remedy with anti-inflammatory and antimicrobial activity [14,16,24].

During the Classical and Hellenistic ages, ancient Greeks used plants, molds, and other natural substances to treat infected wounds. Hippocrates in his authoritative source *'Ιπποκρατικό Σώμα'* (Corpus Hippocraticus) recorded about 400 species of herbs used for their healing properties and included more than 1500 recipes [16,21,23,25,26], although the work authored by Dioklis Karistios (375–295 B.C.) '*Ριζοτομικόν*' (Rizotomikon) might be considered the oldest Greek text on herbal medicines [16,21,23,25,26]. Later, Theophrastos (371–287 B.C.) in his larger botanical work '*Περὶ φυτῶν ἱστορία*' (Historia Plantarum) further enriches the knowledge on herbs, and their healing properties. Hippocrates and his followers believed that the human nature is the main therapeutic agent, and that the physician role should focus on strengthen and correct the body's natural defense effort against all diseases, including infectious ones [16,21]. Thus, by anticipating the concept that enhanced immunity is a cornerstone of health, they promoted the use of several herbs and other natural healing remedies. The *Glycyrrhiza glabra* was employed for its anti-inflammatory and anti-infective (mainly antiviral) activities, and the leave infusion of *Salvia officinalis* was intended to treat oral and larynx infections, alone or with association of other herbal derivatives for the treatment of acute and chronic bronchitis. *Thymus vulgaris* and *Mentha piperita* were recommended for the common cold. The wood extract from *Cedrus Trew* and *Juniperus* (called katrami) demonstrated a strong antibacterial activity, and its external use was indicated as an ointment for wounds treatment and, in diseases of the higher respiratory system [16,25]. *Calendula officinalis* and the essential oil from *Caryophylli floris aetheroleum* was used for mild infections of the mouth and throat, and the bulbs of *Allium cepa* were used for the prevention or adjunctive treatment of mild or moderate upper limb infection, or for relief from cutaneous insect bites, wounds, and minor burns [16,27]. The reasonable doubt that Hippocrates was not in Athens during the plague relies on the fact that Thucydides (460–400 B.C.) does not mention him in his '*Ιστορία του πελοποννησιακού πολέμου*' (History of the Peloponnesian War), but also on the lack of any reference to this disease in the Hippocraticum corpus (although later legends report that the plague was cured by Hippocrates himself). The unique information about the plague comes from Thucydides, who quite accurately described the main signs and symptoms of this disease. The plague broke out during the Spartan siege of Athens (430 B.C.) and until the summer of 428 B.C. decimated the city population. After a short period of recession, the epidemic resumed in 427 B.C. until the winter of 426 B.C. However, today the most accepted hypothesis indicates the typhoid fever involved in etiology of the Athens plague, either exclusively or in combination with some other (currently unknown) contagious factor [28–30].

In the Classical age of the Roman Empire, Greek medicine expanded even more when physicians from the Hellenistic provinces went to Rome and dealt with various infectious diseases and reported treatments in their treatises. Asclepiades of Bithynia or Prusa (124 B.C.-56 A.C.), Dioscorides Pedanius (40–90 A.D.), Themison the Laodiceo (1st century B.C.), Sextius Niger (1st century B.C.), Asclepiades Pharmakion (1st century B.C.), Gajus Plinius secundus (1st century B.C.), Dioscuridis Pedanios, Galinos or Galen of Pergamos (130–200 A.C.), Rufus Ephesius (2nd century A.D.) are among the most renowned physicians of those times [21,31]. In that era, Panfilos Alexandreus (1st century A.D.), who was a manufacturer and merchant of medicines, made-up a preparation based on sandalwood, copper, ash and cantharidin from the officinal beetles *Lytta vesicatoria*, that was used as treatment for a skin disease developing at the hair roots (especially on the mustache and beard) caused by *Staphylococcus* spp [16]. Approximately on 77 A.D. Dioscuridis, in the 5th section of his work '*Περί ιατρικής ύλης*' (On medicine) refers to the main plants that, mixed with wine, were recommended for dermatomycosis and purulent otitis: examples are the μυρσινίτης (mirsinitis) with *Myrtus communis*, the τερμίνθινος (terminthinos) with *Pistacia terebinthus* and the σχίνινος (skininos) with *Pistacia lentiscus* [16,32]. He also described the antiseptic and antimicrobial activity of *Styrax officinalis*, the curative properties of balsam obtained from plants such as *Amyris gileadensis, Balsamodendron gileadense, Commiphora gileadensis*, which indeed contain terpenes, as well as the anti-helminthic properties of the roots from *Punica granatum*. Among inorganic compounds, he used the *Αλός άνθος* (Alos anthos), possibly soda mixed with substances and *Αλός άχνη* (Alos achni), obtained from the sea foam deposited on the rocks (rich in chlorides and dried sulfates) for pneumonia and purulent otitis. Also, the mixture of copper and zinc combustion residues called *διφρυγές* (difriges) with turpentine (from *Pistacia terebinthus*) or with waxy ointment was usually indicated for the treatment of abscesses, while patches of potash foam were applied on lesions of leprosy patients [16]. As a follower of Hippocrates and Aristotles, Galen was among the first to investigate the intensity of a drug action in the human body, and to classify the quality of each compound in four grades. Among the fourth degree warming drugs he included the onion *Allium cepa*, used as an antiseptic. Of note, Dioscourides previously hypothesized that the strength of a drug depends on the quantity (dose) given, suggesting the fundamental concept that each drug may have positive or negative effects (toxicity) according to the amount used, and therefore providing the basis of drug toxicology [32–34].

During the Middle Age, physicians of the Christian Roman Empire (currently Byzantium) handed down the Greece and Hellenistic medicine experiences and contributed with clinical therapeutic novelties and personal experiences. Some of them became teachers at the Imperial University of New Rome (funded in 862 A.D.). Among them, Oribasios of Pergamos (4th century A.D.), Aetius Amidenus, Alexander from Tralles (6th century A.D.), Paul from Nicaea (9th century A.D.), Simeon Seth (century A.D.), Nicholas Myrepsos or Actuarius (around

13th century A.D.), Joannes Zaharias Actuarius (around 14th century A.D.), and others [23,35].

Oribasius refers to the pharmacological treatment of various diseases, including infections, in the *ια΄*(eleven) and *ιε΄* (fifteen) books of his work '*Συναγωγαί ιατρικαί*' (Medical Collections). Simeon Seth, among the herbal remedies and their uses mentioned in the text '*Σύνταγμα κατά στοιχείον περί τροφών δυνάμεων*' (Syntagma de alimentorum facultatibus) recommended *Sinapis* and *Allium sativum* for the leprosy's (as previously reported by Alexander of Tralles). Later, Nicholas Myrepsos wrote the *Δυναμερόν* (Dynameron) that with more than 2,500 pharmacological preparations (including anti-infectious ones) became the most extensive treatise on pharmacognosy until the 18th century [16,36–38].

In medieval Western Europe, Schola Medica Salernitana was the first medical school created in a Benedictine monastery in Salerno, Italy, together with the school of Montpellier (1220 A.D.) in France. The medical school of Salerno's origins should date back to the IX–X centuries (around 802 A.D.) and was likely founded by four medicine experts: the Greek pilgrim Pontus, the Arab Abdela, the Hebrew Elinus, and the Latin Salernus. Thus, its tradition was based on Greek, Arabic, and Jewish medicine [16,35]. Nicolaus (probably Nicolò Aversano) was one eminent doctor dealing with therapeutic activity of plants and minerals in the 'Antidotarium' (early 12th century); similarly, Matthaeus Platearius, in his '*Circa Instans*' (The Book of Simple Medicines) reports numerous therapies for infectious diseases, mostly based on Dioscurides work [16,39].

Arabic medicine paid great attention to the preparation of medical remedies and faithfully followed a written code called Krabasin (prescription), containing a series of recipes against infections. In 9th and 11th century A.D. the work of Avicenna, in texts such as the *'Al-Qanun fi'l-tibb'* (Canon medicae) consisting in 5 books (including the second, that contains pharmacology principles according to Galen) brought Arab medicine to a high level of knowledge [39]. In addition, the focused attention on treatment of infectious diseases leads Muhammad ibn Zakariyā Rāzī (854–923 A.D.) to be the first physician able to differentiate initial symptoms of smallpox from measles. His most important work, entitled '*Al-Hawi fi al-Tibb*' (Liber Continens), consists of thirty-seven books and has an encyclopedic character. Among many others, Ibn Al Wafid (997–1074 A.D.) a pharmacologist who lived in Toledo (Spain), wrote a medical manual entitled '*Liber de medicamentis simplicibus.*'Toledo hosted, at that time, arenowned school of translators converting into Latin the Greek version of Arabian and Hebraical texts [16,39,40].

2.2. Renascence and Dawn of the modern era

During the Renaissance, while the frequency of major plague and leprosy epidemics diminished, new ones appeared, including smallpox, measles, varicella, the English sweating sickness (a dreadful disease whose etiology is unknown that decimated the English population in the early 15th), and more others, such as syphilis, were exacerbated. The spread of gonorrhea and syphilis prompted the use of new treatments such as arsenic, bismuth, and mercury, administered systemically or locally, using specially designed syringes [41,42]. At that time, the alchemist and doctor Paracelsus (Philippus Aureolus Theophrastus Bombastus von Hohenheim, 1493–1541) believed that the proper use of poisons might represent the most effective treatments and introduced lead, mercury, antimony, arsenic, copper sulfate, in small doses in therapeutic remedies based on herbs. He also hypothesized the antibacterial and antiviral properties of the extract of *Chelidonium majus*, whose main constituents are benzophenanthridine alkaloids [16,34]. He was probably influenced by the alchemists of the past, especially the classical ones of the 4th century A.D., such as the Greek Zosimos (Ζώσιμος, 350–420 A.D.) from Panopolis (today Akhmim, Egypt) who created the first and vast encyclopedic text 'Χειροκμήτων' (Chirokmiton = handmade shaped thinks) of chemical procedures, and was indeed the first to use the term χημεὶα (chemistry) [18,19].

The invention of the microscope by Antoine van Leeuwenhoek (1632–1723) and the observations reported with this instrument, as well as the original work in biology by Robert Hooke (1635–1703), pioneered the field of microbiology and made the pathogenesis of infectious diseases better understood [43].

At that time, characterization and potential therapeutic use of plants from the new lands explored began. The dried roots of the *Uragoga ipecacuanha* were utilized to treat dysentery, and the first reference to the treatment of what is now known as amoebic dysentery can be found in the '*Historia naturalis Braziliae*' (1648) from William Piso (1611–1678); however, the compound was not introduced in Europe until 1672 [44,45]. Similarly, the Peruvian balsam derived from the Central American tree *Myroxylon balsamum (*var. *pereirae)* was used as a mild antiseptic in skin diseases accompanied by itching, or against scabies [46]. The Guaiac wood, obtained from the plants *Guaiacum officinale* and *sanctum* (northern coasts of South America and the West Indies, respectively), spread in Europe during the 16th and 17th centuries and was devoted to treat syphilis, tuberculosis, and rheumatism. It was the work *'De Guaiaci Medicina et morbo Gallico, Liber unus*,' published in 1519 by Ulrlich von Hutten (1488–1523) to better explain the potential activity of this natural compound [16,42,47]. Concomitantly, treatment of syphilis and plague was still carried out with antimony and its preparations, as reported by the German alchemist Johann Thölde (1565–1614) in his *'Triumphal chariot of antimony'* in 1604; or with solution of 0.014% of mercuric chloride, as proposed by Gerhard van Swieten (1700–1772). The English pharmacist and physicianThomas Fowler (1736–1801) used arsenic in patients suffering from syphilis or malaria [16]. The *Sarcostemma viminale* native to sub-Saharan Africa, Arabian peninsula, India, Philippines, Oceania and a variety of other plant species including *Cinchona succirubra Pavon*, C. *calisaya* (native to central and southern America) were used for malaria, as well as for patients with smallpox, ocular infections, diarrhea and intestinal disorders, Similarly, the *Shorea robusta* (native to northern India) was useful to heal wounds and ulcers, and also in patients with gonorrhea [16,48].

