Journal of Infection and Public Health 14 (2021) 1750–1766

Contents lists available at ScienceDirect

Journal of Infection and Public Health

journal homepage: http://www.elsevier.com/locate/jiph

Review

Antibiotic resistance in microbes: History, mechanisms, therapeutic strategies and future prospects

Tanvir Mahtab Uddin[a,†], Arka Jyoti Chakraborty[a,†], Ameer Khusro[b], BM Redwan Matin Zidan[a], Saikat Mitra[a], Talha Bin Emran[c,*], Kuldeep Dhama[d], Md. Kamal Hossain Ripon[e], Mário Gajdács[f,*], Muhammad Umar Khayam Sahibzada[g], Md. Jamal Hossain[h], Niranjan Koirala[i]

[a] Department of Pharmacy, Faculty of Pharmacy, University of Dhaka, Dhaka 1000, Bangladesh
[b] Research Department of Plant Biology and Biotechnology, Loyola College, Nungambakkam, Chennai, Tamil Nadu, India
[c] Department of Pharmacy, BGC Trust University Bangladesh, Chittagong 4381, Bangladesh
[d] Division of Pathology, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly 243122, Uttar Pradesh, India
[e] Department of Pharmacy, Mawlana Bhashani Science and Technology University, Santosh, Tangail 1902, Bangladesh
[f] Department of Oral Biology and Experimental Dental Research, Faculty of Dentistry, University of Szeged, 6720 Szeged, Hungary
[g] Department of Pharmacy, Sarhad University of Science and Information Technology, Peshawar, Pakistan
[h] Department of Pharmacy, State University of Bangladesh, 77 Satmasjid Road, Dhanmondi, Dhaka 1205, Bangladesh
[i] Department of Natural Products Research, Dr. Koirala Research Institute for Biotechnology and Biodiversity, Kathmandu 44600, Nepal

ARTICLE INFO

Article history:
Received 23 August 2021
Received in revised form 4 October 2021
Accepted 14 October 2021

Keywords:
Antibiotics
Antimicrobial resistance
Mode of action
Multidrug resistant bacteria
MDR
Preventive strategies

ABSTRACT

eu estou testando!!

Antibiotics have been used to cure bacterial infections for more than 70 years, and these low-molecular-weight bioactive agents have also been used for a variety of other medicinal applications. In the battle against microbes, antibiotics have certainly been a blessing to human civilization by saving millions of lives. Globally, infections caused by multidrug-resistant (MDR) bacteria are on the rise. Antibiotics are being used to combat diversified bacterial infections. Synthetic biology techniques, in combination with molecular, functional genomic, and metagenomic studies of bacteria, plants, and even marine invertebrates are aimed at unlocking the world's natural products faster than previous methods of antibiotic discovery. There are currently only few viable remedies, potential preventive techniques, and a limited number of antibiotics, thereby necessitating the discovery of innovative medicinal approaches and antimicrobial therapies. MDR is also facilitated by biofilms, which makes infection control more complex. In this review, we have spotlighted comprehensively various aspects of antibiotics viz. overview of antibiotics era, mode of actions of antibiotics, development and mechanisms of antibiotic resistance in bacteria, and future strategies to fight the emerging antimicrobial resistant threat.

Contents

* Corresponding authors.
E-mail addresses: tmahtab615@gmail.com (T.M. Uddin), arkwcky@gmail.com (A.J. Chakraborty), armankhan0301@gmail.com (A. Khusro), zidan.redwan88@gmail.com (B.R.M. Zidan), saikatmitradu@gmail.com (S. Mitra), talhabmb@bgctub.ac.bd (T.B. Emran), kdhama@rediffmail.com (K. Dhama), md.kh.ripon@gmail.com (Md.K.H. Ripon), gajdacs.mario@stoma.szote.u-szeged.hu (M. Gajdács), umar.sahibzada@gmail.com (M.U.K. Sahibzada), jamal.du.p48@gmail.com (Md.J. Hossain), koirala.biochem@gmail.com (N. Koirala).
† The authors contributed equally to this work.

https://doi.org/10.1016/j.jiph.2021.10.020

Introduction

Antibiotics are the most significant class of pharmaceuticals and are one of the most influential medical inventions of the twentieth century. Antibiotics have undeniably been a boon to human society in the fight against bacteria, saving millions of lives [1]. However, the number of infections caused by multidrug-resistant (MDR) bacteria is increasing across the world, and the threat of untreatable infections has been looming since the beginning of the 21st century [2]. While antibiotics have allowed the development of several fields of medical practice, including the effective results of several surgical operations and immunosuppressive therapies that rely on antibiotic prophylaxis, and the potential to manage infectious complications, antimicrobial resistance (AMR) presents a significant challenge to all healthcare systems worldwide [3]. As all organisms evolve genetic mutations to prevent lethal selection pressure, AMR is an unavoidable evolutionary result. Bacteria will tend to develop and use resistance strategies as long as antibacterial drugs are used against them (i.e. selection pressure is present in their environment).

According to World Health Organization (WHO) report of 2019, AMR is responsible for the deaths of 700,000 people, while it's estimated that by 2050 the figure will have risen to 20 million, costing over $ 2.9 trillion [4]. As a result, it has become a major problem, posing a serious danger to our lives and economy. In addition to the high expense of antibiotic research and growth, the accelerated evolution of AMR has resulted in lower investment returns for the pharmaceutical R&D industry. Several pharmaceutical companies have already abandoned antibiotic research as well as development of new antibiotics [5]. Though the scenario is grim, a range of new technologies have the ability to change things for the better. There are also a number of scientific advancements that have the potential to aid in the exploration and growth of new antibiotics.

Synthetic biology methods, along with genetic, functional genomic, and metagenomics studies of bacteria, animals, and even aquatic invertebrates, aim to unlock the world's natural products faster than antibiotic discovery [6]. Different therapeutic and preventive approaches viz. bacteriophages [7], monoclonal antibodies [8], and vaccines [9] are used for combating bacterial infections. Novel approaches like 'Defense Advanced Research Projects Agency bionic spleen' [10] provides a potential substitute of treatment. Orthodox synthetic organic and pharmaceutical chemistry remain important instruments in the fight against antimicrobial resistance, in addition to these modern methods [11]. Advancing regulatory initiatives have the potential to prevent the post-antibiotic era from being a reality [6].

This review demonstrates a brief history of antibiotics, global strategies regarding this threat, mechanism of action of antibiotics as well as antibiotic resistance to the readers. Moreover, this review aims to provide a crystal-clear idea about the therapeutic strategies that are being adopted in these days and future research scopes on this particular topic. In light of this, it's critical that proper antibiotic prescription and innovative techniques emerge in order to promote rational therapy and avoid the unintended effects of AMR. This study focuses on the scope and effects of AMR, as well as the relevance and implications of treatments in combating resistance and protecting world health.

Antibiotics: past, present and future

Pre-antibiotic era

During the pre-antibiotic period, the understanding of microbes and infectious diseases were inadequate. The treatment processes and prevention of the transmission of these contagious diseases were futile, which frequently approached epidemic levels, resulting in the death of millions of people [12]. To understand the catastrophic condition of people in the pre-antibiotic period, the outbreak of 'Plague' may be taken as an example. It is caused by *Yersinia pestis,* which was transmitted via infected animal fleas, and was responsible for a number of pandemics throughout history [13], including, the 'Justinian plague' which killed nearly 100 million people [5], the 'Black Death' in the fourteenth century caused over fifty million deaths in Europe [14], and the outbreak between 1895 and 1930 resulted in about 12 million infections. However, plague can be easily treated using antibiotics [15].

In 1676, through the discovery of microscopic living organisms or 'animalcules', Antonie van Leeuwenhoek planted the seeds for the development of antibiotics [16]. In 1871, Joseph Lister discovered that *Penicillium glaucum* has inhibitory effects on bacterial growth, which enabled him to treat a nurse's injury with *P. glaucum* extract and this knowledge sparked the idea that bacteria is responsible for infection [17]. In the second half of 19th century, French bacteriologist Louis Pasteur and German physician Robert Koch independently conducted studies on bacteria. Louis Pasteur worked on *Bacillus anthracis*, while Robert Koch studied *Mycobacterium tuberculosis* and identified a co-relationship between individual species of bacteria and disease. Observations by these two pivotal microbiologists have pushed microbiology and antibiotic development towards its modern era [18].

Early era of antibiotics

The first antibiotic, mycophenolic acid, which was discovered in 1893, by Italian microbiologist Bartolomeo Gosio, was isolated from *P. glaucum*, inhibits the growth of *Bacillus anthracis* [5]. In 1909, Paul Ehrlich and his collaborators discovered Salvarsan (arsphenamine), the first synthetic arsenic-derived antibiotic, which is effective against *Treponema pallidum* [19], the causative pathogen of Syphilis. Neosalvarsan, which was less dangerous and more effective in the treatment of Syphilis than its precursor (Salvarsan), was introduced in 1913 [8]. As both of these drugs had elevated risk factors due to the presence of arsenic, they were overtaken by Prontosil, a broad-spectrum antibacterial sulfonamide (sulfamidochrysoidin) drug discovered by a German bacteriologist Gerhard Domagk in 1930, and was primarily used in the treatment of injured soldiers during World War I. This discovery set another landmark in antibiotic research history [7]. Prontosil exerted bacteriostatic role against different groups of bacteria by inhibiting dihydropteroate synthetase (DHPS) enzyme in folic acid pathway which eventually blocked bacterial nucleic acid synthesis [20], but sulfonamides were eventually superseded by penicillin, as bacteria became resistant to Prontosil [19] due to the occurrence of mutations in the DHPS enzyme [21]. In 1928, Scottish bacteriologist Alexander Flem-

ing inadvertently discovered that a fungus (*Penicillium notatum*) inhibited the development of the colonies of *Staphylococcus aureus*. He postulated that fungus must have excreted a compound that inhibited the bacteria, and in 1929, he was able to isolate the active molecule and named it 'penicillin', the first true antibiotic. However, it was the work of Howard Walter Florey and Ernst Boris Chain who elucidated the structure of penicillin G (the first penicillin to be used in bacterial infection) in 1939 and were able to efficiently purify the antibiotic and scale up the production [22]. The advent of penicillin in treatment in 1945 was the next major breakthrough in antibiotic discovery [23]. The structure of penicillin was elucidated through X-ray crystallographic analysis by Dorothy Crowfoot Hodgkin in the same year, allowing it to be classified as the first member of the β-lactam family of naturally occurring antibiotics [19].

Penicillin, cephalosporins, monobactams, and carbapenems belong to the same β-lactam antibiotic class, because they all contain a β-lactam ring and have a common bactericidal mechanism of action. β-lactam antibiotics inhibit the biosynthesis of cell walls of Gram-positive bacteria [24]. However, certain Gram-negative bacteria, such as *Escherichia coli* and *Klebsiella* spp. can produce β-lactamases enzymes which destroy the drug's β-lactam ring, rendering the bacteria resistant to it [25]. The semi-synthetic derivatives of penicillin including, methicillin, oxacillin, ampicillin, and carbenicillin showed broad spectrum activities against several Gram-positive (*S. aureus, Enterococcus faecalis*) and Gram-negative bacteria *(Haemophilus influenzae*, *E. coli*, and *Proteus mirabilis*). Regardless of the fact that ampicillin is still used in medicine, staphylococci became resistant to methicillin in 1961 and is no longer used in clinical practice [23]. Methicillin-resistant *S. aureus* (MRSA) later was described as the first "superbug" in history.

Golden era of antibiotics

In 1939, French microbiologist René Dubos opened a new chapter on antibiotic discovery when he isolated tyrothricin (a mixture of gramicidin D and tyrocidine) from the soil bacteria *Bacillus brevis*, which effectively inhibited Gram-positive bacteria. However, gramicidin showed high toxicity in humans (and it is currently only used in topical applications) [5]. In 1940s, Selman Waksman conducted a systematic study of the antimicrobial behavior of soil bacteria, especially *Streptomyces* spp. He created the Waksman-framework to showcase bacterial species with antagonistic relationships. He discovered many major antibiotics and antifungals using his platform, including actinomycin (derived from *Streptomyces* spp.), neomycin (derived from *Streptomyces fradiae*), streptomycin (derived from *Streptomyces griseus*), clavacin (derived from *Aspergillus clavatus*), and fumigacin (derived from *Aspergillus fumigatus*) [1]. Waksman's work started the Glorified Era of antibiotic discovery between the 1940s and 1970s [17]. Most of the antibiotics including actinomycin, streptomycin, and neomycin are still in clinical use today [20]. More than 20 antibiotic classes from hundreds of bacterial species and fungi were discovered during that golden period [18]. Following Waksman's platform's culture strategy, several pharmaceutical firms began to use rational screens for the development of new molecules relying on information of antibiotics' established mechanisms of action [16,19]. Unfortunately, only a few new antibiotic groups have been detected: nitrofuran in 1953, macrolides in 1952, tetracyclines in 1948, quinolones in 1960; and oxazolidinones in 1987, and no new classes have been found for the last 50 years [17,18]. The quick and relatively basic development of several types of antibiotics within a brief period of time resulted in their overuse. This along with a stalled antibiotic research pipeline dating back to the 1970s, has resulted in the present condition with few new antibiotics in clinical trials. About 1200 antimicrobial peptides (AMPs) have been discovered from diverse sources since the 1980s, ranging from plants to invertebrates and mammals, but none could have been used as antibiotics [17]. To sum up, the bulk of antibiotics were developed during the golden era of antibiotic research, and following this period, mainly derivatives of already existing agents were marketed [1].