Chewing sticks for cleaning teeth and to prevent oral infections were used by all ancient peoples until the 19th century A. D., and several plant species were chosen for this purpose. To treat or prevent caries and periodontitis, but also as antivirals (herpes virus affecting the oral mucosa), *Azadirachta indica* (native to Bangladesh, India, and Burma), the twigs of *Zanthoxylum alatum* (native to China) and the roots of the *Salvadora persica* (native to Middle East, Africa, and India) were most often employed [16,49].

The ability of microorganisms to produce antibiotics was detected by the end of the 19th century. From the first serendipitous observations, intensive investigations began in search of molecules and drugs that would kill disease-causing bacteria [1,50]. In 1887, the Swiss scientist C. Garre noted that the presence of a *Pseudomonas* (*Bacillus fluorescens*) inhibited the growth of *Staphylococcus* colonies. Few years earlier, in 1871, the physician Joseph Lister reported that urine samples contaminated with fungi did not allow certain bacteria to grow successfully [51]. In 1895, the Italian scientist Vincenzo Tiberio discovered the phenomenon of the antibiosis of some molds and hypothesized their potential anti-infectious activity. Tiberio carried out an entire experimental cycle: from the empirical observation to the verification of the initial hypothesis, to the preparation of the 'antibiotic' substance, to the demonstration of its effect *in vitro*, to the documentation of its efficacy *in vivo*, up to the proposal of a mechanism of action based on the observed changes in the leukocyte structure. It is fascinating to read that, in that work, even the doses and duration of the antibiotic efficacy of Tiberio's extract were evaluated (Figure 3) [52–54].

Since then, inhibition of microbial growth by antimicrobial substances produced by other organisms has been observed by many researchers. An attempt to apply these observations to medicine was made by Rudolf Emmerich (1856–1914) and Oscar Low (1844–1941), who recommended the use of pyocyanin, a substance produced by a *Pseudomonas*, for therapeutic purposes. In the 1890s, the two scientists made Pyocyanase the first antimicrobial drug from the blue pigment of *Pseudomonas aeruginosa* (previously *Bacillus pyocyaneus*) [55]. In 1909, Paul Ehrlich (1854–1915) discovered the successful treatment of syphilis with a compound called Salvarsan, which belongs to the class of arsenobenzole. It was the first truly modern antimicrobial agent and contributed to the introduction of chemotherapy concept [56]. Finally, on the ground of the intense research of Ehrlich Robert Koch (1843–1910) and Emil von Behring (1854–1917) antitoxins became an essential weapon for antibacterial therapy [56,57]. William Osler (1849–1919) described the use of anti-streptococcal serum as a treatment for endocarditis, whereby bacteria isolated from blood cultures were injected into horses, and horse serum was then administered to patients [58]. The first to describe the antibiotic properties of *Penicillium* spp. was the French scientist Ernest Duchesne (1874–1912) in 1897, who preceded the isolation of penicillin by Alexander Fleming (1881–1955). In 1928, while investigating the properties of *Staphylococci* spp [59]., Fleming noticed that several of the microbial culture tablets he had accidentally left unchecked were infected with a fungus and threw them into a detergent container. The subsequent identification of a fungus from the genus *penicillium* isolated in a sample of the mold explains why he called the new substance penicillin. The bactericidal activity of penicillin was observed toward bacteria such as *Staphylococci* and in general Gram-positive pathogens (smallpox, pneumococcus, meningococcus, diphtheria) and some Gram-negative bacteria such as *Neisseria gonorrhea*, but not the germs of typhoid or paratyphoid fever [60]. The therapeutic value of penicillin was confirmed in 1940, when Howard Florey (1898–1968) and Ernst Chain (1906–1979) purified the compound and administered it to treat experimental infections on animals; the importance of penicillin for treating infections in humans was unquestionably established few years later [61,62].

Following the introduction of sulfonamides in the 1930s and the availability of penicillin, the antibiotic revolution accelerated from the late 1940s to the 1960s. These two golden decades were characterized by repeated accomplishments in the development of natural antibiotics that would enhance their intrinsic activity, and in the successful treatment of several infectious diseases [3,63]. Since then, the flow of new antibiotics began to shrink, for at least three main reasons: first, the technical difficulty in discovering new molecules, especially those able to penetrate Gram-negative


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Istituto d'Igiene della R. Università di Napoli

Fascicolo I- Gennaio 1895

SUGLI ESTRATTI DI ALCUNE MUFFE

Dott. VINCENZO TIBERIO

Figure 3. From the work 'On the extracts of some molds' by V. Tiberio (1895): *' … I wanted to observe what action they have on schizomycetes, the cellular products, water-soluble cellular, of some quite common ifomycetes: Penicillium glaucum, mucor mucedo and aspergillus flavescens. My searches they shed especially on pathogenic bacteria in vitro and on these two species the typhus bacillus and the cholera vibrio in the body, as experimental infections … It is clear from these observations that in the cellular substance of the examined molds there are contained water-soluble principles, provided with bactericidal action … Due to these properties, molds would be a strong obstacle to the life and propagation of pathogenic bacteria … The Guinea pigs all survived, except those injected after 10 days, which pointed out a delay in death, compared to controls … As such, this liquid has a preventive action and therapeutic action … '* [54].

bacteria; second, the increasing stringent criteria of relevant legislation; and third, the economic consideration that, as short-term treatment, antibiotics are less profitable than drugs used in chronic diseases for pharmaceutical industry [4]. A brief overview of the most important steps in antibiotic development is summarized below.

In 1932, sulfonamide appeared at the forefront of medical practice. One of the first derivatives was sulfapyridine, known as M&B693. In 1935, Prontosil (sulfacrisoidine) was the first sulfonamide drug developed by Gerhard Domagk (1895–1964) at the Bayer Company department of antibacterial agents. Until 1945, approximately 5,000 sulfonamides were produced and used in urinary tract infections, pneumococcal pneumonia (*Streptococcus pneumoniae),* and meningitis. However, the extensive use of sulfapyridine and other sulfonamides increased bacterial resistance and eventually led to their replacement by other antibiotics [63,64].

The manufacturing process for Penicillin G Procaine was completed by Howard Walter Florey (1898–1968) and Ernst Boris Chain (1906–1979) in 1942 [65]. During the same period, the American microbiologists Selman Waksman (1888–1973) and H. Boyd Woodruff (1917–2017) discovered the *Actinomyces antibioticus*, a Gram-positive bacterium today called *Streptomyces antibioticus*, initiating an extensive production of antibiotics. Bactinomycin (Actinomycin A and Actinomycin B) and Boromycin come from *S. antibioticus,* whereas Virginiamycin comes from *Streptomyces virginiae* [66–68]. In 1943, Waksman made streptomycin, the first molecule of a new group of drugs called aminoglycosides. The streptomycin was effective to treat diseases such as tuberculosis, although at that time side effects were often extremely severe [69]. Waksman was also the first to introduce the term 'antibiotic' in the 1940s and 1950s to differentiate natural remedies produced by fungi or bacteria from 'chemotherapeutic drugs,' now meaning chemicals with antimicrobial activity but synthetically produced [69,70]. Differentiation was consolidated when novel antibiotics were synthesized from natural products by attaching various side groups to their basic structure [3,4].

Vancomycin was discovered at the Elli Lilly company in the 1950s, when one missionary visiting Borneo sent a soil sample to a colleague who isolated the organism *Amycolatopsis orientalis* (formerly called *Streptomyces orientalis* and *Nocardia orientalis*), which was found to produce a substance that inhibits Gram-positive organisms. 'Mississippi Mud,' as it was known for its brown color, was used in clinical trials in the mid-1950s and approved for human use by the U.S. Food and Drug Administration (FDA) in 1958 [71].

In 1955, tetracycline was patented by Lloyd Conover, and in 1957, nystatin started to be prescribed to treat deforming and harmful fungal infections [72].

In 1981, the SmithKline Beecham Company patented amoxicillin tablets (a penicillin-type semi-synthetic molecule), and in 1998, the antibiotic was sold under the brand names Amoxicillin, Amoxil, and Trimox.

Discovered in the 1990s, glycocyclins are derivatives of minocyclines, and the most recent tetracyclines obtained [73].

From the oxazolidinones (cycloserine was used from 1956), linezolid was available in 2000 and tedizolid has recently become an additional option.

Finally, daptomycin was derived from *Streptomyces roseosporus* and launched in the USA in 2003, and glycopeptides such as dalbavancin and oritavancin have become accessible in 2014 [74–78].

When penicillin first appeared in the early 1940s, *S. aureus* was uniformly sensitive to this drug. Starting from the appearance of *S. aureus* methicillin-resistant (MRSA) strains in the 1960s, the scarce sensitivity to new semi-synthetic penicillinase-resistant antimicrobials (e.g. methicillin, oxacillin, and nafcillin), and glycopeptides, especially vancomycin, has become the mainstay of treatment for severe MRSA infections. Over the years, alongside the discovery of various antibiotics with an independent mechanism of action, some bacterial species have developed an increasing resistance to most of them (Table 1) [79].

By the 1990s, resistance to semisynthetic penicillins had spread around the world, compromising the use of these drugs for empirical treatment of *Staphylococcus* infections in several geographic regions. This has led to an increased dependence on vancomycin for treating confirmed MRSA infections, as well as for the empirical treatment of infections in populations where the MRSA prevalence is high. Unfortunately, reports from the 1990s suggested that *S. aureus* susceptibility to vancomycin was changing [80,81].

Table 1. The chronological development of resistance of certain microorganisms to certain types of antibiotics over time.

Timeline from discovery to antibiotics resistance

Antibiotics	Year of discovery	Year of commercial release	Year of efficacy reports release	Resistance mechanisms	Microorganisms
Penicillin G	1940	1943	1940	Penicillinases	*Staphylococcus aureus*
Tetracycline	1948	1952	1952	Extrusion pump	*Shigella dysenteriae*
Erythromycin	1952	1955	1956	23S rRNA methylation	*S. aureus*
Vancomycin	1956	1972	1988 and 2004	D-Ala-D-Ala exchange	*Enterococcus faecalis, S. aureus*
Methicillin	1959	1961	1961	MecA (PPP2a)	*S. aureus*
Gentamicin	1963	1967	1969	Modifying enzymes	*S. aureus*
Nalidixic acid	1962	1964	1966	Topoisomerase mutations	*Escherichia coli*
Cefotaxime	1975	1981	1981 and 1983	AmpC β-lactamases, ESBL	*Enterobacteriaceae*
Imipenem	1976	1987	1986	Acquired carbapenemases	*Pseudomonas aeruginosa, Serratia marcescens*
Linezolid	1979	2000	1999	23S RNA mutations	*S. aureus, E. faecalis*
Daptomycin	1980	2004	2005	Not yet clearly defined	*S. aureus, E. faecalis*

In 1996, the first confirmed infection with *S. aureus* of moderate vancomycin sensitivity (VISA) was reported in a patient in Japan. Subsequently, infections caused by VISA and vancomycin-resistant Staphylococcus aureus (VRSA) strains have been increasingly reported in patients from the United States, Europe, Middle East, and Asia [80–82].

3. Microbiostatic and microbicidal antibiotics

Antimicrobial drugs that inhibit or slow down the growth and proliferation of microorganisms are considered microbiostatic agents and include sulfonamides, tetracyclines, macrolides, chloramphenicol, novobiosin, and thiamulin. Instead, agents that destroy and kill microorganisms are called microbicides and comprehend, among others, penicillins, cephalosporins, aminoglycosides, colistin, bacitracin [1,4,83]. This is not an absolute distinction, as the antibiotic activity of each molecule is related to several factors, including its concentration at the infection site as well as the type and nature of the targeted microorganism. Antibiotics such as benzylpenicillin, lincomycin, or thiamulin (this last for veterinary use) are microbiostatic at low concentrations and microbicidal at high concentrations. Depending on their activity spectrum, antimicrobials can be classified into two main categories: narrow spectrum (active toward either Gram-positive or Gram-negative bacteria) and broad-spectrum (active toward both Gram-positive and Gram-negative bacteria) [84,85]. Antibiotic mechanisms of action vary according to their specific biochemical properties, and include a) selective disruption of bacterial metabolism, b) impaired synthesis of the cell wall (either by direct enzymatic digestion, or by inhibiting the action of enzymes, c) impaired synthesis of proteins, d) altered DNA metabolism, either by directly destroying DNA or by inhibiting the action of enzymes (Table 2) [86,87].

4. Categories of antimicrobial agents

The following paragraphs recapitulate the main characteristics of several antimicrobial drug classes. A more extensive and detailed description of all the drugs currently used is beyond the scope of this review and available elsewhere [88].

4.1. Sulfonamides

As mentioned above, sulfonamides are synthetic drugs, derivatives of sulfanilamide, and, due to their low cost, still widely used.

Mechanism of action – Sulfonamides compete with para-aminobenzoic acid (PABA) to bind the bacterial dihydropteroate synthase, and therefore inhibit the first step in the synthesis of folic acid, which is essential for the growth and proliferation of susceptible bacteria [63].

Bacterial sensitivity – Sulfonamides are broad-spectrum antibiotics effective toward both Gram-positive and Gram-negative bacteria. They are mainly microbiostatic agents.

Representative drugs – The most important molecules are represented by sulfathiazole, sulfadimidine, sulfamerazine and sulfadiazine.

4.2. Diaminopyrimidines

Diaminopyrimidines are synthetic antimicrobials almost always used in combination with sulfonamides for potential synergistic effects on bacterial growth and in the attempt to reduce the development of bacterial resistance [89,90].

Mechanism of action – They bind to the dihydrofolate reductase enzyme and interfere with the conversion of dihydrofolic acid (DHF) to tetrahydrofolic acid (THF) essential for the biosynthesis of the amino acids purine and thiamine and DNA synthesis of susceptible bacteria.

Bacterial sensitivity – These drugs are broad-spectrum antibiotics with mostly bacteriostatic activity.

Representative drugs – They include trimethoprim, pyrimethamine, and baquiloprim (for veterinary use).

4.3. β-lactams

They are characterized by the presence of the lactam ring in their molecules and include various groups of antibiotics, such as penicillins, cephalosporins, monobactams, carbapenems, and lactamase inhibitors. These last molecules, including clavulanic acid, sulbactam, and tazobactam, are used only in combination to enhance the activity of other β-lactam antibacterials against β-lactamase-producing bacteria [91].

Table 2. The various bacterial sites of action of antibiotics. Antibiotics have specific targets and interact with specific elements of the bacterial cell. Three main properties determine the activity of an antibiotic: the affinity between the antibiotic and the cell target, the bacterial cell wall permeability to the antibiotic (determining the amount of drug that will reach the cellular target), and the presence of bacterial enzymes that can inactivate the antibiotic.

MAIN TARGETS OF ANTIBIOTICS						
BACTERIAL WALL SYNTHESIS	**PROTEIN SYNTHESIS**	**MEMBRANE**	**DNA POLYMERASE**	**DNA GYRASE (TOPOISOMERASE)**	**RNA POLYMERASE**	**FOLIC ACID METABOLISM**
CYCLOSERINES	**Inhibitors 30S**:	POLYMIXYNS	NOVOBIOCIN	QUINOLONES	RIFAMPICIN	SULFONAMIDES
PHOSPHOMYCIN	TETRACYCLINES					TRIMETHOPRIM
GLYCOPEPTIDES	SPECTINOMYCIN					
BACITRACIN	STREPTOMYCIN					
PENICILLINES	AMINOGLICOSIDES					
CEPHALOSPORINS	NITROFURANS					
MONOBACTAMS	**Inhibitors 50S**:					
CARBAPENEMS	MACROLIDS					
	CHLORAMPHENICOL					
	LINCOSAMIDES					
	tRNA:					
	MUPIROCINE					
	PUROMYCIN					

4.3.0.1. I. Penicillins.

Mechanism of action – The therapeutic use of penicillins depends on their individual antimicrobial spectrum. Whichever activity they display on bacteria, however, their mode of action is common and lies in the inhibition of the biosynthesis of the mucoproteins constituting the cell wall of susceptible bacteria by interacting with the so-called penicillin-binding proteins (PBP) [92–94]. These PBPs appear to play a role in cell wall peptidoglycan synthesis and cell growth. Through their β-lactam portion, penicillins mimic the terminal portion of the peptide chain and bind to the active site of the enzyme, creating a stable penicillin link to DD-transpeptidase. DD-transpeptidase is therefore inactivated, and peptidoglycan is no longer formed. This weakening of the bacterial cell wall cannot compensate for the osmotic pressure of the cytoplasm. In addition, accumulation of peptidoglycan precursors activates hydrolase and autolysin enzymes that damage the bacterial wall by breaking it during cell division, resulting in digestion of the existing peptidoglycan and subsequent bacterial death [95,96].

Bacterial sensitivity and representative drugs – Penicillins can be categorized into: (a) narrow spectrum, act against Gram-positive bacteria and have in common that can be inactivated by bacteria-produced enzymes termed β-lactamases (penicillinases). The most important agents in this class are benzylpenicillin and the precursor penicillin; (b) narrow spectrum β-lactamase resistant, with cloxacillin and oxacillin being the most representatives. With respect to penicillins of the above category, they are also active vs. *Staphylococci* spp that produce β-lactamases; (c) broad spectrum sensitive to β-lactamases, such as ampicillin and amoxicillin active against Gram-positive and Gram-negative bacteria not producing β-lactamases, including germs such as *E. coli, Salmonella enterica, Shigella* spp., *Proteus mirabilis, Helicobacter pylori,* and *Haemophilus influenzae*; (d) broader spectrum penicillins of the newer generation, such as carbenicillin, ticarcillin, azlocillin, meslocillin and piperacillin less sensitive to β-lactamases, but active also toward Gram-negative organisms, including *Pseudomonas aeruginosa* [92,93,97].

4.3.0.2. Ii. Cephalosporins.

Mechanism of action – They are related to penicillins and classified in a common category under the term β-lactams, since they have a similar structure and, mode of action to penicillins. Cephalosporins are semi-synthetic antimicrobial drugs, derivatives of cephalosporin C, a substance found in *Cephalosporium acremonium*.

Bacterial sensitivity and representative drugs – Based on their resistance to the β-lactamases (in this case termed cephalosporinases) and their effectiveness to treat infections by Gram-positive or Gram-negative bacteria, they are divided into five groups or generations: (a) first generation includes cephalosporins such cephalexin, cefadroxil, all sensitive to cephalosporinases, and mostly effective against Gram-positive bacteria; (b) second-generation cephalosporins, such as cefuroxime, which are more resistant to cephalosporinases, may be effective toward Gram-negative bacteria but less active in Gram-positive-dependent infections; (c) third-generation cephalosporins, also resistant to β-lactamases, such as ceftiofur, cefquinom, and cefoperazone, are more effective against Gram-negative bacteria compared to both the first and second generations (d) fourth-generation cephalosporins, such as cefepime and cefpirome, resistant to β-lactamases and used for more severe bacterial infections; (e) ceftaroline is one fifth-generation cephalosporin used to treat infections, including MRSA infections, that are resistant to other antibiotics [91,98].

4.3.0.3. Iii. Carbapenems.

Carbapenems (formerly called thienamycins) are a broad-spectrum class of β-lactams and considered 'essential antibiotics' according to the WHO list of essential medicines. They are natural antibiotics produced by *Streptomycetes* and contain a carbon atom in the fourth position of the central a β-lactam ring.

Mechanism of action – Their mechanism is shared with all other β–lactams, thus consisting in inhibition of the synthesis of peptidoglycan, the major component of the bacteria cell wall.

Bacterial sensitivity – Carbapenemic structure confers high resistance to most β-lactamases, except for β-lactamases produced by the *Stenotrophomonas maltophilia* and *Bacteroides* strains. They can be hydrolyzed by renal dipeptidases [99,100].

Representative drugs – The first member of the carbapenem family is thienamycin, whose instability in water limited its clinical use. This instability was overcome by the semisynthetic production of its N-formimidoyl derivative, called imipenem. Other agents are ertapenem, meropenem, and doripenem [91,100,101].

4.3.0.4. Iv. Monobactams.

They are semi-synthetic antibiotics produced by Gram-negative bacteria and then synthetically modified. With respect to other β-lactam antibiotics, they contain a monocyclic-lactam ring, without additional (cyclization) rings. Monobactams are stable to β-lactamases but are not absorbed from the gastrointestinal tract, and therefore they should be administered parenterally. Therapeutic use is usually limited to subjects allergic to penicillins and cephalosporins.

Bacterial sensitivity – Importantly, monobactams work only against aerobic Gram-negative bacteria (*Neisseria, Pseudomonas*) and are not effective against anaerobic and Gram-positive pathogens.

Representative drugs – Aztreonam is the first monobactam component released for clinical use [102,103].

4.4. Tetracyclines

Mechanism of action – Tetracyclines antimicrobial activity results from drug binding to the 30S subunit of the ribosome in susceptible bacteria, with subsequent interference with bacterial protein synthesis [104].

Bacterial sensitivity – These broad-spectrum, bacteriostatic antimicrobial drugs have been widely used against Gram-positive and Gram-negative bacteria, and for treatment of infectious diseases due to chlamydia, mycoplasmas, rickettsiae, and protozoan parasites.

Representative drugs – Tetracyclines are mainly represented by tetracycline, oxytetracycline, chlorotetracycline, methacycline, and doxycycline. Depending on the duration of their activity, they are classified into short-term (tetracycline,

oxytetracycline, and chlorotetracycline), medium-term (metacycline), and long-term (doxycycline) drugs. Glycocyclins, derivatives of minocyclines, were discovered in the 1990s and represent a subgroup of tetracyclines. Tigecycline, which belongs to this category, is the most recent semi-synthetic glycocycline

4.5. Aminoglycosides

Molecules of this class are characterized by a core structure of amino sugars connected via glycosidic linkages to a dibasic aminocyclitol, which is most commonly 2-deoxystreptamine.

Mechanism of action – In position 5 of the 2-deoxyptreptamine ring they carry a hydroxyl, while in positions 4 and 6 they bind to amino sugars. The remaining positions of the ring carry free amino groups that bind with high affinity to the A-site on the 16S ribosomal RNA of the bacterial 30S ribosome. Because of this interaction, the antibiotics promote codon misreading on delivery of the aminoacyl transfer RNA and subsequent mistranslation. This results in error prone protein synthesis, incorrect amino acids assembly into a polypeptide and ultimately damage to the membrane and to other components of the bacterial cell. Because of their chemical structure and high surface charge, aminoglycosides are poorly absorbed via the gastrointestinal tract and are, thus, administered via the intravenous or intramuscular route for systemic infections.