Present situation

At present, antibiotics are being developed in small numbers [26], and only 5 of the 20 pharmaceutical firms that participated in antibiotic exploration in the 1980s are still active today, and the majority of the big pharmaceutical companies have now abandoned the area of antibiotic discovery; this responsibility has been since taken up by smaller, start-ups, and biotechnology firms [18]. According to a database from 2018, only 2 of the 45 new antibiotic candidates in clinical trials for the US market belonged to major pharmaceutical firms, with the majority being undertaken by research laboratories and small to medium sized companies [19]. Resistance towards antibiotics is the prime cause of this issue. In the early days of antibiotic use, resistant bacteria existed as well, but a constant stream of experimental antibiotics offered alternate remedies, and it was simple switch treatment once resistance against a specific antibiotic was developed [3]. However, antibiotics ceased arriving in larger numbers in the 1980s. The last time a new antibiotic class was discovered and brought to market was in 1987, and the last group of broad-spectrum agents (i.e. the fluoroquinolones) were discovered in the 1980s as well [17]. Since then, there has been a dearth of creativity in the area, and there are currently only a few new antibiotic groups in development that are capable of combating current levels of AMR [27].

In addition, companies are facing financial and regulatory challenges as a result of this resistance, and a lack of understanding of how to produce antibiotics against these resistant bacteria has resulted in a large investment in science, which has now caused pharmaceutical companies to decrease and/or abandon antibiotic development [28], according to experts we are approaching towards a 'post antibiotic era'. On the other hand, antibiotic innovation is steadily increasing again. Antibiotic discovery and disease diagnostics methods have received scaled-up funding in recent years. New antibiotics and diagnostics are being developed by collaborations between universities and pharmaceutical companies e.g., New Drugs 4 Bad Bugs: ENABLE and CARB-X [11,29]. Alternatives to antibiotics, such as bacteriophages (viruses that destroy bacteria) and antimicrobial peptides are still being investigated [11]. Despite the importance of these methods, their applications have certain drawbacks, and they are yet to be translated into medical devices. Nevertheless, they may be a useful addition to complement and/or antibiotics.

Future approaches

Various novel approaches, based on a re-conceptualization of the dynamics of resistance, illness, and prevention are being explored by scientists [6,30,31]. Whole genome sequencing (WGS) is one of the methods, and has been a crucial method for drug discovery because it allows for the fast detection of resistance pathways and the regulation of bacterial resistance [32]. Another promising technique is the newly discovered quorum-quenching (QQ) method, which works by interacting with microbial cell-to-cell contact to prevent bacterial infections [33]. Bacteriophages, also known as viral phage therapy, have lately gained popularity because they are more effective than antibiotics, as they are harmless to the host organisms, including gut flora, thus lowering the risks of opportunistic infections [7]. Phages have been actively used to treat bacterial infections even before the boom

of antibiotics – especially in Russia and Georgia – and currently are being re-discovered as a promising alternative [32]. Humanized monoclonal antibodies are the fastest expanding community of biotechnology-derived molecules in clinical trials, thanks to rapid advances in genetic sequencing. Injections of monoclonal antibodies or white blood cells that target bacteria carry promise for treating pathogens, despite their high cost [34]. Furthermore, a group of scientists used X-ray crystallography to obtain the 3D structures of ribosomal fragments from *Staphylococcus aureus*, which disclosed the unique structural patterns specific to this bacterial strains that could be used to design environmentally friendly novel degradable pathogen-specific drugs [30].

Global reports on antibiotic resistance

Antibiotics are known as the "wonder medicines" for fighting bacteria [35], and their development and eventual therapeutic application are the miracle in medical history [4]. Antibiotics have been employed for decades not just for medicinal uses, but also as a preventative measure in a variety of fields, including animal husbandry and agriculture [2]. AMR refers to bacteria and other microorganisms' capacity to withstand the impact of an antibiotic to which they were previously susceptible, allowing germs to survive and thrive [35]. AMR is an inevitable phenomenon because microbes develop genetic mutations to mitigate its lethal effect [36]. Resistance was initially observed in staphylococci, streptococci and gonococci; after the very first commercial antibiotic, penicillin was introduced in market in 1941, and penicillin-resistant *S. aureus* emerged just a year later, in 1942 [3]. Again, methicillin, a penicillin related semi-synthetic antibiotic which was introduced in the market in 1960 to combat penicillin resistant *S. aureus* became resistant to methicillin the very same year [17]. AMR has been a major source of concern over the years, as it takes no time for an antibiotic to establish resistance, and with more than 70% of pathogenic bacteria being resistant to at least one antibiotic [37], it has now become one of the most serious challenges to public health, food protection, and sustainable healthcare.

According to Center for Disease Control (CDC's) Antibiotic Resistance Threats Report 2019 in the United States, more than 2.8 million antibiotic-resistant infections arise in the United States per year, resulting in more than 35,000 deaths [38]. According to a report, in India, a child dies from an antibiotic resistant bacterial infection in every 9 min, and more than 50,000 newborns are likely to die from sepsis because of microbes being resistant to common antibiotics in India [36]. According to the report of European Antimicrobial Resistance Surveillance Network (EARS-Net) between 2015–2019 [39], there were alterations in the frequency of antimicrobial resistance throughout the European Union based on the species of bacteria, antibiotics' class, and geographical location. *Escherichia coli* (44.2%), followed by *S. aureus* (20.6%), *K. pneumoniae* (11.3%), *E. faecalis* (6.8%), *Pseudomonas aeruginosa* (5.6%), *S. pneumoniae* (5.3%), *E. faecium* (4.5%), and *Acinetobacter* spp. (1.7%) were widely studied. According to a report, MRSA accounts for between 13 and 74% of all *S. aureus* infections worldwide. *S. aureus* affected an estimated 119,247 individuals in the United States, resulting in 19,832 fatalities [40]. The WHO's latest Global Antimicrobial Surveillance System (GLASS) [41] shows widespread AMR among 500,000 people with reported bacterial infections in 22 countries. *E. coli*, *S. aureus, S. pneumoniae*, and *K. pneumoniae* were the most widely identified resistant bacteria. Resistance to ciprofloxacin, an antibiotic widely used to treat urinary tract infections (UTIs), ranged from 8.4 to 92.9% for *E. coli* and from 4.1 to 79.4% for *K. pneumoniae*, with penicillin resistance ranging up to 51% among countries stating to GLASS. In 2019, GLASS received data on MRSA bloodstream infections from 25 nations, regions, and zones, and *E. coli* bloodstream infections from 49 countries. The median incidence of methicillin-resistant *S. aureus* was 12.11% (IQR 6.4–26.4), and the median rate of *E. coli* resistant to third generation cephalosporins was 36% (IQR 15.2–63) [42].

According to the most recent anti-tuberculosis (TB) drug resistance monitoring results, 3.5% of current TB cases and 18% of previously treated TB cases worldwide are expected to have multidrug-resistant (MDR)- or rifampicin-resistant (RR)-TB [43]. In 2017, an approximate 558,000 new cases of MDR/RR-TB were reported worldwide, and took the lives of 230,000 people that year [44]. To summarize, antimicrobial resistance has become a major danger to humanity, causing an estimated 700,000 deaths worldwide per year [45], and it is predicted that if this problem is not resolved adequately, millions of mortalities will be reported by 2050 [36].

Reasons behind antibiotic resistance

Microorganisms, such as bacteria are living organisms that adapt over time. Their main objective is to replicate, survive, and spread as rapidly as possible. As a result, microbes adjust to their surroundings and evolve in ways that guarantee their continued existence [46]. If something stops their ability to grow, such as an antibiotic, genetic modifications may arise, making the bacteria immune to the medication and allowing them to survive [47]. It is the natural process of bacteria to develop drug resistance. However, several elements remain currently at stake in the multifaceted etiology of antibiotic resistance. This involve antibiotic overuse and abuse, inexact diagnosis and improper antibiotic prescribing, patient sensitivity loss and self-medication, bad healthcare environments, poor personal hygiene, and widespread agricultural use [48–50].

Microbial (natural) reasons

AMR is primarily caused by alterations within the bacteria [51], and may occur in a variety of ways:

Genetic mutation

Changes in few base pairs may occur during bacterial replication (point mutations), resulting in the replacement of one or a few amino acids in a critical target (enzyme, cell wall, or cell structure), as well as control genes or chromosomal structural, resulting in new resistant strains. The newly developed defense may render antibiotics ineffective, which were meant to be able to handle the organism for years [54].

Genetic material transfer

Resistance from another species or genus may be accumulated by a formerly susceptible strain. Most of the antibacterial resistance genes are carried on plasmids and other types of mobile genetic elements, which may and do spread to bacteria of different genus and species. Drug-resistant bacteria may pass on a copy of their genes to other non-resistant bacteria. The non-resistant bacteria accumulate the new DNA and develop drug resistance [54].

Selective pressure

Selective pressure may be defined as the environmental conditions that allow the survival and proliferation of organisms with novel mutations or newly developed characteristics [52]. When treated by an antimicrobial, microbes are either destroyed or if they resistance genes they survive [53]. These survivors will multi-

ply, and resistant microbes that are newly developed, will rapidly overtake the microbial population as the dominant form [55].

Inaccurate diagnosis

While diagnosing an infection, healthcare professionals sometimes rely on unreliable or inaccurate knowledge, prescribing an antibiotic "just in case" or a wide-spectrum antibiotic when a particular narrow spectrum antibiotic might be more appropriate. These circumstances exacerbate selective pressure and hasten antimicrobial resistance [48,56].

Inappropriate prescription of antibiotics

When doctors are unclear if an infection is exacerbated by bacteria or a virus, they may prescribe antibiotics. Antibiotics, on the other hand, do not act against viral infections, and resistance may develop [57,58].

Self-medication

In Southeast Asian region of the world, antibiotics are widely used without physician's prescription. Self-medication with antibiotics (SMA) is linked to the possibility of improper drug usage, which puts patients at risk for adverse drug reactions, masking signs of underlying diseases, and development of drug resistance in microbes [59].

Inadequate and overuse of antibiotics

If an individual does not finish a course of antibiotics, some bacteria may thrive and develop resistance to that antibiotic. Again, in the year 1945, the discoverer of antibiotics, Alexander Fleming, had issued a public warning against the overuse of antibiotics, as he had realized the dangers associated with the inappropriate use of these drugs. Taking antibiotics too often for the wrong reasons can develop modifications within the bacteria that antibiotics do not work against them [50,60].

Poor hospital environment

Thousands of patients, staff, and visitors arrive at hospitals every day, each with their own set of microbiome and colonizing bacteria on their clothing and on/inside their bodies. Bacteria can spread if hospitals do not have adequate procedures and protocols in place to help maintain spaces clean. As a result, the emergence and spread of AMR is aided [61].

Extensive use in agriculture

In both the industrialized and emerging parts of the world, antibiotics are used as growth supplements and growth promoters for animals. Treatment of livestock with some antibiotic, much like in humans, will result in the appearance of antibiotic-resistant bacteria. The antibiotic-resistant bacteria found in the livestock can be pathogenic to humans, readily spread to humans by food chains, and is widely circulated in the ecosystem by animal waste. In humans, this may lead to complex, untreatable, and long-term infections [50,62].

Availability of few new antibiotics

The pharmaceutical industry's invention of new antibiotics, which had previously been effective in combating antibiotic-resistant bacteria, had largely slowed due to technical challenges, a lack of knowledge, important difficulties in combating bacterial physiology (e.g., the complex Gram-negative cell wall) and financial and regulatory hurdles. However, when new antibiotics become widespread, the development of resistance (and under a relatively short period of time) is almost unavoidable. As a result of this fear, doctors frequently restrict this latest medications for only the most severe conditions, continuing to administer older agents (often generic drugs) that have demonstrated similar effectiveness, raising the likelihood that older agents will become ineffective owing to the development of resistance within bacteria [50,58].