Bacterial sensitivity – Aminoglycosides are particularly active against aerobic Gram-negatives, although they may act synergistically against certain Gram-positive organisms. An active oxygen/ATP-dependent pump is needed for the drug to enter the bacterial cell, and this explains their limited activity toward anaerobic bacteria. All aminoglycosides are rapidly bactericidal and typically produce a prolonged post-antibiotic effect. However, bacteria may become resistant to aminoglycosides by producing enzymes able to acetylate, phosphorylate, or adenylate amino or hydroxyl groups found at various positions around the aminoglycoside core scaffold. In case of streptomycin, resistance may also be due to an established resistance mechanism that is genetically linked to other genes in an integron, or selected by other antimicrobial agents utilizing the same endurance mechanism.

Representative drugs – Aminoglycosides are divided according to the categories into narrow-spectrum aminoglycosides, such as streptomycin and dihydrostreptomycin, and broad-spectrum aminoglycosides, such as neomycin, gentamicin, spectinomycin, and apramyc (Figure 4).

The newest aminoglycosides such as plazomicin and arbekacin are currently evaluated in an attempt to overcome the rising AMR of several bacterial strains [105–107].

4.6. Macrolides

Their name is strictly related to the structure, derived from a lactone ring of 14, 15, or 16 members.

Mechanism of action – These antimicrobial agents inhibit protein synthesis by binding to the 50S subunit of the ribosome of susceptible bacteria, are usually well tolerated and can be easily absorbed by oral administration.

Bacterial sensitivity – Macrolides display a wide range of antimicrobial activity, which includes mainly Gram-positive but also Gram-negative bacteria, anaerobic bacteria, *Spirochetes, Chlamydia,* and *Mycoplasma*. After the 1980s, with the introduction of modern macrolides and subsequent extensive use, resistant strains have emerged, mainly *Pneumococcus* spp., *Staphylococcus* spp., and *Streptococcus* spp. but also resistant Gram-negative bacteria such as enterobacteria and *Haemophilus influenzae* [108,109].

Representative drugs – The class of macrolides embraces older molecules such as picromycin (1950), erythromycin (1952), spiramycin, carbomycin, kitasamycin, oleandomycin, josamycin, and more recent ones including clarithromycin, azithromycin, roxithromycin, dirithromycin, flurithromycin, darithromycin, tylosin, and tilmicosin [108].

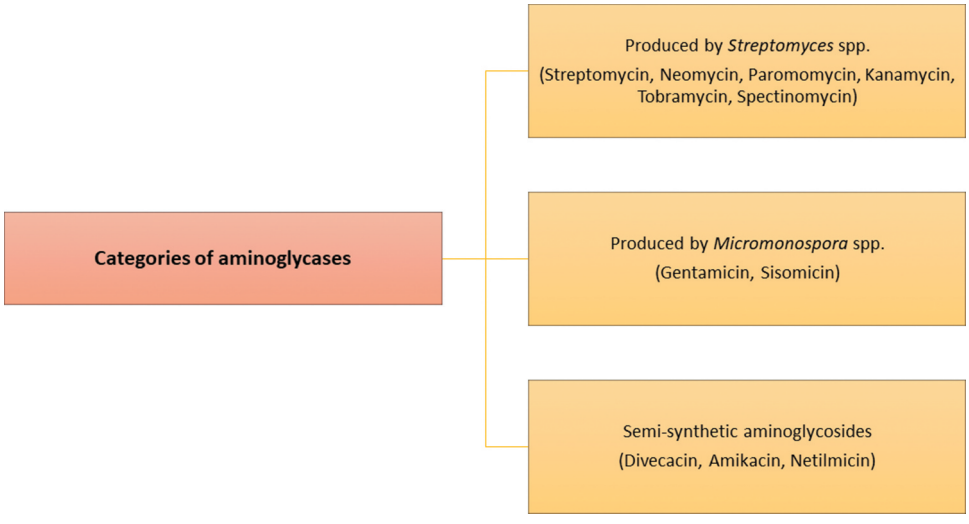

Figure 4. The three categories of aminoglycosides according to bacterial origin.

4.7. Lincosamides

Mechanism of action – Lincosamides mechanism of action is similar to that of macrolides and consists in the inhibition of the protein synthesis process at the level of amino acid transport, during the peptide chain elongation process. Molecules of this class display a large distribution in human tissues, including bones, and accumulate in phagocytes, which may explain why they can be found in pus. Their activity is strictly dependent on the concentration reached at the infection site and on the nature of the microorganisms involved, and therefore they may exert either a microbiostatic or a microbicidal effect [110,111].

Bacterial sensitivity – Lincosamides primarily target Gram-positive bacteria as they are unable to pass through the outer membrane of Gram-negative organisms.

Representative drugs – Two substances belong to this group: lincomycin and clindamycin, which are used in infections by Gram-positive bacteria and mycoplasma.

4.8. Polymyxins

Polymyxins are cationic polypeptides that consist of a cyclic heptapeptide possessing a tripeptide side chain acylated at the N terminus by a fatty acid hydrophobic tail.

Mechanism of action – Polymyxins interact with phospholipids in the cell membrane of sensitive bacteria, resulting in disruption of their function and impairment of defensive cell wall structure [112,113].

Bacterial sensitivity – Their antimicrobial spectrum embraces mainly Gram-negative bacteria, while polymyxins have lower potential efficiency toward Gram-positive strains. These germicidal drugs have recently regained significant interest due to the increasing incidence of infections due to multidrug-resistant Gram-negative bacteria.

Representative drugs – The group includes polymyxin A, B, C and D, E and colistin.

4.9. Rifamycins

Rifamycins are part of the ansamycin class of natural products. They are characterized by their basket-like structure that is formed when the ends of the naphthalene aromatic moiety are bridged by a polyketide chain decorated with different chemical moieties to form a loop.

Mechanism of action – Rifamycins interfere with the RNA synthesis of susceptible bacteria by inhibiting RNA polymerase activity. They may have microbiostatic or microbicidal activity, depending on their concentration at the infection site and on the nature of the microorganism involved [114,115].

Bacterial sensitivity – Rifamycins exhibit antibacterial activity against many Gram-positive and Gram-negative bacteria, and are known for their use in treating tuberculosis.

Representative drugs – Rifampicin, rifabutin, rifapentine, and rifaximin are the main molecules of this class.

4.10. Quinolones and Fluoroquinolones

This large class of synthetic microbicide molecules includes older compounds, such as oxolinic acid and nalidixic acid, and the currently available group of fluoroquinolones, derived from nalidixic acid with the introduction of a fluorine atom in their chemical structure to enhance their stability, bioavailability, and antimicrobial activity.

Mechanism of action – By targeting and inhibiting the bacterial DNA-gyrases of susceptible bacteria, they are able to prevent bacterial DNA from unwinding and duplicating. At higher concentrations, these drugs may also directly interfere with RNA [116].

Bacterial sensitivity – Fluoroquinolones are broad-spectrum antibiotics that are active against both Gram-positive and Gram-negative bacteria, including mycobacteria, and anaerobes.

Representative drugs – They can be classified into 4 groups: 1) naphthyridines do not contain a fluoro-substituent in their ring and are used in lower urinary tract infections caused by *Enterobacteriaceae* such as *E.coli* b) oxacins (e.g. cinoxacin) diffuse well in most tissues and penetrate cells, 3) 4-quinolones with a piperazinyl group (e.g. pipemidic acid), 4) fluorinated derivatives (the majority of quinolones in clinical use) such as norfloxacin, ciprofloxacin, levofloxacin, gatifloxacin and moxifloxacin [117–119].

4.11. Nitroimidazoles

Nitroimidazoles are a class of antimicrobial derivatives of 5-nitroimidazole. These compounds are prodrugs, meaning that a bioactivation of the nitro group is required to exert an antimicrobial effect.

Mechanism of action – When the nitro group is reduced to reactive radical species, these radicals react with cellular components such as DNA or protein. Under anaerobic conditions, the redox potential of the electron-transport system in microbes is sufficiently negative to reduce the nitro group. However, when oxygen is present, the cytotoxic nitro radical anion is rapidly reoxidized to its parent drug, and the bactericidal effects may be reduced.

Bacterial sensitivity – Nitroimidazoles have a relatively narrow range of action and are more effective against Gram-negative anaerobic bacteria and some protozoa such as *Giardia lamblia*, and *Entamoeba histolytica*. They are usually reserved as additional therapy in severe anaerobic infections, mixed bacterial infections with other antibiotics and, as mentioned above, against protozoan infections [14,120,121].

Representative drugs – They include metronidazole, dimetridazole, ornidazole and tinidazole

4.12. Glycopeptides

These drugs are of microbial origin derived from glycosylated cyclic or polycyclic non-ribosomal peptide complexes.

Mechanism of action – Instead of inhibiting an enzyme involved in peptidoglycan biosynthesis (as β-lactams), glycopeptides bind to a substrate, the lipid-linked disaccharopentapeptide [122]. The stable, heteropolar complex formed by drugs binding to the C-terminus D-Ala-D-Ala residue of the peptidoglycan precursors prevents their insertion into the cell wall, thereby inhibiting the final step of peptidoglycan

synthesis outside the cytoplasmic membrane. This, in turn, increases the intracellular accumulation of UDP-bound MurNAc-bound pentapeptide precursors and triggers the activation of bacterial autolytic processes.

Bacterial sensitivity – Vancomycin and other molecules of this class have been able to strongly inhibit cell wall synthesis in *S. aureus* and other Gram-positive organisms, and have been considered an effective alternative when MRSA infection is suspected. However, any modification in the bacterial structure that may interfere with the binding of glycopeptides to the D-Ala-D-Ala residues in the cell wall reduces the activity of these drugs [72,123–125].

Representative drugs – Glycopeptides include vancomycin (prototypical molecule, produced by *Streptomyces* spp), teicoplanin, telavancin, ramoplanin, decaplanin, corbomycin, complestatin, and bleomycin, this last used in cancer therapy.

4.13. Amphenicols

Mechanism of action – These broad-spectrum antibacterial agents target the 50s subunit of bacterial 70S ribosome. More specifically, they inhibit the activity of peptide transferase, hindering the growth of the peptide chain, thereby preventing protein synthesis. At high concentrations, they also exhibit a bactericidal effect against highly sensitive bacteria [126].

Bacterial sensitivity – Amphenicols generally have a stronger effect on Gram-negative than Gram-positive bacteria. Susceptible species include Enterobacteriaceae, Bacillus anthracis, Streptococci and Staphylococcus aureus and, to a lesser extent, anaerobic bacteria.

Representative drugs – Chloramphenicol and amphenicol are the main components of this class.

4.14. Other antimicrobial drugs

Antimicrobial agents that do not belong to the above described groups include, among others, bacitracin, novobiocin, oxazolidinones, and streptogramins. Bacitracin has a similar antimicrobial spectrum to benzylpenicillin and acts by inhibiting the biosynthesis of the cell wall of susceptible bacteria [124]. Novobiocin is a broad-spectrum antimicrobial drug that binds to magnesium ions, which are essential for the stability of the cell wall of susceptible bacteria and causes cell membrane damage, concomitantly interfering with DNA synthesis [125].

Oxazolidinones include d-cycloserine, whose antibacterial activity mainly depends on the inhibition of d-Ala-d-Ala ligase activity – thus interfering with cell-wall biosynthesis – and the relatively more recent linezolid, active against Gram-positive bacteria, which is especially useful for the treatment of infections caused by MDR streptococci, VRE, and MRSA.

Streptogramin antibiotics such as pristinamycin and quinupristin/dalfopristin are unique, in the sense that the producer strains synthetize two structurally unrelated antibiotics, streptogramin A, which is a cyclic hybrid peptide-polyketide macrolactone compound, and streptogramin B, which is a cyclic depsipeptide compound. The combination of these two acts synergistically to induce a rapid bacterial cell death by binding to the ribosomal exit tunnel and blocking it.

5. The bacterial antibiotic resistance

A bacterial strain is resistant to a drug when it can multiply in the presence of drug concentrations inhibitory for most of the stems of the same species, or equal to the maximum ones achievable during therapeutic use. The incidence and selection of resistance-inducing mutations in several bacterial strains has progressively increased due to the misuse of antibiotics, depending on their empirical use (treatment of a disease from unknown etiological agents), prophylaxis in surgery, inappropriate use of molecules with a broad spectrum of action, auxinic use in farm animals, administration in pediatric patients with viral infections and improper patient compliance [3]. Clinical significance of resistance implies that resistant strains survive in the presence of antibiotic serum concentrations reached with administration of standard therapeutic doses. Resistance predicts the possible failure of antibiotic therapy (Table 3) [126].

According to the report on 'Antibiotic Resistance Threats' issued by the Centers for Disease Control and Prevention (CDC) [127] more than 2.8 million antibiotic-resistant infections occur each year in the United States and more than 35,000 people die per year. The MRSA infections have become a major health issue in the United States and all over the western world, with 119,000 infections and nearly 20,000 deaths in 2017, and with rates of decline from hospital-onset slowed since 2012 [126].

5.1. Biochemical mechanisms of bacterial resistance to antibiotics

Bacteria growing in environments with low levels of antibiotics are generally sensitive to antibiotics, whereas bacteria exposed to constant antibiotic pressure will develop a high resistance to the administered substances. When a new antibiotic is introduced for therapeutic or nutritional use, resistance rapidly develops. Such effects have been observed in common food-borne pathogens (Figure 5).

Table 3. The origin of antibiotic resistance.

Type of antibiotic resistance	
Natural (intrinsic)	**Acquired**
Condition of general non-susceptibility to a drug that extends to all the strains of a given species. The microorganism may lack the structure on which the antibiotic acts (e.g. penicillins Gram-; *Chlamydiae* and *Mycoplasma* are devoid of cell wall and therefore insensitive to β-lactams). The cell wall structure or cytoplasmic membrane of a microorganism may be impermeable to an antibiotic.	Generally selected from a previous exposure of the pathogen to the antibiotic. It is implemented according to different mechanisms of which the main ones are: (1) modification of the bacterial target (2) production of enzymes that inactivate the antibiotic (3) reduced permeability to the antibiotic (4) efflux of the antibiotic from the cell thanks to a system of active pumps.

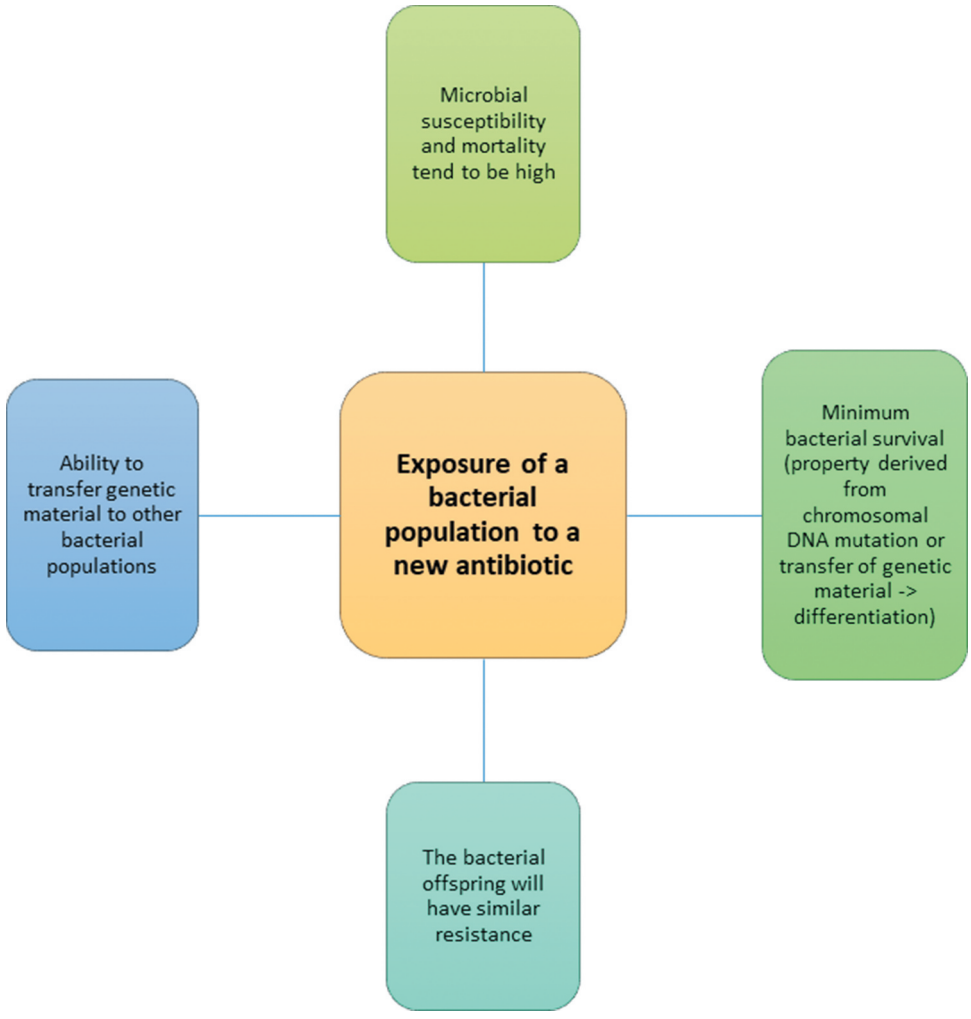

Figure 5. Possible mechanisms occurring in a microorganism in contact for the first time with the antibiotic.

Genes conferring resistance to antibiotics and their molecular mechanisms of transport across species have been shown to be the same in bacteria derived from food and pathogenic (animal and human) samples. Chronic exposure of microorganisms to the action of antimicrobial agents results in the selection of strains which remain unaffected by having developed one or more of the following defense mechanisms: (a) antibiotic inactivation; (b) target protein mutation; (c) acquisition of genes for less sensitive target proteins from other species; and (d) inhibition of drug access to target and target bypass (Figure 6) [13,127,128]

(a) Antibiotic inactivation – The mechanism of inactivation is common in conferring resistance to the naturally occurring antibiotics, such as β-lactams, which are inactivated by enzymatic hydrolysis from β-lactamases, usually in the periplasm, and aminoglycosides, which are inactivated by enzymatic phosphorylation (by aminoglycoside fosforyltransferase), acetylation (by aminoglycoside acetyltransferase), or by adenylation (by aminoglycoside adenyltransferase or nucleotide transferase). Aminoglycosides are also inactivated through modifications that reduce the net positive charges of these polycationic antibiotics [129–135].

After the introduction of β-lactam antibiotics, resistant strains of *S. aureus* were identified that carried enzymes able to hydrolyze β-lactams. More than 200 different types of β-lactamases have been described so far. The beta-lactamase gene blaZ has been involved in these activities. BlaZ is an 846-bp gene controlled by two regulatory genes (antirepressor regulatory protein blaR1 and repressor blaI) [136–140]. After exposure to β-lactams, blaR1 (which is a transmembrane sensor–transducer) undergoes autocatalytic cleavage, promoting the cleavage of blaI and leading to the transcription of blaZ. Importantly, blaZ encoding enzymes can be found in either plasmids or chromosomes, transported horizontally through plasmids, and spread across different strains. The genes encoding these inactivating enzymes can easily produce resistance as additives [141–146]

(b) Target protein mutation – Synthetic antibiotics are unlikely to be inactivated by the enzyme mechanisms described above. However, bacteria can become resistant through mutations that make the target protein less sensitive to the agent. The likelihood that such resistance mechanisms can be transferred to other cells with plasmids depends on the mode of action of the drug. Fluoroquinolone resistance, for

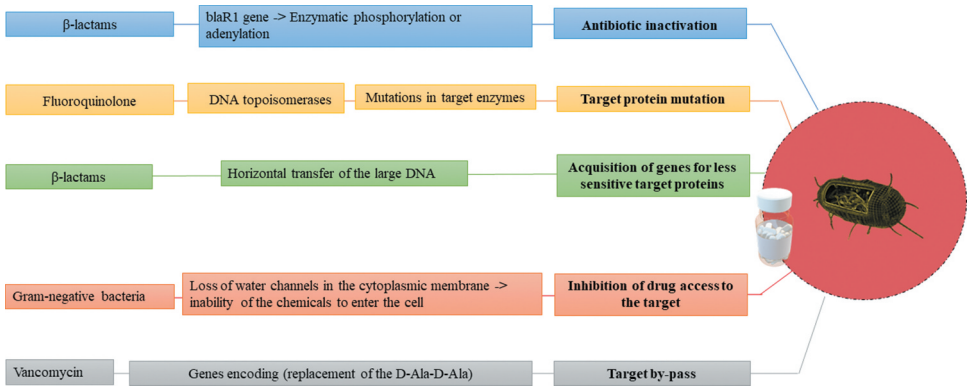

Figure 6. The figure demonstrates the main biochemical mechanisms of bacteria that lead to defense against some antibiotics, thus acquiring the 'shield' of antibiotic resistance (macrolides, beta-lactams and aminoglycosides through inactivation of their action. The vancomycin, tetracyclines, trimethoprim, and sulfonamides through a bypass of the drug target. The aminoglycosides, fluoroquinolones and penicillins through the inhibition of the target. The β-lactams through the acquisition of genes for less sensitive target proteins and fluoroquinolones with the target protein mutation).

example, is mainly due to mutations in target enzymes, represented by DNA topoisomerases. For these drugs, which kill bacterial cells containing the drug-sensitive enzyme, the addition of the gene encoding a drug-resistant enzyme does not make the bacterium fully resistant, nor plasmid transport completely efficient. However, when sulfonic drugs are used in combination, they help to select the mutations of the respective enzymes for drug resistance. In this case, the high levels of drug-resistant enzymes carried by plasmids can make bacteria resistant, and resistance genes may spread widely. Thus, mutations will become more prevalent with clonal selection in the presence of selection pressure [147,148].

(c) Acquisition of genes for less sensitive target proteins – This mechanism occurs also in case of resistance to β-lactams: indeed, sequences of genes encoding for penicillin targets such as DD-transpeptidase or penicillin binding protein (PBPs) have been observed in *Streptococcus*. *Streptococcus pneumoniae* is capable of natural transformation and can introduce foreign DNA [149–151]. A similar mechanism of resistance to penicillin has been found in *Neisseria meningitidis*. Since the *S. aureus* is not naturally transformable, it is not clear how the horizontal transfer of the large DNA fragments took place. However, when this happens, it results in Methicillin-resistant *S. aureus* (MRSA) strains that contain a new penicillin-binding protein-mediated resistance, called PBP-2A. Methicillin resistance results when foreign DNA, containing The methicillin resistance mecA gene encoding the alternative PBP2a, a low-affinity protein for β-lactams antibiotics, is integrated into a specific chromosome (Figure 7) [150,151].

If PBP2a is not inhibited by β-lactamases, *S. aureus* correctly synthesizes peptidoglycan, keeping a structurally stable cell wall. In addition to mecA, mecI and mecR1 genes may be involved in the regulation of methicillin resistance. Like blaI and blaR1 mechanisms, MecI suppresses the transcription of mecA and mecR1 for switching. Additional factors, such as the fem genes (essential for methicillin resistance), the llm genes (lipophilic proteins), and the aux (helper) genes also affect the expression of methicillin resistance [151,152]. On the same line, some *S. aureus* strains possess erythromycin-resistant methylase genes (ermA, ermB, and ermC) that encode a change in ribosomal RNA resulting in impaired macrolides binding and a high level of resistance [152–155]. The resistance mechanism for lincosamides is the enzymatic mutation of ribosomal RNA [154].