Mechanism of action of antibiotics

Antibacterial activity is usually classified as one of five mechanisms: interfering with bacterial cell wall synthesis, inhibition of bacterial protein biosynthesis, inhibition of bacterial nucleic acid synthesis, inhibition of metabolic pathways, and inhibition of bacterial membrane function (Fig. 1 and Table 1) [63].

Antibiotics inhibiting cell wall synthesis

Bacterial cell walls are made of cross-linked peptidoglycan [64]. Antibiotics, such as β-lactams (penicillin and its derivatives, cephalosporins, and carbapenems) and glycopeptides (vancomycin) inhibit peptidoglycan biosynthesis, rendering the cell vulnerable to osmotic pressure and autolysis. As a result, bactericidal antibiotics inhibit the synthesis of cell wall. Because animal cells lack peptidoglycan, the mechanism of action is selective in nature [25].

β-lactam antibiotics

Peptidoglycan, a necessary component of the bacterial cell wall that generates mechanical support, is found in both Gram-positive and Gram-negative bacteria. However, peptidoglycan is thick (ten to forty layers) in Gram-positive bacteria but thin (one or two layers) in Gram-negative bacteria [65]. Peptidoglycan is made up of glycan chains made up of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) disaccharide subunits, crosslinked by pentapeptide chains [64].

Through acylating the transpeptidase engaged in cross-linking peptides to assemble peptidoglycan, β-lactam antibiotics block the last stage in peptidoglycan synthesis. Penicillin-binding proteins (PBPs) are the main targets of the activities of β-lactam antibiotics. This, in turn, disrupts the terminal transpeptidation mechanism, resulting in the microorganisms' loss of viability and lysis [66].

Glycopeptides

Glycopeptides such as vancomycin blocks cell wall synthesis by attaching to the D-Ala-D-Ala terminal of the expanding peptide chain during cell wall synthesis, resulting in transpeptidase inhibition, preventing subsequent elongation, and cross-linking of the peptidoglycan chain [67].

Antibiotics inhibiting protein synthesis

The 70S ribosome of bacteria (based on the protein sedimentation rates, expressed as "Svedberg" units) constitutes of 30S and 50S subunits. Antibiotics inhibits protein synthesis by targeting the 30S (aminoglycosides and tetracyclines) or 50S (chloramphenicol, macrolides, and oxazolidinones) subunit [68].

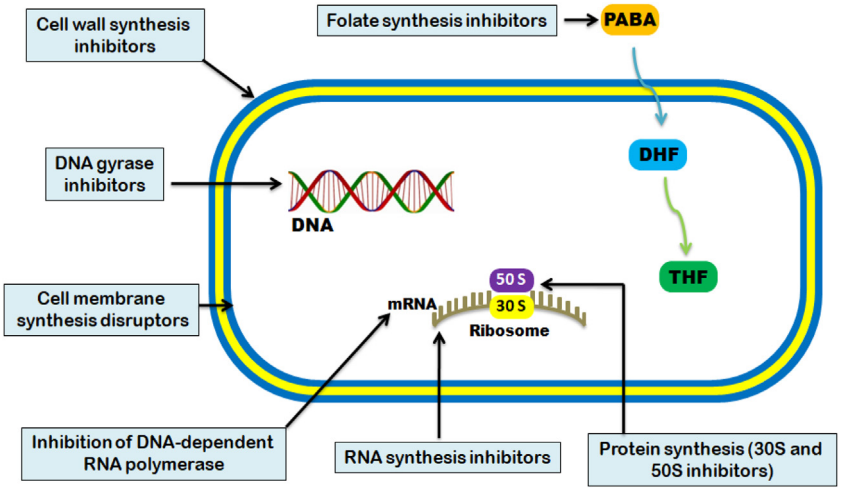

Fig. 1. Mode of action of antibiotics.

Table 1
Mode of action of different classes of antibiotics [63].

Mode of action	Targets	Drug class	Specific drugs example
Cell wall synthesis inhibition	Penicillin-binding protein	β-lactams	Penicillin G, amoxicillin, and cephalosporin C
	Peptidoglycan subunits	Glycopeptides	Vancomycin
Inhibition of protein synthesis	30 s subunit	Aminoglycosides and tetracyclines	Streptomycin, gentamicin, neomycin, tetracycline, and doxycycline
	50 s subunit	Macrolides, chloramphenicol, and oxazolidinones	Erythromycin, azithromycin, chloramphenicol, and linezolid
Inhibition of nucleic acid synthesis	RNA	Rifamycin	Rifampin
	DNA	Fluoroquinolones	Ciprofloxacin and ofloxacin
Anti-metabolites	Folic acid synthesis enzymes	Sulfonamides and trimethoprim	Sulfamethoxazole, dapsone, and trimethoprim.
Disrupt membranes	Lipopolysaccharides	Polymyxins	Polymyxin B and colistin

Inhibitors of 30S subunit

Aminoglycosides

Aminoglycosides, such as streptomycin, neomycin, and gentamicin prevent protein synthesis by binding with high affinity to the A-site on the 16S ribosomal RNA (rRNA) of 30S rRNA. Thus, the antibiotics facilitate misreading of codons when the aminoacyl-transfer RNA is delivered. This leads to erroneous protein synthesis, allowing incorrect amino acids to compile into a polypeptide, which is then released, causing noxa to the cell membrane [68].

Tetracyclines

Tetracycline antibiotics diffuse passively in the bacterial cell membrane via porin channels and binds reversibly to the 30S ribosomal subunit, inhibiting protein synthesis by blocking tRNA binding to the mRNA-ribosome complex [69].

Inhibitors of 50S subunit

Macrolides

Macrolides, such as azithromycin, bind to the 23S rRNA of bacterial 50S ribosomal subunits. They inhibit the transpeptidation or translocation process of protein synthesis, resulting in the premature separation of incomplete peptide chains, preventing bacterial protein synthesis [70].

Chloramphenicol

As chloramphenicol is lipid-soluble, it presents with good absorption properties *in vivo* and it can pass through bacterial cell membranes. It then binds reversibly to the L16 protein of the 50S subunit of bacterial ribosomes, inhibiting peptide bond formation and resulting protein synthesis by preventing amino acid transfer to expand peptide chains, possibly by suppressing peptidyl transferase activity [71].

Oxazolidinones

Oxazolidinones, such as linezolid and tedizolid work as an antibacterial agent by interfering with the translation of bacterial proteins. They connect to a site on the 50S subunit of the bacterial 23S ribosomal RNA, preventing the development of a functional 70S initiation complex, which is required for bacterial replication, and thus preventing bacteria from multiplying [72].

Antibiotics inhibiting nucleic acid synthesis

Some antibacterial drugs e.g., rifamycin and fluoroquinolones function by inhibiting RNA and DNA, respectively [73].

Rifamycin

Rifamycin inhibit bacterial DNA-dependent RNA polymerase, by attaching tightly to the polymerase subunit deep inside the DNA/RNA pathway, allowing direct blocking of the elongating RNA. Bacterial RNA polymerase enzymes vary structurally from eukaryotic RNA polymerase enzymes, allowing for selective toxicity against bacterial cells [73,74].

Fluoroquinolones

Quinolones block DNA synthesis by inhibiting DNA gyrase and topoisomerase IV, two basic type II topoisomerases. Both targets cause one double stranded DNA molecule to move through another, with the initial strand religated afterward [75]. Quinolones have a

strong affinity for the A subunit of DNA gyrase, interfering with its strand splitting and resealing role, preventing normal cell division. Topoisomerase IV, which nicks and separates the daughter DNA strand after DNA replication, is the main focus of action in Gram-positive bacteria. Drugs with higher affinity for this enzyme can confer greater potency against Gram-positive bacteria [76].

Inhibition of metabolic pathways

Some synthetic antibiotics function as anti-metabolites, or competitive inhibitors of bacterial metabolic enzymes, to control bacterial infections. Sulfonamides and trimethoprim inhibit different steps in folic acid metabolic pathway [77].

Sulfonamides

By competing with para-aminobenzoic acid (PABA) for binding to dihydrofolate synthetase, a step in the synthesis of tetrahydrofolic-acid (THF), sulfonamides inhibit the enzymatic conversion of pteridine and PABA to dihydropteroic acid. THF is necessary for purine and dTMP synthesis, and its inhibition restricts bacterial growth [78].

Trimethoprim

Trimethoprim reversely inhibits dihydrofolate reductase, which is one of the main enzymes involved in the conversion of dihydrofolate (DHF) to THF. THF is needed for the synthesis of bacterial proteins and nucleic acids, as well as for bacterial survival; thus, inhibiting its synthesis results in bactericidal action [77,79].

Trimethoprim is sometimes used in addition with sulfamethoxazole (sulfonamide), which prevents the phase before bacterial protein synthesis. When used in combination, sulfamethoxazole and trimethoprim inhibit two stages in bacterial nucleic acid and protein biosynthesis. Trimethoprim is singularly bacteriostatic, although when combined with sulfamethoxazole, it is believed to be bactericidal [79].

Inhibition of cell membrane function

A small class of antibiotics such as polymyxins (polymyxin B and E) lyse cell membrane of bacteria [80]. They are detergent-like lipophiles, which destroy the membrane by interfering with the lipopolysaccharide portion of Gram-negative bacteria [81–83].

Mechanisms of antibiotic resistance

Natural and acquired resistances to antibiotics are the two main forms of antibiotic resistance. Normal resistance may be innate (it is often expressed in the organisms), or mediated (the genes are normally present in the bacteria but are only activated to resistance levels following antibiotic treatment) [82]. On the other side, acquired resistance may be the result of the bacteria acquiring genetic material by translation, conjugation, or transposition [83], or mutations in its own chromosomal DNA [84]. AMR mechanisms may be divided into four categories: (1) drug uptake limitation; (2) drug target modification; (3) drug inactivation; and (4) drug efflux [63]. Owing to the structural differences and others, Gram-negative bacteria can use all four mechanisms, while Gram-positive bacteria are less likely to use limiting the uptake of a drug (the lipopolysaccharide in the outer membrane is absent) and drug efflux mechanisms [85].

Limiting drug uptake

Gram-negative bacteria are intrinsically less permeable to certain antibiotics than Gram-positive bacteria, owing their outer membrane creates a permeability shield due to the existence of a lipopolysaccharide (LPS) layer. The fact that glycopeptide antibiotics e.g., vancomycin is not effective against Gram-negative bacteria because of the lack of penetration through the outer membrane is a prime illustration of the efficiency of this natural barrier. Hydrophilic molecules including β-lactams, tetracyclines, and certain fluoroquinolones are highly affected due to the modifications in the permeability of outer membrane [86]. Polar molecules have difficulties entering the cell wall of enterococci due to porin channel downregulation or even substitution with non-selective channels confers inherent tolerance to aminoglycosides. Furthermore, recent studies suggest that reductions in porin expression greatly lead to resistance to drugs such as carbapenems in members of the Enterobacterales order, *Acinetobacter* spp., and *Pseudomonas* spp. For example, resistance to carbapenems in Enterobacterales will emerge in the absence of enzymes of carbapenemase activity, if mutations decrease porin production or if mutated porin alleles are present [87]. Biofilm formation is another mechanism which helps in the colonization of bacteria [88]. The biofilm matrix includes polysaccharides, proteins, and DNA, making antimicrobial agents difficult to enter the bacteria and thereby providing defense [89].

Drug efflux

Many antibiotics are actively transported out of the cell by bacterial efflux pumps, which are important contributors to Gram-negative bacteria's intrinsic resistance. Efflux pumps come in a variety of forms of most bacteria. The ATP-binding cassette (ABC) family, small multidrug resistance (SMR) family, multidrug and toxic compound extrusion (MATE) family, resistance-nodulation-cell division (RND) family, and large facilitator superfamily (MFS) are the five primary families of efflux pump, which are categorized considering its structure and energy supply [82]. Except for the RND family which are multi part pumps that efflux substrate across the cell envelope, all other efflux pump families are singular pumps that transfer substrates across the cytoplasmic membrane [47]. Tetracycline resistance is a textbook example of efflux-mediated resistance, in which Tet efflux pumps (of the MFS family) use proton exchange as a source of energy to extrude tetracyclines. Several MDR efflux pumps, such as MexAB-OprM in *P. aeruginosa* and AcrAB-TolC in Enterobacterales (of the RND family) can extrude tetracyclines as part of their contribution to MDR [69]. Resistance to macrolides is another clinically relevant phenotype induced by the efflux mechanism. The mef genes, which extrude the macrolide class of antibiotics, encode the most well-characterized efflux pumps (e.g., erythromycin). MacB, an ABC family member, acts as a tripartite pump (MacAB-TolC) for extruding macrolide drugs [47].