(d) Inhibition of drug access to target and target bypass – Decreased permeability of the drug through the cell membrane is observed in Gram-negative bacteria, where the loss of porins (water channels of the cytoplasmic membrane) leads to inability of the chemical to enter the cell. In other cases, while the drug has been able to enter the bacterial cell, an enhanced drug efflux effect occurs. Some lipophilic drainage pumps are activated, reducing the intracellular concentration of the antimicrobial agent, and consequently inhibiting its antibacterial activity [132]. The protective action of a microorganism community on individual bacterial cells, in particular the structure of a biofilm, involves many microorganisms, as well as their products, and creates a protective wall without allowing external factors to approach the interior. In this case, the drug does not even reach the target and the biocommunity becomes antibiotic resistant [133]. Increased activity of outflow transporters has been identified among mechanisms to prevent the accumulation of antibiotics, such as macrolides inside the bacterial cell. The msrA genes encoding effluent pumps are in transposable plasmids and provide a low level of resistance compared to the erm genes. Moreover, the genetic mechanism of vancomycin resistance in *S. aureus* is phenotypically related to accelerated synthesis of cell wall components, leading to thinning of the cell wall that, in theory, could be capable of trapping large amounts of vancomycin and protecting the target site [156]. The development of linezolid resistance in *S. aureus* and enterococcal clinical strains, on the other hand, involves mutations in one of the multiple 23S rRNA copies (drug target), followed by homologous recombination between the remaining 23S rRNA copies of the gene in a so-called gene conversion process. In addition to linezolid resistance, the gene conversion has also been implicated in the development of macrolide resistance of *Staphylococci*, which, like linezolid resistance, appears to be the result of changes in rRNA operons [156–159].

The most renowned example of antibiotic resistance due to target bypass is vancomycin resistance. The mechanism here

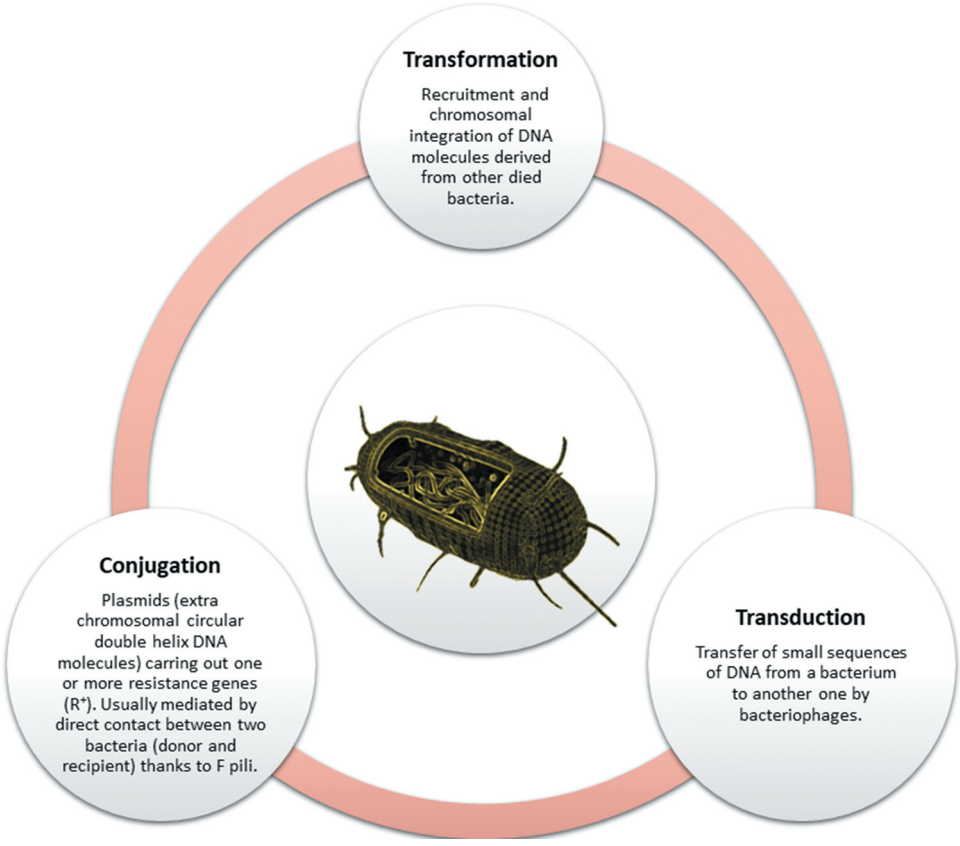

Figure 7. Modalities for exogenous genetic material entry in bacteria.

depends on the replacement of the D-Ala-D-Ala site at the end of the pentapeptide where vancomycin binds, by an ester structure, D-Ala-D-lactic acid, which does not bind to vancomycin. The production of this altered structure requires the participation of many importers' genes [160]. Vancomycin resistance is prevalent in *Enterococci*, common in our intestinal tract. Because *Enterococci* are naturally resistant to β-lactams, aminoglycosides, macrolides, and tetracyclines, these vancomycin-resistant *Enterococci* have become prevalent in the hospital setting, colonizing patients, and causing infections that are not easily treated [161].

5.2. How bacterial resistance to antibiotics is acquired

While in several cases described above bacterial resistance is acquired to one or at most two groups of antimicrobials, which are usually chemically related, in some other cases multi-drug resistant strains are created, making them particularly difficult to treat [2]. Among the defensive modalities reported by bacteria, some differences in the function of the bacterial cell may result in the acquisition of resistance to few antibiotics and/or multi-resistance to several classes of distinct antibiotics. As mentioned, there are basically two ways of occurrence: a) mutagenesis, although this is a less frequent mechanism b) horizontal transfer of genetic material (HGT, Horizontal Gene Transfer). In this last case, genes of the host bacterium may undergo a spontaneous mutation, or genes may belong to bacteria producing the same antibiotic substances and therefore they have self-defense mechanisms, or finally genes may belong to soil bacteria possessing inherited resistance [2,131–135]. Resistance mechanisms may depend on mutations in bacterial chromosomal genes encoding antibiotic susceptibility, or on the transfer of extrachromosomal elements, such as plasmids, integrons, and transposons. Data can be transferred between bacteria via conjugation (cell-to-cell contact), transduction (bacteriophage insertion), or transformation (naked DNA uptake). Transfer of genes occurs when bacterial cells are near (as in the intestinal tract, in starter cultures, in fermented foods, etc.) [2].

Plasmids are small extrachromosomal DNA fragments that multiply independently from the chromosomal material and can be transferred both intracellularly (i.e. integrated into the bacterial chromosome), but also from cell to cell, in this case imparting properties to a bacterial cell that did not possess them previously. This phenomenon, called conjugation, is mainly responsible for HGT and is directly involved in the development of resistance to many antibiotics [136–139]. This is particularly problematic, since plasmids can overcome barriers between species and genera, and the proportion of plasmid transport seems to increase proportionally in more heterogeneous communities. The dose of antibiotics and the interval between treatments are likely to affect the development of plasmid-transmitted resistance [2,140,141]. Transposons are pieces of genetic material that contain a gene or a small group of genes that can move both intracellularly and between

neighboring cells. Transposons have been considered among the main mechanisms to confer the *S. aureus* resistance to tetracyclines via the following mechanisms: a) protection of ribosomal RNA, b) increased effluent pump activity, and c) enzymatic inactivation of the antibiotic [142]. Indeed, the resistance genes encoding these mechanisms have been found in transposons, in conjugate plasmids or conjugate transducers. A class of conjugate transducers can self-transport from *Streptococci* to a variety of Gram-positive and Gram-positive negative bacteria. The predominant gene in *Staphylococci*, which encodes the active outflow of tetracycline, is TetA (K) and is located on a plasmid [143]. Finally, integrons consist of a gene encoding an integrase (that contributes to the process of integration into a DNA molecule) and a group of genes that make up the gene cassette. An example is the integron of *Salmonella enteric*, that leads to multidrug resistance. Thus, bacterial multidrug resistance results from the accumulation, in plasmids or transposons, of genes, each of which encodes resistance to a specific agent and/or by the action of multipurpose effluent pumps, each of which can pump out more than one type of drug [144].

5.3. Sources of resistance genes

Flexibility in the exchange of genetic information between different organisms is fundamental to promote biodiversity and biological innovations, it contributes to a correct adaptation to the changing environment and allows subsequent optimal physiological performance of the host. However, exchange of genetic material can cause both beneficial and/or adverse biological consequences, and it represents one of the most important mechanisms for antimicrobial resistance to antibiotics. In most cases, resistance genes come from organisms that produce antibiotics or by microorganisms of the environment (especially of the soil). Indeed, some of the genes conferring resistance to aminoglycoside derive from *Streptomycetes*, that naturally produce similar antibiotic substances. Genes that encode resistance to vancomycin appear to have a similar origin. In this last case, resistance requires the production of several new enzymes, and it seems unlikely that the genes encoding these enzymes evolved in the few decades following the discovery of vancomycin [162]. Indeed, the genes in vancomycin-resistant *Enterococci* are homologous and organized in the same way as those found in vancomycin-producing *Streptomycetes*. Moreover, some resistance genes have been discovered in the chromosome of environmental bacteria, such as the ampC gene in the environmental genus of *Enterobacteriaceae* (*Enterobacter, Proteus, and Serratia*), as well as in the soil *P. aeruginosa*. Since none of these genes show signs of being introduced into the recent past [163,164], is reasonable to infer that transfer of these genes may have occurred naturally. Nonetheless, the rising pressure of antimicrobial use remains the most likely explanation for the accelerated transferring of multiple antibiotic-resistant genes among bacteria strains.

5.4. The current issue of the resistant strains

The multidrug resistance phenomenon implies that some bacteria strains may show resistance to all commonly available antimicrobial agents [164]. Unfortunately, the list of pathogens showing low sensitivity or full resistance to most antibiotics currently available is growing worldwide. AMR strains of *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter species* (globally defined as ESKAPE pathogens) represent a universal threat to human health, and since 2017, according to its published list, the WHO designated ESKAPE pathogens as a 'priority status' to solicit and guide research of potentially effective new antibiotics [165,166].

Among the first to be isolated, the MRSA appearance in Intensive Care Units has greatly impaired the successful treatment of those patients. The genes that control antibiotic resistance are usually in transposons, which confer resistance not only to methicillin (developed to treat penicillinase-producing *S. aureus*) but also to aminoglycosides, macrolides, tetracycline, chloramphenicol, and lincosamides [165–169]. Since these dreadful strains are also resistant to disinfectants, they may also become a major source of nosocomial infections. Similarly, to treat infections from vancomycin-resistant (VRSA) strains, drugs such as linezolid and quinopristine/dalfopristine have been developed, but their initial effectiveness has been progressively diminished [167]. For some Gram-positive bacteria, the transfer of resistance genes takes place via plasmids, and resistance may be reinforced by a combination of changes in the outer membrane barrier and in the potentiating mechanisms of drug efflux [167]. The spread of *Enterococci*, known for their intrinsic resistance to vancomycin, might have been facilitated by the inappropriate use of antibiotics in veterinary practice in past years [170]. Indeed, survival and multiplication of vancomycin-resistant strains of *E. faecium* (derived from birds) and virginiamycin-resistant strains *E. faecium* (derived from pigs) has been observed in the human intestinal tract [171–173]. Antibiotic-resistance to some strains of *Listeria* spp. seems to result from transferring of genes via plasmids and transposons [174].