Drug inactivation

Bacteria inactivate antibiotics in one of the two ways: by destroying the drug, or by the chemical alteration of the drug [86].

Chemical modification of the drug

Bacteria may generate enzymes that can attach various chemical groups to the drugs. This prevents the antibiotic from binding to its target in the bacterial cell. Transfer of phosphoryl, acetyl, and adenyl groups to the compound is the most effective method of drug inactivation by chemical group transfer [90]. Acetylation is the most often employed mechanism, with aminoglycosides, chloramphenicol, streptogramins, and fluoroquinolones both being considered to use it. The aminoglycosides are considered to be targeted through adenylation and phosphorylation. The involvement of aminoglycoside modifying enzymes (AMEs) covalently alters the hydroxyl or amino groups of the aminoglycoside molecule and

makes it inactive. It is one of the best examples of resistance through drug modification [47].

Destroying the drug

The β-lactam medicines, such as penicillin and cephalosporins, are the most commonly employed antimicrobial agents [66]. The central structure of this drug class is a four-sided β-lactam loop, which is shared among all members. The activity of β-lactamases destroys the β-lactam loop, which is the key mechanism of β-lactam resistance. The β-lactamases hydrolyze β-lactam ring formation, thus, inhibiting its binding to penicillin-binding proteins (PBP) [25].

Drug target modification

The modification of the antibiotic's target is a common mechanism by which bacteria become resistant to antibiotics [82]. Changes in the arrangement and/or amount of PBPs are one of the mechanisms of resistance towards β-lactam drugs. The amount of drug that can attach to the target is affected by changes in the number of PBPs [25]. A structural alteration e.g. the development of the mecA gene in *S. aureus* will reduce or completely prevent drug binding [91]. Another example is the erythromycin ribosome methylase (erm) gene family, which methylates 16S rRNA and changes the drug-binding site, blocking macrolides, streptogramins, and lincosamines from binding [92]. Resistance to drugs that inhibit nucleic acid synthesis e.g. fluoroquinolones, is mediated by changes in DNA gyrase or topoisomerase IV. These mutations alter the composition of gyrase and topoisomerase, reducing or excluding the drug's ability to attach to these components [75].

Therapeutic strategies against AMR

With the rapid and global emergence of MDR bacteria, new antibiotic techniques are required. Despite the fact that a range of novel small molecule antibiotics are now in progress, and even others in pre-clinical testing, infection management clinical options and procedures must be increased. This potential for expansion is provided by biologics and non-antibiotic adjuvants. Nonetheless, intelligent hybrid methods with various parallel and complementary treatments must be planned to avoid established pathways of resistance. Combination techniques can involve clever directed distribution strategies in addition to biologically active molecules. Antimicrobial stewardship, novel antibiotic molecules, biologics, and distribution mechanisms must all be integrated into successful combined treatments that a) combat the infection, b) prevent resistance, and c) secure and maintain the normal microbiome [93]. There are a few approaches to be adapted to combat against AMR as discussed below:

Approaches targeting several pathways in combination

The most researched and promising methods for developing multidrug cocktails to tackle AMR are dual drug-delivery strategies [94]. Combining a multidrug combination method with local drugs distribution can be the most effective protocol for restoring the effectiveness of the existing antibiotics while minimizing required drug concentrations [95]. Several successful combinations that act through various pathways often provide non-antibiotic adjuvants. Because of the variety of pathways, combining antibiotics with adjuvants are leading approach for tackling multidrug resistant process. Rifampin, minocycline, and chlorhexidine are only a few examples of such variation. Antiseptic inhibitor and other natural (e.g., plant-derived) or biological (e.g., bacteriophage) moieties are three typical adjuvants that have had some therapeutic success. Tranquilizers, antihistamines, antispasmodics, anti-hypertensives, and anti-inflammation medications are among the recognized groups of substances being investigated as antibiotic adjuvants [96].

Strategies functioning on similar pathways in combination

Varied biomolecules in the similar pathway may be targeted using a hybrid technique. While this is a less diversified approach than attacking various pathways, it may be a very successful strategy if the right route is selected. The option of direction is limited by two factors. To begin, the targeted pathway must be an utter survival prerequisite, such as the need for folate to synthesize dTMP, a precursor to DNA synthesis. Second, the pathway cannot be redundant, since this makes the technique vulnerable to opposition [97]. In the face of rising antibiotic resistance, focusing on different steps in the similar pathway is a risky approach, but it is also more successful than monotherapies in certain situations [98–100].

Approaches operating on similar target in combination

While focusing two separate biomolecules in the similar pathway is less variable than targeting two entirely different pathways, it is more diversiform than utilizing two monotherapies to suppress the similar biomolecules. Antibiotics that target the bacterial ribosomes are its classical example. Synercid (quinupristin/dalfopristin), a semi-synthetic two-drugs hybrid in which the constituents adhere to neighboring region of 50S ribosomal sub-units, demonstrates the effectiveness of this strategy, since it is 10–100 times more efficient than any drug alone [97]. Because of the bacterial ribosome's essential and conserved structure, combining drugs that act on ribosome could be useful. As a result, it's obvious how important goal selection is in this approach [93].

Polymicrobial infections in multifaceted approaches

Combination treatments are pivotal for treating polymicrobial infections (e.g., abdominal infections, where strict anaerobes and gut bacteria are both present), along with the combination that are successful against a single pathogen [101]. Peri-prosthetic joint infection is caused by the involvement of multiple pathogens in between 4–27% of cases; therefore, the potential to treat multiple pathogens simultaneously and rapidly is evolving as a critical weapon in the battle against infections. Importantly, hybrid treatments may not have to be restricted to only two medication combinations. As opposed to two antibiotic combinations, triple antibiotic combinations have proved to be even more successful against extensively drug resistant MRSA strains [102]. In addition, antibiotic combinations show different modes of actions.

Drug formulations of synergistic effects and resistance

Despite multiple selective pressures, a drug combo could actually encourage the development of drug resistance, notwithstanding the initial appearance that utilizing a drug combination will help to bypass several resistance mechanisms [103–106]. It might be due to the reason that *in vitro* tolerance to some antibiotics improves susceptibility towards other antimicrobials, as shown by increased minimum inhibitory concentrations (MICs) [107]. Owing to the evolution of MDR variants, drugs cocktails will also raise the risk of extreme-infection [101]. To combat this challenge, creating successful drug formulations is a critical step in mitigating the proliferation of antibiotics resistant microbes while enhancing medication effectiveness and extending the usefulness of individual agents [93].

Combination medication delivery's drawbacks

The effect of interaction between drugs, bioactive component concentrations, and dose regimens on adsorption, absorption, and excretion of drug separately must be closely considered in all contemplated drug formulations [108–110]. The relatively simple technique of simultaneously administering few synergistic antibiotics may not only change the pharmacokinetics of drugs but also it may cause toxicity to the host cells. Nisin and lantibiotic can help to prevent any of these popular pitfalls [104,111]. Notably, such medication formulations (e.g., antibiotics and metallic nanoparticles) will potentially reduce the toxic effect of individual drug, owing to lower dosing and concentration requirements [112]. Furthermore, the mechanism of action of these drugs should be considered in order to avoid antagonistic reactions [113]. Finally, a deep analysis of antibiotics combination with adjuvant is needed [93].

Molecular application and antibiotic resistance

Antibiotic-resistant bacteria have emerged quickly, making it more difficult to fight infectious diseases and produce new antibiotics. As a bacterial adaptive immune system, the clustered frequently interspaced short palindromic repeats – CRISPR-associated (CRISPR-Cas) system is known as one of the recent methods for combating antibiotic-resistant strains. This system's programmable Cas nuclease, when used against bacterial genomic sequences, may be lethal or help decrease AMR in bacteria [114].

CRISPR-Cas system has been discovered to be a form of adaptive immune system in bacteria. They've been used for genome editing and a variety of other uses, including the treatment of hereditary disorders [115]. CRISPR systems work in a similar way to RNA interference (RNAi) in eukaryotic cells [116–118]. About 50% of bacterial genome and 87% of archaeal genome include the framework [119]. The Cas proteins are important functional elements that are encoded up-stream of CRISPR array and control how the mechanism works [120,121]. In bacterial species, CRISPR-Cas attacks virulent genes and genes encoding antibiotics resistance [127]. In several researches, the CRISPR mechanism was shown to have a substantial negative relationship with resistance in some bacteria, such as enterococci. CRISPR1-Cas, orphan CRISPR2, absence of Cas mutations, and CRIS PR3-Cas are the three CRISPR loci found in *E. faecalis*. Pheromone-response plasmids are found to be essential in enterococci genome plasticity and virulence [122–126]. The Inc18 plasmid family lacks any spacer. Tn916, as a vector of antibiotic resistance, is yet to be detected with CRISPR spacers. Streptococcus thermophiles strains that carry the CRISPR gene have gained new spacers originating from the virus, making them immune to phage infection [127]. In *E. coli*, there are 4 CRISPR loci: CRISPR1, CRISPR2, CRISPR3, and CRISPR4 [134]. CRISPR has no impact on the plasmid in *E. coli* or the distribution of antibiotics resistant genes. These observations contradict those of Palmer and Gilmore, who discovered that CRISPRs are inversely related to antibiotic tolerance in enterococci [124].

The amount of repetitions is a strong measure of the system's potential efficiency and honesty. Cas proteins are guided to directly target and cleave DNA by RNA-based spacers flanked by partial repeats. Recent research has shown that attacking the sequences of the bacterial genome using CRISPR-Cas system, whether intentionally or accidentally, is cytotoxic and can result in cell death. For the formation of RNA-guided nucleases, extraction and production of distribution carrier and vector will be needed. Furthermore, because of the delivery mechanism used in higher species, RNA-guided nucleases could be able to modulate the presence of specific gene in wild-type population, viz. antibiotics tolerance genes and virulence determinants [128,129]. For treating the therapeutic strain of *S. aureus* USA300, cas9, and crRNA of the methicillin resistance gene mecA90 in the phagemid (pDB121:mecA) were prepared [130]. Cell death was not reported in any of the Cas9 plasmids that target tetracycline-resistant plasmids. Following the delivery of CRISPR-Cas system, the bacteria can be re-sensitized to antibacterial or destroyed, depending on the antibacterial resistance gene target or critical gene target. Conjugative-based distribution, phage-based delivery, and polymeric-nanoparticles-based delivery are among the possible delivery pathways. The treatment with the Cas9-bPEI complex could dramatically reduce growth by 32% as compared to the treatment with Castex complex without sgRNA. This discovery could pave the way for the development of CRISPR-based antimicrobial drugs.

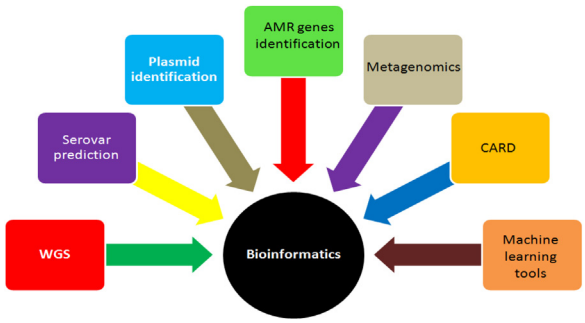

Fig. 2. Role of different bioinformatics approaches to combat AMR microbes.

The CRISPR-Cas method may also be utilized for changing the structure of diverse groups of bacteria. The existing treatments that utilize a medication to alter the human microbiota have the ability to alleviate pain from a variety of illnesses, but Citorik and his collaborators argue that the processes through which they work are still poorly understood. An *in vivo* mouse model was used to assess the efficacy of Cas9 phagemid against bacteria [123]. In situations where bacterial species are particularly immune to current antimicrobial agents, phagemids may be a suitable substitute. For planktonic and biofilm environments, most reports propose a mixture of phage, conjugative, and polymeric nanoparticle-based delivery systems. Furthermore, full extinction of the intended organism(s) and the absence of special methods to monitor pathogenic organisms are major issues in microbiome modulation, microbiology, and infectious disease control. CRISPR-Cas-based antibacterial can face challenges from CRISPR-Cas distribution vectors or automobiles, as well as complex bacterial communities [114,131].

Bioinformatics in combating antibiotic resistance

Bioinformatics is revolutionizing and fascinating the realm of science and technology. Nowadays, homology modeling is used to create 3D models in order to test or validate our desired outcomes. Bioinformatics has revolutionized molecular biology research by understanding the structures of macromolecules. Bioinformatics has provided basic tools to develop ideal drugs strategies for tackling the growing issue of antibiotics resistance microbes. Bioinformatics is concerned with the study and understanding of different forms of macromolecules' data and their interactions [132]. Some prominent methods in the field of bioinformatics are shown in Fig. 2.

Whole genome sequencing (WGS)

The WGS of a pathogen genetic material has recently become a vital method for genotyping [32,133]. The use of WGS to analyze the whole bacteria genome could offer insight into associated bacterial lineages and revolutionize outbreak research in hospitals

[134]. Genome sequencing offers a strong framework for scientific advancement, especially in biomolecular modeling and drug design, with a focus on antibiotic resistance. High-throughput technological platforms and bioinformatics may offer fresh insights into virulence, disease propagation, and antimicrobial tolerance, in addition to detecting pathogens more quickly and accurately than conventional approaches [135]. Sequencing of DNA is an excellent tool for protein modeling and drug development. Furthermore, significant advancements in protein expression, gene sequencing, nuclear magnetic resonance, and high-throughput crystallography have changed the possibilities for using protein three-dimensional architectures to speed drug discovery. This is one of the most important techniques for combating bacterial resistance because it allows for the detection of putative relatives, alignment of sequences, and modeling of three-dimensional structures [136].

Serovar, serogroup, and antigenic profile study in silico

As classical serotyping has been replaced by molecular serotyping, the development of WGS technologies has taken center stage in genotyping. Surface antigen encoding gene constituting core genome multi-locus sequence typing (MLST) and serovar-specific gene marker are used in current *in silico* serovar prediction approaches. However, only a few serovars can be distinguished using serovar-specific gene marker. Zhang et al. developed computational serovar prediction method by comparing 1089 genomes indicating 106 serovars to a range of 131 serovar-specific gene markers. According to the researchers, this technique was a valuable screening instrument for genomic studies. It can be used not only to classify a particular class of gene marker but also create inexpensive assays for detecting specific gene marker in major serovars [137].

In silico plasmid identification

cBar [138], PLACNET [139], plasmidSPAdes [140], and Recycler are only a few of the tools that can be used to remove and assemble plasmids from high throughput sequencing (HTS) results [141]. These methods may be used to look for certain markers in plasmid data or to investigate the unique structure of plasmid sequences [142]. Since plasmids may occur in single-copy [143] or linear DNA molecules, the methods are dependent on relative failure [144–146]. PlasmidFinder [147], MOB-suite, and other tools (regardless of which tool is implied for reconstructing or detecting plasmid in HTS data) [142], consumers would have to struggle to read the list of hits and assess the effect of potential plasmids on bacteria. In the National Center for Biotechnology Information (NCBI) reference sequence (RefSeq) plasmid database [148], there are approximately 13,924 entries, with a relative lack of essential resources for accessing massive data sets of plasmids sequences [142].

In silico identification of AMR genes and their association with plasmids

Kudirkiene et al. used WGS to select 16 plasmids bearing antimicrobial resistance (AMR) genes of *Salmonella enterica* in one of their promising experiments [149]. Prediction of plasmids and resistance gene sites were performed by a combination of PlasmidFinder, ResFinder, plasmidSPAdes, and BLAST [150,151]. After using S1-PFGE to analyze plasmid profile, the WGS verified the existence of antimicrobial resistance genes in *Salmonella* spp. [151]. In *S. enteritidis*, resistance genes were mostly found on IncN plasmids. However, in *S.* Typhimurium, they were mostly found on IncFII(S)/IncFIB(S)/IncQ1 plasmids. Resistance genes in *S. virchow* and *S. poona* were also found on IncX1 and TrfA/IncHI2/IncHI2A form plasmids, respectively. The use of genomic tools provided to study complete mapping of resistant plasmids in all *Salmonella* isolates studied. Findings indicated that enhanced technique might be used to classify plasmids in WGS that are directly correlated with resistance phenotypes. With this expertise, fast MDR monitoring tools in *Salmonella* communities may be developed using WGS [149].

Metagenomics for antimicrobial surveillance

Metagenomics strategies depend on short-read next-generation sequencing (SR-NGS) data, which allows for the quantification of thousands of transmissible resistance genes in a single sample without the use of any preset genes. As a result, it may provide more knowledge about the existence of bacterial organisms, pathogens, and virulence genes, and the data obtained can then be re-analyzed if new genes of interest are discovered [152]. Metagenomics has recently been shown to be more effective than traditional techniques of AMR surveillance in pig herds [153], and has been successfully utilized to compare AMR in animals [154], as well as in epidemiological evidence investigations [155]. Because of its several advantages, metagenomics has a promising future as a method for AMR surveillance. This could lead to a single-point surveillance of AMR, thereby allowing for the identification of all resistance genes [152].

Comprehensive AMR database (CARD)

The CARD is a meticulously assembled platform that provides detection models, macromolecule sequences, and computational resources for understanding the molecular mechanism of AMR [156]. CARD is a database that focuses on providing high-quality reference data and molecular sequences within a specified vocabulary. CARD's Resistance Gene Identifier (RGI) program is used in the Antibiotic Resistance Ontology (ARO), which was created by the CARD biocuration team to integrate with software creation activities for resistome analysis and prediction [156]. CARD's usage grew in 2017 as a result of widespread reference sequence curation, amendment of ontological framework, the introduction of novel classification methodology, and the extension of bio-analytical methods [156]. The latest resistomes and variants module, for example, includes interpretation and statistical summaries of expected resistance variants from 82 microbes and over 100,000 genomes [156]. The expected resistance could be summarized using the data in CARD and patterns in AMR mobility could be identified by including these resistance variants in CARD [157].

Machine learning tools for predicting antibiotics resistance

AMR was usually detected in the above mentioned reports by analyzing certain recognized determinants established through genome sequencing, which necessitates prior understanding of the pathways involved. Machine learning algorithms were then utilized for predicting resistance towards 11 components across 4 groups of antibiotics from known and novel whole-genome sequences of 1936 *E. coli* isolates, in order to address this constraint. The authors looked at a variety of methods as predictors, including population structure, isolation year, gene material, and polymorphism detail [153]. Finally, decision trees with gradient boosted have greater performance than alternative models, with an overall precision of 0.91 on held-out data (range 0.81–0.97) relative to alternative models. Although the better simulations used gene material the most, utilizing just population structure knowledge yielded an overall accuracy score of 0.79. Single nucleotide variation data, on the other hand, were less valuable, and only two antibiotics, including ciprofloxacin, showed a substantial improvement

in prediction [150]. The findings show that WGS can be utilized for forecasting antibiotics resistance in *E. coli* without knowing the pathways in advance. It shows the necessity of machine learning methods to be integrated into diagnostic software in clinics [153].

Bacteriophages: a therapeutic strategy

The development of resistant bacteria to antimicrobial drugs is now the most challenging issue in the treatment of bacterial infections, thus attention is being given to alternative possible targets. Many advantages have been stated that use phage treatment over chemotherapy, and it appears to be a potential drug to replace antibiotics, based on the good findings of phage therapy [158,159]. Bacteriophages may be distinguished from other antibacterial agents in several ways, including the production of virolysin, the encoding of antimicrobial peptides, the delivery mechanism for genes that encode antimicrobial compounds, and the ability to infect susceptible bacteria as a living phage [159]. Effective viral delivery methods are now being developed to deliver the correct genome to the target cells [160]. Phages can also be used to deliver therapeutics. Phages transfer genes encoding antimicrobials or harmful antimicrobials into target bacteria throughout this procedure. Additionally, filamentous phages have the capacity to deliver therapeutic genes to mammalian cells [161]. Mammalian cells are transduced via receptor-mediated endocytosis during this process. This method is not antibacterial in and of itself, but it can be further developed to deliver antibiotic genes to intracellular bacterial pathogens [162]. Bacteriophage treatment, which was originally utilized almost a century ago, is experiencing resurgence, mostly due to the AMR issue. Strictly lytic phages, proven antibacterial efficacy against the target pathogen, and elimination of contaminated bacterial debris and endotoxins are recommended regulatory conditions for the therapeutic use of phages [163]. In addition, the identification of any therapeutic phage's bacterial host receptor should be determined, as this will give crucial information on the establishment of phage resistance, evolutionary trade-offs, and the adoption of combinatorial treatments that are less likely to create phage-resistant hosts [164]. Adsorption to particular receptors on the bacterial host's surface is the first step in lytic phage infection. These receptors can be found on Gram-positive or Gram-negative cell walls, polysaccharide capsules, and even appendages like pili and flagella. The host range that a phage may infect is generally determined by the lock-and-key interaction between the phage and the bacterial receptors, and the list of described phage receptors is continuously expanding. The virus will expel its genetic material into the host after adsorption. The bulk of lytic phages have been linked to human diseases [165].

Avian egg model as a therapeutic strategy against AMR

Avian immunoglobulin is a "superdrug" that should be evaluated immediately in the battle against antibiotic resistance across the world. Avian antibodies have been demonstrated to meet all of the above criteria, and they have the potential to be a useful alternative to antibiotics and other antimicrobials in the fight against AMR. The immune system has traditionally been the strongest defense against diseases in humans, animals, and birds. Polyclonal antibodies specific to a range of infectious organisms, including viruses, bacteria, and parasites, have been produced using eggs as a source. These do not harm normal flora too [166]. The virus then takes over the bacterial replication machinery, resulting in the production of new phage offspring. Replication will continue until phage-encoded proteins are triggered to lyse the cell and kill the host, enabling freshly manufactured viruses to escape and restart the cycle. The lysis time, also known as the latent period, is the time it takes a phage to complete an intracellular life cycle [167].

Cytokines in AMR therapy

Antibodies play a key role in maintaining body homeostasis and the appropriate functioning of the immune system [168]. T cells, which secrete numerous interleukins (ILs) that activate antimicrobial activities in mononuclear phagocytes, are largely responsible for acquired resistance to intracellular microorganisms. The role of ILs in antimicrobial infection has been clarified to experimental infection of mice with *Mycobacterium bovis* and *Listeria monocytogenes*. A research indicated that IFN-γ decreases the quantity of *L. monocytogenes*, and anti-IFN-γ antibody therapy worsens listeriosis; moreover IFN-γ, IL-4, and IL-6 activate tuberculostatic and listericidal macrophage activities *in vitro*. Anti-TNF antibodies exacerbate listeriosis and interfere with *M. bovis*-induced granuloma development; simultaneous application of mycobacterial products, and TNF produces necrotic responses. At least part of IFN-γ's actions on macrophages in humans may be mediated by 1,25-dihydroxyvitamin D3. These findings highlight the complex involvement of ILs in antimicrobial resistance, with IFN-γ playing a key role [169].

Nanomedicine against antibacterial resistance

Because of its favorable physicochemical characteristics, drug targeting effectiveness, increased absorption, and biodistribution, nanomedicine-based drugs have attracted a lot of attention from scientists and pharmaceutical companies [170]. Drug loading efficiency of both lipophilic and hydrophilic antibiotics is improved by nanometer-sized particles, resulting in improved antibacterial activity [171]. In addition, crossing the reticulo-endothelial system allowed a more anticipated cellular uptake of the antibiotic-loaded nanosystems [172]. Nanosystems' surface charge and zeta-potential drive interactions with proteins, tissues, and different tissue components, altering cellular biodistribution and uptake. Anionic host cells, such as macrophages, attract positively charged nanosystems over uncharged and negatively charged ones [170]. Nanoparticles produce deadly alterations in bacterial cell shape and structure; nanophotothermal treatment uses inorganic NPs such as AuNPs to kill harmful bacterial cells.

Probiotics as a means of treating AMR

Probiotics are living organisms that have a positive impact on the host, most often belonging to the genera *Bifidobacterium* and *Lactobacillus*, however strains of other species are also marketed [173]. Probiotics have been specifically chosen such that they do not contribute to the spread of antibiotic resistance and do not carry antibiotic resistance that may be passed from person to person. Antibiotics and probiotics used together have been shown to lessen the severity, duration, and occurrence of antibiotic-associated diarrhea. This encourages people to follow their antibiotic prescriptions more closely, which slow down the spread of resistance. The extent to which probiotics directly prevent the transmission of antibiotic resistance is still being investigated; nevertheless, maintaining a healthy microbiome while taking antibiotics may give chances for decreasing resistance spread [174].