The resistance of *Streptococci* and *Enterococci* to several classes of antimicrobials is increasing worldwide [175]. Reduced sensitivity or durability to β-lactams has been reported with alarming frequency for *S. pneumoniae* and *S. viridans*. Resistance to macrolides and fluoroquinolones has been described in *S. viridans* and *β-hemolytic Streptococcus*. Glycopeptide resistance is a growing threat among enterococcus species, especially *E. faecium* and *E. faecalis* [176, -177].

Efficacy of cephalosporin- and carbapenem-class antibiotics for serious infections caused by Enterobacterales, such as *Klebsiella pneumoniae*, has been compromised by the widespread acquisition of genes encoding enzymes, such as extended-spectrum β-lactamases (ESBLs) and carbapenemases (CRE), which mediate the respective resistance to these critical drugs [178]. Since the first observation of carbapenem-resistant *K. pneumoniae* (CRKP) strains in 2001 in the U.S., genes encoding these β-lactamases have spread among other Gram-negative bacterial species in Europe, in locations

across the Indian Ocean rim, and in China, demonstrating extremely high transmission rates in health-care settings [178].

The spread of MDR and carbapenem-resistant *A. baumannii* (CRAB) isolates in Europe as well as in North America, Central, and South America underlines the emergence of pandrug-resistant strains toward which last-resort drugs such as carbapenems and polymyxins are no longer effective,

The Gram-negative opportunistic human pathogen *P. aeruginosa* is intrinsically resistant to a wide range of antimicrobial agents, allowing the pathogen to chronically persist in the host and evade antibiotic treatment. The current observation of strains resistant to multiple classes of antibiotics explains how pulmonary infections by *P. aeruginosa*, already responsible for 10% of all nosocomial infections, have become a common cause of community-acquired infections.

Since the clinical strains of all previously mentioned bacteria species are responsible for a variety of severe infections (including sepsis, endocarditis, periodontitis, gastritis, meningitis), rapid and accurate identification, and control of antimicrobial susceptibility are particularly relevant. For these bacteria, the evaluation of antibiotic susceptibility by disk diffusion methods is questionable, as the technique does not provide quantitative values [179–186]. In this context, the International Organization of the Clinical and Laboratory Standards Institute (CLSI) in USA has established that strains of *S. pneumoniae* should not be simply reported as penicillin-resistant or only moderately susceptible, and instead the minimum inhibitory concentration (MIC) values for penicillin and cefotaxime/ceftriaxone or meropenem are mandatory [187,188]. Proper detection of vancomycin-resistant *Enterococci* is also critical and the MIC values may provide valuable information to distinguish *Enterococcus* spp. carrying various vancomycin (van)-type determinants [189,190]. Available data suggest that the infections by *S. aureus* strains whose vancomycin MICs are ≥ 4 μg/ml are not sensitive to vancomycin therapy. Patients may not show any clinical improvement under this treatment, especially if they carry permanent catheters or when the source of infection has not been recognized. According to the CLSI, breakpoints for vancomycin treatment for *S. aureus* infections should consider vancomycin-susceptible *S. aureus* (VSSA) if the MIC values are ≤2 μg/ml, vancomycin-intermediate or moderate sensitivity *S. aureus* (VISA) when the MIC are 4–8 μg/ml and vancomycin-resistant *S. aureus* (VRSA) for MIC values ≥16 μg/ml [188]. Since many VISA isolates are also resistant to teicoplanin, the term glycopeptide intermediate *S. aureus* (GISA) has been considered the most appropriate in these cases; however, the acronyms VISA and GISA come from CLSI interpretative criteria. Thus, while the term GISA may be more specific for strains of moderate sensitivity to vancomycin and teicoplanin, it does not mean that all VISA strains are moderately sensitive to teicoplanin, and therefore the term VISA remains the most accurate and widely used term. Nowadays, VISA strains are characterized by a resistance mechanism that cannot be transferred to susceptible strains and is usually associated with vancomycin exposure. This implies that, in the absence of pressure from vancomycin, the probability of transmission and maintenance of the VISA phenotype is expected to be low. In contrast, VRSA strains are characterized by the expression of the vanA gene, obtained from an Enterococcus spp, suggesting that this kind of resistance can most likely be transmitted to susceptible strains and other organisms [191–193].

6. The study and choice of the suitable antibiotic: focus on novel molecules

As mentioned above, the emergence of MDR bacteria has been paralleled by a progressive decline in antibiotic development pipeline, accompanied in the last years by the universally recognized need to urgently fill this gap. In 2018, three new antibiotics with the potential to treat serious bacterial infections were approved by the U.S. FDA and the European Medicines Agency (E.U., EMA), and all included compounds targeting the 30S subunit of the bacterial ribosome: the aminoglycoside analog plazomicin, and the tetracycline analogs eravacycline and omadacycline [194–197]

In 2019, four additional antimicrobial drug therapies, all demonstrating efficacy against ESKAPE pathogens [198], were approved by FDA, EMA, or the Japanese PMDA Agency: imipenem-cilastatin-relebactam, lefamulin, lascufloxacin and cefiderocol. Imipenem-cilastatin-relebactam contains a novel β-lactamase inhibitor, relebactam, like the other group components avibactam and vaborbactam [199]. Lefamulin represents a new oral and intravenous treatment for pneumonia caused by CA-MRSA [200]. Lascufloxacin has been approved by the Japanese PMDA for the treatment of CA bacterial pneumonia caused by a range of bacterial pathogens, including quinolone-resistant *Staphylococcus* and *Klebsiella* spp [201]. Cefiderocol is a novel siderophore–cephalosporin conjugate approved for the treatment of infections caused by susceptible Gram-negative pathogens (i.e. CRE, CRAB, and carbapenem-resistant *P. aeruginosa*) [202]

All these recently approved drugs are molecular analogs of antibiotic classes with established mechanism of action. Thus, the possibility that novel MDR strains will emerge in parallel with their increasing clinical use is unfortunately very likely.

On the other hand, murepavadin is one of the few new antibiotics whose mechanism of action is different: this molecule selectively targets outer membrane proteins such as the protein transporter LptD, which mediates the transport of LPS (lipopolysaccharide) to the outer leaflet. Murepavadin has shown a potent activity against carbapenemase-producing and polymyxin-resistant *P. aeruginosa* strains. Brilacidin, as the prototypical drug of synthetic defensin mimetics, is currently undergoing a phase II clinical trial, for the treatment of *S. aureus*-mediated infections [203].

6.1. Side effects and antibiotic toxicity

When appropriately used, most antibiotics are relatively safe with few side effects. However, each class of drugs might elicit side effects with potentially severe and debilitating adverse consequences, based on their specific mechanism of action and/or unwanted interference with host targets.

Gastrointestinal disturbances (such as nausea, stomach upset, vomiting, flatulence, diarrhea, or malabsorption) are the most frequent side effects shared between antibiotic

classes, since they result from the drugs activity on 'innocent-by-stander' strains of the host microbiota. For this reason, the use of probiotics is nowadays considered a necessary adjuvant support under any antibiotic treatment, which may help reduce the long-term toxicity of antibiotics, facilitate the eubiosis recovery of the gut microbiota, and even prevent several infectious-related conditions [9].

Common side effects of antibiotics include allergic reactions, which may range from mild-to-moderate rash, skin, or vaginal itching to rhinitis, and to lips, facial, or tongue swelling (angioedema), occasionally becoming life-threatening if it affects breathing [204]. Although penicillins and cephalosporins are among the least toxic antibiotics, the incidence of allergies and rashes has been reported with a high incidence for these classes of β-lactams [205,206]

Specific class-related side effects include pro-arrhythmic disturbances from macrolides such as azithromycin and quinolones such as levofloxacin, which have been associated with cardiac arrhythmias and sudden death. Therefore, they should be avoided, or their use carefully considered in patients with cardiac rhythm disturbances [205]. Tetracyclines may induce kidney toxicity over time, and photosensitivity to ultraviolet radiation. They can also cause allergic reactions and should not be used during pregnancy or in children because they can interfere with calcium-rich tissues, including bones and cause tooth discoloration [207]. Aminoglycosides are well known for reversible nephrotoxicity and potentially irreversible ototoxicity at the vestibule (streptomycin and gentamicin), or at the cochlear level (amikacin, neomycin, and kanamycin) [208]. Fluoroquinolones are generally well tolerated but may induce nausea, vomiting, diarrhea, abdominal pain and headaches, confusion, dizziness, and convulsions. They tend to deposit in tendons and may increase vulnerability of these tissues under solicitation and should be avoided in aged patients, in subjects with epilepsy or during pregnancy [209].

In patients with infections sustained by ESKAPE bacteria, the compromised state of the patient, combined with the need for high-dose therapeutic regimens and with the innate toxicity of certain antibiotics, may help to explain the high rate of drug-induced adverse effects. For example, the nephrotoxic effects of last-resort antibiotics of the polymyxin-class (that should be used to treat infections sustained by carbapenem resistant ESKAPE pathogens) represent a severe limitation for treatment options. For these reasons, in addition to the development of novel drugs, the current research is also facing structural modification of available molecules that may significantly improve the safety and dose-limiting toxicity issues associated with some of the afore-mentioned antibiotics [198,209]

6.2. Laboratory investigations: the central role of the antimicrobial susceptibility tests

As previously described, each antibiotic is characterized by an antibacterial spectrum, which makes it possible to predict its effectiveness in specific infections. This prediction, of course, is feasible only for those infections sustained by bacteria uniformly and constantly sensitive to certain antibiotics (*Haemophilus influenzae, Salmonella*, etc.) [210]. Beyond other diagnostic techniques, the laboratory bacteriological examination is fundamental to identify the etiological agent, and the subsequent choice of suitable antibacterial drug. When the bacteriological exam highlights the presence of bacterial species with different sensitivity to various drugs (such as *Pseudomonas, Streptococci, Enterococci, Enterobacteria* of the *Coliform* or *Proteus* groups), the identification of each strain in each sample must be completed with the evaluation of their respective 'susceptibility' to different antibiotics. The same applies when the observed pathogen species are known to give rise to antibiotic-resistant mutants (as for *Staphylococcus* spp., or *Mycobacteria*) [210–212].

Antibiotic sensitivity tests are performed *in vitro*, measure the response (growth) of an isolated microorganism to one or more antibiotics, and must be carried out under standardized conditions (the technical implementation must meet perfectly standardized criteria periodically updated by various National Committees, CLSI, etc.) to guarantee the reproducibility of the results. Cumulative data from individual antibiotic susceptibility tests are used to create an antibiogram, whose aim is to predict appropriate empiric antimicrobial therapy prior to the availability of specific information on the patient's isolates and help the choice of the antibiotic together with clinical information and professional experience [212].

In clinical practice, this laboratory test has become essential not only to choose the most effective drug but also to provide an estimate of the most appropriate therapeutic dose for the successful treatment of the infectious disease. As previously outlined, treatment modalities might vary according to the geographic areas the patient comes from, and the likelihood that the pathogen might show high or low resistance (<15%) to the evaluated agent, or the potential presence of multi-drug-resistant strains [43,213,214].

An antibiogram screening can be performed on samples obtained from blood (must be performed promptly in positive samples), urines, CSF, biological material from the respiratory tract (sputum, bronchoalveolar lavage), bone or joint specimens, pleural effusions, or fluids from body cavities (when a bacterial etiology is suspected) [215,216]. Besides guiding the correct treatment in single patients, antibiogram can be useful for epidemiological purposes and help to identify hospital infections, which may be antibiotic resistant. On the other hand, results from antibiogram may be meaningless, for example, when the isolated microorganism can reasonably be excluded as responsible for an infection being instead a contaminant or a commensal population (e.g. oral *Streptococcus* spp. or *H. parainfluenzae* in a bronchial specimen), or when the number of reported CFU/ml is below a significant threshold in the respective fluid or secretion examined (e.g. urine or bronchial secretions). The second reason is that the isolated pathogen belongs to a species constantly sensitive to standard treatments, or for which there is no correlation between the *in vitro* and *in vivo* activity of the drug [216].