Herbal medicines used against AMR

Both infectious and non-infectious illnesses are and have been routinely treated using traditional herbal remedies. Antimicrobials used to treat bacterial infections caused by multiple drug resistant (MDR) and total drug resistant (TDR) strains, on the other

hand, are becoming increasingly frequent in the clinical environment, and the world is seeking for new ways to treat such illnesses. Herbal medications are thought to be superior options for present and developing antimicrobial drug resistant (ADR) bacteria, thus they are expected to protect humans against infections. Herbal antimicrobials work in a similar way as antibiotics in that they kill bacteria or limit their development. Similar to antibiotic resistance in microorganisms, herbal medicine resistance might have mechanisms which is not clearly understood. Recent research on the antibacterial effects of herbal medicines on clinical isolates has revealed that some microorganisms are insensitive or resistant to several popular herbal antimicrobial components [175].

Global and national action to resist AMR

Antibiotics, safe water, hygiene, and vaccination, have contributed towards vast changes in wellbeing and survival in developed countries. The key challenge is to expand antibiotic accessibility in developing countries without extensively developing antimicrobial resistance, which may lead to catastrophic consequences [176]. Regardless of the effectiveness of attempts to minimize antibiotic use, new antibiotics are needed, but even more should be achieved in terms of diagnostics, vaccinations, and infection prevention systems in terms of formulation and distribution innovation to mitigate the necessity of antibiotics. The development of any new vaccines decreases the requirement of antibiotics [176].

Research and development can help in eradicating antibiotic resistance. Unsurpisingly, financing also plays a key role here. The Affordable Medicines Facility for Malaria (AMFm) is an indicator of drug funding, with the goal of increasing accessibility to anti-malarial agents and lowering the probability of developing anti-malarial resistance [177]. In Asia and major parts of Africa, chloroquine had lost its potency against malaria by the early 2000s. The only similarly successful and robust first-line treatments were relatively costly artemisinin derivatives. It became a global priority and a humanitarian objective to preserve their usefulness and ensure access to them. AMFm was proposed by a US Institute of Medicine committee tasked with addressing the problems of antimalarial therapy at the dawn of the twenty-first century [178]. A comprehensive assessment of an eight-country pilot study showed that AMFm was performing as expected [179]. However, there are some national responsibilities to be addressed about this issue.

AMR is well-known among doctors and researchers on the ground in most countries; however, governments and policymakers are yet to identify it as a priority or develop a strategy to counter it. AMR must be brought to the national agenda in a process that allows specialists to assemble around the problem [176]. Antibiotic intelligence may be shared by scientific professionals and partners from all related fields – veterinary, agricultural, medicine, industries, academics, and government. This 'working party' establishes itself as a reliable and impartial source of intelligence and guidance. Volunteers are welcome, but a paid organizer is needed [176].

AMR development and associated policies at the national level requires time. National anti-AMR plans must take into account country-specific objectives based on socioeconomic demographics. In addition, the strategy should prioritize i) improving human and animal health surveillance for resistant microorganisms, ii) establishing human and veterinary AMS and infection-control programs, iii) conducting research on novel diagnostic and therapeutic approaches, and iv) implementing educational programs aimed at professional groups and the general public [180]. Stakeholders must become acquainted with the issue, believe that it must be addressed, and compromise on how to do so. Before national-level intervention is possible, it can take many years to build knowledge, understanding, and confidence, but it will then become deeply entrenched [176]. Situation assessments and analysis provide the foundation for prospective policies. Developing a partnership, advisory, or internally integrated arrangement with the ministry of health and agriculture would ensure a national effect that is permanent. Based on their circumstance review, the GARP working group in Vietnam is embedded inside a Ministry of Health Hospital and is informing the Ministry on the establishment and execution of a National Action Plan on Antimicrobial Resistance [181]. Implementing projects, creating resources, and other activities to improve antibiotics usage increases the awareness of working group participants while still benefiting them directly [176]. To prevent and control antibiotic resistance, WHO advised some steps [182]:

Individuals

Individuals may only use antibiotics as prescribed by a certified healthcare professional, and they should never demand antibiotics if a health practitioner says that they are not necessary. Preventing infections by preparing food hygienically, never use or share leftover antibiotics, avoid close contact with sick people, regularly wash hands, stay up to date on vaccinations, and practice safer sex, keep vaccinations up to date, avoid close contact with sick people, and practice safer lifestyles [182].

Health professional

In order to avoid and to monitor the spread of antibiotic resistance, healthcare professionals should keep their equipment, palms, and surroundings clean and hygienic. Antibiotics may only be prescribed and dispensed as absolutely necessary, according to existing recommendations. Tracing antibiotic-resistant diseases to monitoring teams as well as educating the patients about how to take antibiotics properly and risk of antibiotic misuse are suggested [182].

Agricultural sectors

Antibiotics should be administered under the supervision of veterinarians, in order to avoid the emergence of AMR microbes. Vaccination should be used as primary option instead of antibiotics-based treatment [182].

Policymakers

Policymakers should strengthen the policies, implement the prevention strategies of infections prevention, and make information more accessible to avoid the emergence of AMR microbes [182].

Healthcare sector

The health sector should invest in research and production of vaccinations, diagnostics, novel drugs development, and other methods to avoid and monitor the spread of AMR [182].

Conclusions and future perspectives

AMR is an increasingly common occurrence, and bacteria have evolved to combat antibacterial products' activity for centuries. The emergence in antibiotic resistance, along with a scarcity of innovative antibiotics, paints a grim vision. Again, the value of antibiotic stewardship in clinical practice cannot be understated. Antibiotic usage must be better regulated both on a local and from a global scale, including in developed countries. Stopping the usage

of over-the-counter antibiotics in these countries and educating prescribers regarding antimicrobial resistance could further reduce antibiotic use. To minimize inappropriate demand, increased global public awareness is also needed. Agricultural application must be restricted to contaminated animal care rather than development stimulation. Surveillance of antibiotic usage and resistance must be greatly improved to permit antibiotic stewardship. If the production of new anti-infectives is to keep pace with increasing resistance, significant global intervention and expenditure, from both public and private sector financing, is expected.

Funding

The article processing charge (APC) was funded by the University of Szeged Open Access fund (ID: 5524). M.G. was supported by the János Bolyai Research Scholarship (BO/00144/20/5) of the Hungarian Academy of Sciences. The research was supported by the ÚNKP-21-5-540-SZTE New National Excellence Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund. M.G. would also like to acknowledge the support of ESCMID's "30 under 30" Award.

Ethics statement

Not applicable.

Competing interests

The authors declare no competing interests.

Ethical approval

Not required.

Data availability

The datasets supporting the conclusions of this study are included within the article.

Acknowledgements

The authors would like to thank Cambridge Proofreading & Editing LLC. (https://proofreading.org/) for editing a draft of this manuscript.

References

[1] da Cunha BR, Fonseca LP, Calado CRC. Antibiotic discovery: where have we come from, where do we go? Antibiotics 2019;8, http://dx.doi.org/10.3390/antibiotics8020045.
[2] Gajdács M, Albericio F. Antibiotic resistance: from the bench to patients. Antibiotics 2019;8, http://dx.doi.org/10.3390/antibiotics8030129.
[3] Dodds DR. Antibiotic resistance: a current epilogue. Biochem Pharmacol 2017;134:139–46, http://dx.doi.org/10.1016/j.bcp.2016.12.005.
[4] Watkins RR, Bonomo RA. Overview: global and local impact of antibiotic resistance. Infect Dis Clin North Am 2016;30:313–22, http://dx.doi.org/10.1016/j.idc.2016.02.001.
[5] Mohr Kathrin I. History of antibiotics research. Curr Top Microbiol Immunol 2016;358:3–32.
[6] Fortman JL, Mukhopadhyay A. The future of antibiotics: emerging technologies and stewardship. Trends Microbiol 2016;24:515–7, http://dx.doi.org/10.1016/j.tim.2016.04.003.
[7] Golkar Z, Bagasra O, Gene Pace D. Bacteriophage therapy: a potential solution for the antibiotic resistance crisis. J Infect Dev Ctries 2014;8:129–36, http://dx.doi.org/10.3855/jidc.3573.
[8] DiGiandomenico A, Sellman BR. Antibacterial monoclonal antibodies: the next generation? Curr Opin Microbiol 2015;27:78–85, http://dx.doi.org/10.1016/j.mib.2015.07.014.
[9] Thanawastien A, Cartee RT, Griffin TJ, Killeen KP, Mekalanos JJ. Conjugate-like immunogens produced as protein capsular matrix vaccines. Proc Natl Acad Sci U S A 2015;112:E1143–51, http://dx.doi.org/10.1073/pnas.1425005112.
[10] Bumbaširević M, Lesic A, Palibrk T, Milovanovic D, Zoka M, Kravić-Stevović T, et al. The current state of bionic limbs from the surgeon's viewpoint. EFORT Open Rev 2020;5:65–72, http://dx.doi.org/10.1302/2058-5241.5.180038.
[11] Czaplewski L, Bax R, Clokie M, Dawson M, Fairhead H, Fischetti VA, et al. Alternatives to antibiotics—a pipeline portfolio review. Lancet Infect Dis 2016;16:239–51, http://dx.doi.org/10.1016/S1473-3099(15)00466-1.
[12] Jayachandran. Pre-antibiotics era to post-antibiotic era. J Indian Acad Oral Med Radiol 2018;30:100, http://dx.doi.org/10.4103/JIAOMR.JIAOMR_29_18.
[13] Yang R. Plague: recognition, treatment, and prevention. J Clin Microbiol 2018;56, http://dx.doi.org/10.1128/JCM.01519-17.
[14] Twigg G. The black death and DNA. Lancet Infect Dis 2003;3:11, http://dx.doi.org/10.1016/S1473-3099(03)00480-8.
[15] Galy A, Loubet P, Peiffer-Smadja N, Yazdanpanah Y. The plague: an overview and hot topics. Rev Med Interne 2018;39:863–8, http://dx.doi.org/10.1016/j.revmed.2018.03.019.
[16] Gould K. Antibiotics: from prehistory to the present day. J Antimicrob Chemother 2016;71:572–5, http://dx.doi.org/10.1093/jac/dkv484.
[17] Durand GA, Raoult D, Dubourg G. Antibiotic discovery: history, methods and perspectives. Int J Antimicrob Agents 2019;53:371–82, http://dx.doi.org/10.1016/j.ijantimicag.2018.11.010.
[18] Nicolaou KC, Rigol S. A brief history of antibiotics and select advances in their synthesis. J Antibiot (Tokyo) 2018;71:153–84, http://dx.doi.org/10.1038/ja.2017.62.
[19] Pallasch TJ. Antibiotics: past, present, and future. CDA J 1986;14:65–8.
[20] Sykes JE, Papich MG. Antibacterial drugs. In: Canine and feline infectious diseases. Elsevier Inc.; 2013. p. 66–86, http://dx.doi.org/10.1016/B978-1-4377-0795-3.00008-9.
[21] Sköld O. Sulfonamide resistance: mechanisms and trends. Drug Resist Updat 2000;3:155–60, http://dx.doi.org/10.1054/drup.2000.0146.
[22] Tan SY, Tatsumura Y. Alexander Fleming (1881–1955): discoverer of penicillin. Singapore Med J 2015;56:366–7, http://dx.doi.org/10.11622/smedj.2015105.
[23] Gaynes R. The discovery of penicillin—new insights after more than 75 years of clinical use. Emerg Infect Dis 2017;23:849–53, http://dx.doi.org/10.3201/eid2305.161556.
[24] Khanna NR, Gerriets V. Beta lactamase inhibitors. StatPearls Publishing; 2021.
[25] Bush K, Bradford PA. β-lactams and β-lactamase inhibitors: an overview. Cold Spring Harb Perspect Med 2016;6, http://dx.doi.org/10.1101/cshperspect.a025247.
[26] WHO. Antibacterial agents in clinical development. Annu Rep Med Chem 2017;16:113–23.
[27] Frieri M, Kumar K, Boutin A. Antibiotic resistance. J Infect Public Health 2017;10:369–78, http://dx.doi.org/10.1016/j.jiph.2016.08.007.
[28] Plackett B. Why big pharma has abandoned antibiotics. Nature 2020;586:S50–2, http://dx.doi.org/10.1038/d41586-020-02884-3.
[29] O'Neill J. Publications | AMR review. Rev Antimicrob Resist Antimicrob Resist Tackling a Cris Heal Wealth Nations 2016:1–16.
[30] Matzov D, Bashan A, Yonath A. A bright future for antibiotics? Annu Rev Biochem 2017;86:567–83, http://dx.doi.org/10.1146/annurev-biochem-061516-044617.
[31] Weledji EP, Weledji EK, Assob JC, Nsagha DS. Pros, cons and future of antibiotics. New Horizons Transl Med 2017;4:9–14, http://dx.doi.org/10.1016/j.nhtm.2017.08.001.
[32] Quainoo S, Coolen JPM, van Hijum SAFT, Huynen MA, Melchers WJG, van Schaik W, et al. Whole-genome sequencing of bacterial pathogens: the future of nosocomial outbreak analysis. Clin Microbiol Rev 2017;30:1015–63, http://dx.doi.org/10.1128/CMR.00016-17.
[33] Hemmati F, Salehi R, Ghotaslou R, Kafil HS, Hasani A, Gholizadeh P, et al. Quorum quenching: a potential target for antipseudomonal therapy. Infect Drug Resist 2020;13:2989–3005, http://dx.doi.org/10.2147/IDR.S263196.
[34] Motley MP, Banerjee K, Fries BC. Monoclonal antibody-based therapies for bacterial infections. Curr Opin Infect Dis 2019;32:210–6, http://dx.doi.org/10.1097/QCO.0000000000000539.
[35] Bin Zaman S, Hussain MA, Nye R, Mehta V, Mamun KT, Hossain N. A review on antibiotic resistance: alarm bells are ringing. Cureus 2017, http://dx.doi.org/10.7759/cureus.1403.
[36] Subramaniam G, Girish M. Antibiotic resistance—a cause for reemergence of infections. Indian J Pediatr 2020;87:937–44, http://dx.doi.org/10.1007/s12098-019-03180-3.
[37] Economou V, Gousia P. Agriculture and food animals as a source of antimicrobial-resistant bacteria. Infect Drug Resist 2015;8:49–61, http://dx.doi.org/10.2147/IDR.S55778.
[38] European Centre for Disease Prevention and Control. Antimicrobial resistance in the EU/EEA (EARS-Net) - Annual Epidemiological Report 2019. Trop Doct 2020;30:114–6.
[39] Antimicrobial resistance in the EU/EEA (EARS-Net) - Annual Epidemiological Report for 2019 n.d.
[40] Shahriar A, Akter T, Kobra AT, Emran TB, Mallick J, Dutta M. Isolation of pathogenic and non-pathogenic microbial stains from different types of sea fish samples and their quality assessment with antibiogram properties. J Adv Microbiol 2019;19:1–10, http://dx.doi.org/10.9734/jamb/2019/v19i130185.
[41] GLASS | Global Antimicrobial Resistance Surveillance System (GLASS). WHO 2020.

[42] de Kraker MEA, Wolkewitz M, Davey PG, Koller W, Berger J, Nagler J, et al. Burden of antimicrobial resistance in European hospitals: excess mortality and length of hospital stay associated with bloodstream infections due to Escherichia coli resistant to third-generation cephalosporins. J Antimicrob Chemother 2011;66:398–407, http://dx.doi.org/10.1093/jac/dkq412.

[43] Migliori GB, Tiberi S, Zumla A, Petersen E, Chakaya JM, Wejse C, et al. MDR/XDR-TB management of patients and contacts: challenges facing the new decade. The 2020 clinical update by the Global Tuberculosis Network. Int J Infect Dis 2020;92:S15–25, http://dx.doi.org/10.1016/j.ijid.2020.01.042.

[44] Ballestero JG de A, Garcia JM, Bollela VR, Ruffino-Netto A, Dalcolmo MMP, Moncaio ACS, et al. Management of multidrug-resistant tuberculosis: main recommendations of the brazilian guidelines. J Bras Pneumol 2020;46:1–9, http://dx.doi.org/10.36416/1806-3756/e20190290.

[45] Woolhouse M, Waugh C, Perry MR, Nair H. Global disease burden due to antibiotic resistance—state of the evidence. J Glob Health 2016;6, http://dx.doi.org/10.7189/jogh.06.010306.

[46] MacGowan A, Macnaughton E. Antibiotic resistance. Med (United Kingdom) 2017;45:622–8, http://dx.doi.org/10.1016/j.mpmed.2017.07.006.

[47] Munita JM, Arias CA. Mechanisms of antibiotic resistance. In: Virulence mechanisms of bacterial pathogens. wiley; 2016. p. 481–511, http://dx.doi.org/10.1128/9781555819286.ch17.

[48] Chokshi A, Sifri Z, Cennimo D, Horng H. Global contributors to antibiotic resistance. J Glob Infect Dis 2019;11:36–42, http://dx.doi.org/10.4103/jgid.jgid_110_18.

[49] Mahmoud MA, Aldhaeefi M, Sheikh A, Aljadhey H. Community pharmacists perspectives about reasons behind antibiotics dispensing without prescription: a qualitative study. Biomed Res 2018;29, http://dx.doi.org/10.4066/biomedicalresearch.29-18-1112.

[50] Sreeja MK, Gowrishankar NL, Adisha S, Divya KC. Antibiotic resistance-reasons and the most common resistant pathogens – a review. Res J Pharm Technol 2017;10:1886–90, http://dx.doi.org/10.5958/0974-360X.2017.00331.6.

[51] Ventola CL. The antibiotic resistance crisis: causes and threats: part 1: causes and threats. Pharm Ther 2015;40:277–83.

[52] Holmes AH, Moore LSP, Sundsfjord A, Steinbakk M, Regmi S, Karkey A, et al. Understanding the mechanisms and drivers of antimicrobial resistance. Lancet 2016;387:176–87, http://dx.doi.org/10.1016/S0140-6736(15)00473-0.

[53] Martínez JL, Coque TM, Baquero F. What is a resistance gene? Ranking risk in resistomes. Nat Rev Microbiol 2015;13:116–23, http://dx.doi.org/10.1038/nrmicro3399.

[54] Von Wintersdorff CJH, Penders J, Van Niekerk JM, Mills ND, Majumder S, Van Alphen LB, et al. Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. Front Microbiol 2016;7, http://dx.doi.org/10.3389/fmicb.2016.00173.

[55] Zhao R, Feng J, Liu J, Fu W, Li X, Li B. Deciphering of microbial community and antibiotic resistance genes in activated sludge reactors under high selective pressure of different antibiotics. Water Res 2019;151:388–402, http://dx.doi.org/10.1016/j.watres.2018.12.034.

[56] Denning DW, Perlin DS, Muldoon EG, Colombo AL, Chakrabarti A, Richardson MD, et al. Delivering on antimicrobial resistance agenda not possible without improving fungal diagnostic capabilities. Emerg Infect Dis 2017;23:177–83, http://dx.doi.org/10.3201/eid2302.152042.

[57] Marc C, Vrignaud B, Levieux K, Robine A, Le Guen CG, Launay E. Inappropriate prescription of antibiotics in pediatric practice: analysis of the prescriptions in primary care. J Child Health Care 2016;20:530–6, http://dx.doi.org/10.1177/1367493516643421.

[58] Ventola CL. The antibiotic resistance crisis: causes and threats. P T J 2015;40:277–83, https://doi.org/Article.

[59] Nepal G, Bhatta S. Self-medication with antibiotics in WHO Southeast Asian region: a systematic review. Cureus 2018;10, http://dx.doi.org/10.7759/cureus.2428.

[60] Sulis G, Daniels B, Kwan A, Gandra S, Daftary A, Das J, et al. Antibiotic overuse in the primary health care setting: a secondary data analysis of standardised patient studies from India, China and Kenya. BMJ Glob Heal 2020;5:3393, http://dx.doi.org/10.1136/bmjgh-2020-003393.

[61] Almagor J, Temkin E, Benenson I, Fallach N, Carmeli Y. The impact of antibiotic use on transmission of resistant bacteria in hospitals: insights from an agent-based model. PLoS One 2018;13:e0197111, http://dx.doi.org/10.1371/journal.pone.0197111.

[62] Manyi-Loh C, Mamphweli S, Meyer E, Okoh A. Antibiotic use in agriculture and its consequential resistance in environmental sources: potential public health implications. Molecules 2018;23, http://dx.doi.org/10.3390/molecules23040795.

[63] Garima K, Saurabh S, Ashok E. Action and resistance mechanisms of antibiotics: a guide for clinicians. J Anaesthesiol Clin Pharmacol 2018;34:46–50.

[64] Levin PA, Angert ER. Small but mighty: cell size and bacteria. Cold Spring Harb Perspect Biol 2015;7:1–11, http://dx.doi.org/10.1101/cshperspect.a019216.

[65] Mai-Prochnow A, Clauson M, Hong J, Murphy AB. Gram positive and gram negative bacteria differ in their sensitivity to cold plasma. Sci Rep 2016;6, http://dx.doi.org/10.1038/srep38610.

[66] Page MGP. Beta-lactam antibiotics. Antibiotic discovery and development, vol. 9781461414. Springer US; 2012. p. 79–117, http://dx.doi.org/10.1007/978-1-4614-1400-1_3.

[67] Wang F, Zhou H, Olademehin OP, Kim SJ, Tao P. Insights into key interactions between vancomycin and bacterial cell wall structures. ACS Omega 2018;3:37–45, http://dx.doi.org/10.1021/acsomega.7b01483.

[68] Krause KM, Serio AW, Kane TR, Connolly LE. Aminoglycosides: an overview. Cold Spring Harb Perspect Med 2016;6, http://dx.doi.org/10.1101/cshperspect.a027029.

[69] Grossman TH. Tetracycline antibiotics and resistance. Cold Spring Harb Perspect Med 2016;6, http://dx.doi.org/10.1101/cshperspect.a025387.

[70] Parnham MJ, Haber VE, Giamarellos-Bourboulis EJ, Perletti G, Verleden GM, Vos R. Azithromycin: mechanisms of action and their relevance for clinical applications. Pharmacol Ther 2014;143:225–45, http://dx.doi.org/10.1016/j.pharmthera.2014.03.003.

[71] Dinos GP, Athanassopoulos CM, Missiri DA, Giannopoulou PC, Vlachogiannis IA, Papadopoulos GE, et al. Chloramphenicol derivatives as antibacterial and anticancer agents: historic problems and current solutions. Antibiotics 2016;5, http://dx.doi.org/10.3390/antibiotics5020020.

[72] Papich MG. Linezolid. In: Saunders handbook of veterinary drugs. Elsevier; 2016. p. 451–2, http://dx.doi.org/10.1016/B978-0-323-24485-5.00339-9.

[73] Bhattacharjee MK. Antibiotics that inhibit nucleic acid synthesis. In: Chemistry of antibiotics and related drugs. Springer International Publishing; 2016. p. 109–28, http://dx.doi.org/10.1007/978-3-319-40746-3_5.

[74] Saito K, Warrier T, Somersan-Karakaya S, Kaminski L, Mi J, Jiang X, et al. Rifamycin action on RNA polymerase in antibiotictolerant *Mycobacterium tuberculosis* results in differentially detectable populations. Proc Natl Acad Sci U S A 2017;114:E4832–40, http://dx.doi.org/10.1073/pnas.1705385114.

[75] Nainu F, Masyita A, Bahar MA, Raihan M, Prova SR, Mitra S, et al. Pharmaceutical prospects of bee products: Special focus on anticancer, antibacterial, antiviral, and antiparasitic properties. Antibiotics 2021;10:822, http://dx.doi.org/10.3390/antibiotics10070822.

[76] Aldred KJ, Kerns RJ, Osheroff N. Mechanism of quinolone action and resistance. Biochemistry 2014;53:1565–74, http://dx.doi.org/10.1021/bi5000564.

[77] Fernández-Villa D, Aguilar MR, Rojo L. Folic acid antagonists: antimicrobial and immunomodulating mechanisms and applications. Int J Mol Sci 2019;20, http://dx.doi.org/10.3390/ijms20204996.

[78] Akter T, Shahriar A, Alo M, Rahman T, Emran TB. Survival assessment of pathogenic bacteria with antibiotic resistance traits from fresh summer royal grape: In vitro microbial challenge test. J Microbiol Biotechnol Food Sci 2020;10:344–9, http://dx.doi.org/10.15414/jmbfs.2020.10.3.344-349.

[79] Wróbel A, Maliszewski D, Baradyn M, Drozdowska D. Trimethoprim: an old antibacterial drug as a template to search for new targets. Synthesis, biological activity and molecular modeling study of novel trimethoprim analogs. Molecules 2020;25, http://dx.doi.org/10.3390/molecules25010116.

[80] Poirel L, Jayol A, Nordmanna P. Polymyxins: antibacterial activity, susceptibility testing, and resistance mechanisms encoded by plasmids or chromosomes. Clin Microbiol Rev 2017;30:557–96, http://dx.doi.org/10.1128/CMR.00064-16.

[81] Trimble MJ, Mlynárčik P, Kolář M, Hancock REW. Polymyxin: alternative mechanisms of action and resistance. Cold Spring Harb Perspect Med 2016;6, http://dx.doi.org/10.1101/cshperspect.a025288.