The most correct method to determine the efficacy of an antibiotic against a microorganism is to establish, for each antibacterial drug, the minimum inhibitory concentration (MIC), and the minimum bactericidal concentration (MBC).

This last measure allows to establish an antibiotic activity scale for different bacterial species. The MBC is defined as the lowest concentration of antibiotic capable of inhibiting bacterial growth by at least 99.9% of the initial population [217,218]. In this respect, the CLSI publishes the criteria for interpreting the results of sensitivity tests, by providing the categories (S-sensitivity, I-intermediate/increased rate sensitivity, and R-resistance) identified according to MIC values called breakpoints (threshold, limit). The standard sensitivity values vary for each organism and depend on the plasma concentration that can be reached with a drug without the appearance of toxic effects. The breakpoints for categorization (S, I, R) are therefore identified by CLSI and determined based on the pharmacokinetic and pharmacodynamic parameters of the single antibiotics, corresponding to the levels reached *in vivo* (in blood and tissues) and on respective clinical activity (correlation between *in vitro* results (MIC) and *in vivo* results, meaning resolution of the clinical case) (Table 4) [219].

Two methods are in place for the evaluation of antibiotic sensitivity in vitro: broth dilution (micro and macro-method) and agar diffusion disc (Kirby–Bauer method). In antibiotic-susceptibility dilution tests, the susceptibility of the microorganism is evaluated according to its growth in a culture medium (solid or liquid) containing different concentrations of the antibiotic. This is a precise quantitative method and allows to accurately determine both the MIC and the MBC values. However, being expensive and quite complicated (it requires the setting of antibiotic panels), its use is generally limited to those cases of serious diseases, in which the MBC evaluation is mandatory (for example, bacterial endocarditis, or osteomyelitis) or when sensitivity evaluation is required for slow-growing microorganisms (Mycobacteria and Actinomycetes) [220]. The diffusion in agar, instead, represents a qualitative-quantitative, simple, rapid, and cost-effective method, accurate and sustainable for fast growing aerobic microorganisms. Currently, the most widely used disc diffusion test is still the Kirby–Bauer method, developed in the early 1960s. Flexibility in the choice of antibiotics and straightforward accomplishment are among the advantages of this standardized technique, which might provide a correlation between *in vitro* results (antibiogram) and clinical resolution (*in vivo* results). The disadvantages and limitations include the unfeasible adoption of fully automated procedures and the qualitative nature of results (sensitivity categories). The epsilon or epsilometer (E)-test is a quantitative method for MIC determination. It consists of 5 × 50 mm plastic strips containing a gradient corresponding to 15 doubling concentrations of a given antibiotic (in mg/ml). According to the different antibiotic used, the range may vary between 0.00025–4, 0.002–32, 0.016–256, 0.032–512, and 0.064–1024. The full plate seeding technique, similar to the Kirby-Bauer diffusion antibiogram, requires the placement of antibiotic strips on the surface of the inoculated plate and subsequent incubation at 37°C for 18–24 hours. The MIC value is read for bacterial growth at the intersection of the strips [221].

Another parameter that can be used to evaluate antibiotic therapy efficacy *in vitro* is the Mutant Prevention Concentration (MPC, the concentration of the drug that blocks the growth of mutant cells), which determines the antimicrobial concentration needed to block the growth of the least susceptible cell in the sample, and is independent of the resistance mechanisms. According to Hesje et al., the MPC could provide further information on the real dynamics of bacterial populations exposed to certain antimicrobial compounds. Furthermore, MPC estimates the concentration of mutants and not that of mutations, and it is similar to a MIC test that uses a substantially higher bacterial inoculum [222].

Finally, the susceptibility test may have a potential problem for the definition of susceptibility/resistance in certain cases such as for cefazolin and methicillin-resistant *S. aureus* (MRSA), but there is not yet an optimal remedy for therapy. This is because the susceptibility results were substantially different for these cefazolin-degrading strains [223].

These susceptibility (and therefore resistance) tests are performed *in vitro*, which is an important limitation because they cannot override *in vivo* factors (e.g. pharmacodynamics and pharmacokinetics, site-specific drug concentrations, host immune status, site-specific host defenses) that influence the success of the treatment. Thus, the results of *in vitro* susceptibility tests do not always predict the outcome of the treatment and the clinical course of the patient [222].

Table 4. The breakpoints categorization identified by CLSI – Clinical and Laboratory Standards Institute (formerly NCCLS – National Committee for Clinical Laboratory Standards).

BREAKPOINTS		
S	**I**	**R**
The infection caused by that strain can be adequately treated with the dosage of an antimicrobial agent commonly recommended for that type of infection.	The bacterial growth is inhibited only at the maximum recommended dosage; bacterial isolates show MICs corresponding to serum and tissue levels of antibiotic for which efficacy may be lower than that recorded for susceptible isolates. Currently, has been proposed that an increased dose of the tested antibiotic can warrant the therapeutic effect.	Category that predicts the possible therapeutic failure of the antibiotic tested. The antibiotic should be used at dosages that would be toxic for the host as the strains are not inhibited using drug concentrations usually achievable with normal dosages.

7. Conclusive remarks

Antibiotics are among the most successful drugs developed and employed in the treatment of human and animal pathologies. From the empirical use of several natural substances to the increasing knowledge on mechanisms and properties characterizing modern drugs, antibiotics have undoubtedly contributed to the evolution and achievements of human civilization. Unfortunately, the extensive misuse of these – often life-saving – molecules has created the appropriate selective pressures for bacteria, whose resilience mechanisms have spread among species and represent nowadays a dreadful menace.

8. Expert opinion

In the present article, a panel of experts in medical microbiology, pharmacology and toxicology, intensive care, medical and dental care examined the historical development of antibiotics up to their discovery, underlying the most relevant biochemical aspects of antibiotics' mode of action and reviewing the biomolecular mechanisms conferring bacterial resistance to antibiotics.

Toward 2050, according to the WHO's alert for superbugs, the research for new and effective antimicrobial molecules must be strengthened, as well as antimicrobial stewardship programs.

In the current scenario of increasing AMR bacteria strains, the most recent accomplishments in the research and development of novel effective drugs, and the implementation of rapid and cogent diagnostic strategies are reviewed, to streamline a multidisciplinary approach to the management of patients with infectious diseases. This requires a shared effort from all health workers and patients to warrant to avoid the emergence of new and more aggressive harmful bacteria, difficult to control, as has happened in recent months for the unexpected COVID-19 pandemic.

8.1. What is the current strategy to prevent and control AMR-microorganism infections?

The WHO Access, Watch, Reserve (AWaRe) classification of antibiotics has been developed to reduce antimicrobial resistance. According to this list, antibiotics are classified into different groups to emphasize the importance of their appropriate use. Although how to select the most appropriate interventions for each setting remains challenging, antimicrobial stewardship programs may represent the core strategies to tackle AMR and rely on the shared effort of several health workers. These programs aim to:

- overcome clinician knowledge deficits regarding the optimal use of antibiotics;
- improve access to reliable clinical diagnostic or microbiologic testing;
- guarantee reliable access to quality-assured antimicrobials;
- reassure on the possibility that withholding or delaying antibiotics not always leads to poor outcomes;
- improve communication between health-care providers, and allow access to antimicrobial prescribing trends, as well as to data regarding the prevalence of AMR in the community;
- warning on risks of public access, without prescriptions, to antibiotics.

8.2. What is the best approach for a correct use of currently available antibiotics?

Antimicrobial therapy should be performed with molecules that target pathogenic microorganisms showing sensitivity to the agent administered. In general, the correct choice of the appropriate antibiotic agent must consider five basic criteria: effectiveness, appropriateness, cost, ease of use, and avoidance of side effects. Host factors like age, physiological state (e.g. pregnancy and lactation), organ function (e.g. renal or hepatic function), genetic variation (e.g. G6PD deficiency), allergy, or intolerance must be considered while prescribing antimicrobial therapy. Clinicians should keep in mind that drugs indicated as 'first choice treatment' represent the best option in terms of effectiveness, harms, and potential for resistance. This implies that, under specific circumstances, a second-choice antibiotic would be the right alternative. Nevertheless, in general, these broader-spectrum antibiotics display a higher resistance potential or less favorable risk–benefit ratios.

8.3. Is there any difference in terms of efficacy between access, watch and reserve antibiotics?

The AWaRe classification of antibiotics is based on their impact on antibiotic resistance and need for surveillance, and is not based on differences in clinical effectiveness. Thus, antibiotics in the Access group remain the 'strongest,' most effective antibiotics for many infections and should be preferred whenever possible, according to the appropriate protocol steps described above.

8.4. When is empirical antibiotic therapy still appropriate?

While definitive therapy depends on the microbiologic diagnosis by isolation, empirical therapy should be based on a clinical diagnosis combined with literature evidence and physician experience. Empirical use of antibiotics should be justified in patients with life threatening infections, in ICU settings and while awaiting results of culture. To optimize an accurate microbiological diagnosis, clinicians should ensure that properly obtained specimens are promptly submitted to the microbiology laboratory. Antibiotics work by eliminating the majority of bacteria while allowing the immune system to handle the remaining germs. Besides choosing the right antibiotics (based on their activity spectrum and mode of action), the proper duration of the correct antibiotic therapy is a priority, since not finishing the full course increases the likelihood of recurrence, and also promotes the development of drug resistance. This is particularly relevant when considering the bactericidal or bacteriostatic nature of the antimicrobial agent used. Concomitantly, factors affecting antibiotic activity such as poor bioavailability for incorrect route of administration, renal excretion, other drugs' interactions, and allergy must be considered before prescribing the chosen antibiotic, to avoid or limit long-term toxicities in specific patients. Therefore, antibiotic therapy should be reevaluated in order to escalate or deescalate doses according to the efficacy achieved and to increased risk of side effects.

8.5. What is the research perspective for novel antimicrobial molecules?

The urge for novel and effective antibiotic agents is particularly relevant for infections sustained by AMR pathogens. Indeed, among the few new molecules approved, the β-lactam/β-lactam inhibitor combinations target ESBL-producing, or *K. pneumoniae* carbapenemase (KPC) and oxacillinase-48 (OXA-48)-producing *Enterobacteriaceae*. There are only two agents (cefiderocol and durlobactam [ETX-2514] + sulbactam) that are active against MDR *A. baumannii* and one (cefiderocol) that is active against MDR *P. aeruginosa*. Unfortunately, the lack of differentiation against existing treatments, their non-inclusion in clinical guidelines, and their higher prices in comparison to existing generic treatments make it difficult to predict the place in the treatment landscape for these newly approved products. Moreover, since the majority of these novel drugs belong to existing classes where multiple resistance mechanisms are well established, the possibility of fast emergence of resistance should be considered.

In recent years, some biological products, comprising monoclonal and polyclonal antibodies, and phage endolysins have been tested, but only bezlotoxumab (that targets *C. difficile toxins*) is currently approved. Although all these products target new structures through new modes of action and should therefore be considered innovative, their potential use for single-agent therapy remains to be proven.

Author contributions

All the authors equally contributed for conceptualization, writing and final approval of the manuscript. L Bottalico and I Alexandros Charitos collected, analyzed, and discussed the historical features discussed here, MA Potenza and M Montagnani accounted for the pharmacological and biochemical data, and L Santacroce for the microbiological insights. M Montagnani and L Santacroce provided resources and financial support for this study.

Declaration of interest

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