[82] Reygaert WC. An overview of the antimicrobial resistance mechanisms of bacteria. AIMS Microbiol 2018;4:482–501, http://dx.doi.org/10.3934/microbiol.2018.3.482.

[83] Lerminiaux NA, Cameron ADS. Horizontal transfer of antibiotic resistance genes in clinical environments. Can J Microbiol 2019;65:34–44, http://dx.doi.org/10.1139/cjm-2018-0275.

[84] Culyba MJ, Mo CY, Kohli RM. Targets for combating the evolution of acquired antibiotic resistance. Biochemistry 2015;54:3573–82, http://dx.doi.org/10.1021/acs.biochem.5b00109.

[85] Hoffman SB. Mechanisms of antibiotic resistance. Compend Contin Educ Pract Vet 2001;23:464–72, http://dx.doi.org/10.1128/microbiolspec.vmbf-0016-2015.

[86] Blair JMA, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJV. Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol 2015;13:42–51, http://dx.doi.org/10.1038/nrmicro3380.

[87] Iredell J, Brown J, Tagg K. Antibiotic resistance in *Enterobacteriaceae*: mechanisms and clinical implications. BMJ 2016;352, http://dx.doi.org/10.1136/bmj.h6420.

[88] Pang Z, Raudonis R, Glick BR, Lin TJ, Cheng Z. Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and alternative therapeutic strategies. Biotechnol Adv 2019;37:177–92, http://dx.doi.org/10.1016/j.biotechadv.2018.11.013.

[89] Hall CW, Mah TF. Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. FEMS Microbiol Rev 2017;41:276–301, http://dx.doi.org/10.1093/femsre/fux010.

[90] Lin J, Nishino K, Roberts MC, Tolmasky M, Aminov RI, Zhang L. Mechanisms of antibiotic resistance. Front Microbiol 2015;6:34, http://dx.doi.org/10.3389/fmicb.2015.00034.

[91] Foster TJ. Antibiotic resistance in *Staphylococcus aureus*. Current status and future prospects. FEMS Microbiol Rev 2017;41:430–49, http://dx.doi.org/10.1093/femsre/fux007.

[92] Peterson E, Kaur P. Antibiotic resistance mechanisms in bacteria: relationships between resistance determinants of antibiotic producers, environmental bacteria, and clinical pathogens. Front Microbiol 2018;9:2928, http://dx.doi.org/10.3389/fmicb.2018.02928.

[93] Brooks BD, Brooks AE. Therapeutic strategies to combat antibiotic resistance. Adv Drug Deliv Rev 2014;78:14–27, http://dx.doi.org/10.1016/j.addr.2014.10.027.

[94] Hamoud R, Zimmermann S, Reichling J, Wink M. Synergistic interactions in two-drug and three-drug combinations (thymol, EDTA and vancomycin) against multi drug resistant bacteria including *E. coli*. Phytomedicine 2014;21:443–7, http://dx.doi.org/10.1016/j.phymed.2013.10.016.

[95] Smith JK, Moshref AR, Jennings JA, Courtney HS, Haggard WO. Chitosan sponges for local synergistic infection therapy: a pilot study. Clin Orthop Relat Res 2013;471:3158–64, http://dx.doi.org/10.1007/s11999-013-2988-5.

[96] Ejim L, Farha MA, Falconer SB, Wildenhain J, Coombes BK, Tyers M, et al. Combinations of antibiotics and nonantibiotic drugs enhance antimicrobial efficacy. Nat Chem Biol 2011;7:348–50, http://dx.doi.org/10.1038/nchembio.559.

[97] Fischbach MA. Combination therapies for combating antimicrobial resistance. Curr Opin Microbiol 2011;14:519–23, http://dx.doi.org/10.1016/j.mib.2011.08.003.

[98] Phougat N, Khatri S, Singh A, Dangi M, Kumar M, Dabur R, et al. Combination therapy: the propitious rationale for drug development. Comb Chem High Throughput Screen 2014;17:53–67, http://dx.doi.org/10.2174/1386207313166660065.

[99] Read AF, Huijben S. Evolutionary biology and the avoidance of antimicrobial resistance. Evol Appl 2009;2:40–51, http://dx.doi.org/10.1111/j.1752-4571.2008.00066.x.

[100] Payne DJ, Gwynn MN, Holmes DJ, Pompliano DL. Drugs for bad bugs: confronting the challenges of antibacterial discovery. Nat Rev Drug Discov 2007;6:29–40, http://dx.doi.org/10.1038/nrd2201.

[101] Ahmed Z, Saeed Khan S, Khan M. In vitro trials of some antimicrobial combinations against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Saudi J Biol Sci 2013;20:79–83, http://dx.doi.org/10.1016/j.sjbs.2012.10.005.

[102] Wood K, Nishida S, Sontag ED, Cluzel P. Mechanism-independent method for predicting response to multidrug combinations in bacteria. Proc Natl Acad Sci U S A 2012;109:12254–9, http://dx.doi.org/10.1073/pnas.1201281109.

[103] Peña-Miller R, Lähnemann D, Schulenburg H, Ackermann M, Beardmore R. The optimal deployment of synergistic antibiotics: a control-theoretic approach. J R Soc Interface 2012;9:2488–502, http://dx.doi.org/10.1098/rsif.2012.0279.

[104] Hegreness M, Shoresh N, Damian D, Hartl D, Kishony R. Accelerated evolution of resistance in multidrug environments. Proc Natl Acad Sci U S A 2008;105:13977–81, http://dx.doi.org/10.1073/pnas.0805965105.

[105] Yeh PJ, Hegreness MJ, Aiden AP, Kishony R. Drug interactions and the evolution of antibiotic resistance. Nat Rev Microbiol 2009;7:460–6, http://dx.doi.org/10.1038/nrmicro2133.

[106] Pena-Miller R, Laehnemann D, Jansen G, Fuentes-Hernandez A, Rosenstiel P, Schulenburg H, et al. When the most potent combination of antibiotics selects for the greatest bacterial load: the smile-frown transition. PLoS Biol 2013;11, http://dx.doi.org/10.1371/journal.pbio.1001540.

[107] Lázár V, Pal Singh G, Spohn R, Nagy I, Horváth B, Hrtyan M, et al. Bacterial evolution of antibiotic hypersensitivity. Mol Syst Biol 2013;9, http://dx.doi.org/10.1038/msb.2013.57.

[108] Goldberg DE, Siliciano RF, Jacobs WR. Outwitting evolution: fighting drug-resistant TB, malaria, and HIV. Cell 2012;148:1271–83, http://dx.doi.org/10.1016/j.cell.2012.02.021.

[109] Kalan L, Wright GD. Antibiotic adjuvants: multicomponent anti-infective strategies. Expert Rev Mol Med 2011;13, http://dx.doi.org/10.1017/S1462399410001766.

[110] Roemer T, Schneider T, Pinho MG. Auxiliary factors: a chink in the armor of MRSA resistance to β-lactam antibiotics. Curr Opin Microbiol 2013;16:538–48, http://dx.doi.org/10.1016/j.mib.2013.06.012.

[111] Hasper HE, Kramer NE, Smith JL, Hillman JD, Zachariah C, Kuipers OP, et al. An alternative bactericidal mechanism of action for lantibiotic peptides that target lipid II. Science (80-) 2006;313:1636–7, http://dx.doi.org/10.1126/science.1129818.

[112] Allahverdiyev AM, Kon KV, Abamor ES, Bagirova M, Rafailovich M. Coping with antibiotic resistance: combining nanoparticles with antibiotics and other antimicrobial agents. Expert Rev Anti Infect Ther 2011;9:1035–52, http://dx.doi.org/10.1586/eri.11.121.

[113] Bollenbach T, Quan S, Chait R, Kishony R. Nonoptimal microbial response to antibiotics underlies suppressive drug interactions. Cell 2009;139:707–18, http://dx.doi.org/10.1016/j.cell.2009.10.025.

[114] Gholizadeh P, Köse Ş, Dao S, Ganbarov K, Tanomand A, Dal T, et al. How CRISPR-Cas system could be used to combat antimicrobial resistance. Infect Drug Resist 2020;13:1111–21, http://dx.doi.org/10.2147/IDR.S247271.

[115] Goren MG, Yosef I, Edgar R, Qimron U. The bacterial CRISPR/Cas system as analog of the mammalian adaptive immune system. RNA Biol 2012;9:549–54, http://dx.doi.org/10.4161/rna.20177.

[116] de Olivera FT, Chanda A, Banerjee D, Shan X, Mondal S, Que L, et al. Small CRISPR RNAs guide antiviral defense in prokaryotes. Science (80-) 2006.

[117] Makarova KS, Grishin NV, Shabalina SA, Wolf YI, Koonin EV. A putative RNA-interference-based immune system in prokaryotes: computational analysis of the predicted enzymatic machinery, functional analogies with eukaryotic RNAi, and hypothetical mechanisms of action. Biol Direct 2006;1, http://dx.doi.org/10.1186/1745-6150-1-7.

[118] Makarova KS, Wolf YI, Iranzo J, Shmakov SA, Alkhnbashi OS, Brouns SJJ, et al. Evolutionary classification of CRISPR–Cas systems: a burst of class 2 and derived variants. Nat Rev Microbiol 2020;18:67–83, http://dx.doi.org/10.1038/s41579-019-0299-x.

[119] Grissa I, Vergnaud G, Pourcel C, Ishino Y, Shinagawa H, Makino K, et al. An updated evolutionary classification of CRISPR-Cas systems. Nat Rev Microbiol 2015;36:1–10.

[120] Horvath Philippe, Barrangou Rodolphe. CRISPR/Cas, the immune system of bacteria and archaea. Science (80-) 2010.

[121] Makarova Kira S, Wolf Yuri I, Alkhnbashi Omer S, Fabrizio C, Shah Shiraz A, Saunders Sita J, et al. An updated evolutionary classification of CRISPR-Cas systems. Nat Rev Microbiol 2015;13:722–36.

[122] Hale CR, Majumdar S, Elmore J, Pfister N, Compton M, Olson S, et al. Essential features and rational design of CRISPR RNAs that function with the cas RAMP module complex to cleave RNAs. Mol Cell 2012;45:292–302, http://dx.doi.org/10.1016/j.molcel.2011.10.023.

[123] Bikard D, Euler CW, Jiang W, Nussenzweig PM, Goldberg GW, Duportet X, et al. Exploiting CRISPR-cas nucleases to produce sequence-specific antimicrobials. Nat Biotechnol 2014;32:1146–50, http://dx.doi.org/10.1038/nbt.3043.

[124] Touchon M, Charpentier S, Pognard D, Picard B, Arlet G, Rocha EPC, et al. Antibiotic resistance plasmids spread among natural isolates of *Escherichia coli* in spite of CRISPR elements. Microbiol (United Kingdom) 2012;158:2997–3004, http://dx.doi.org/10.1099/mic.0.060814-0.

[125] Shahriar A, Kobra AT, Shomi FY, Emran TB, Mallick J, Dutta M. Presumptive correlation between phenotypic, genotypic and symbiotic diversities with antibiotic susceptibility traits of rhizobial strains from plant legumes. J Adv Biotechnol Exp Ther 2020;3:59–69, http://dx.doi.org/10.5455/jabet.2020.d108.

[126] Gholizadeh P, Aghazadeh M, Asgharzadeh M, Kafil HS. Suppressing the CRISPR/Cas adaptive immune system in bacterial infections. Eur J Clin Microbiol Infect Dis 2017;36:2043–51, http://dx.doi.org/10.1007/s10096-017-3036-2.

[127] Moineau S, Barrangou R, Boyaval P, Deveau H, Romero DA, Horvath P, et al. CRISPR provides acquired resistance against viruses in prokaryotes. Science (80-) 2007;315:1709–12.

[128] Touchon M, Rocha EPC. The small, slow and specialized CRISPR and anti-CRISPR of *Escherichia* and *Salmonella*. PLoS One 2010;5, http://dx.doi.org/10.1371/journal.pone.0011126.

[129] Díez-Villaseñor C, Almendros C, García-Martínez J, Mojica FJM. Diversity of CRISPR loci in *Escherichia coli*. Microbiology 2010;156:1351–61, http://dx.doi.org/10.1099/mic.0.036046-0.

[130] Ubukata K, Nonoguchi R, Matsuhashi M, Konno M. Expression and inducibility in *Staphylococcus aureus* of the mecA gene, which encodes a methicillin-resistant *S. Aureus*-specific penicillin-binding protein. J Bacteriol 1989;171:2882–5, http://dx.doi.org/10.1128/jb.171.5.2882-2885.1989.

[131] Roach DR, Leung CY, Henry M, Morello E, Singh D, Di Santo JP, et al. Synergy between the host immune system and bacteriophage is essential for successful phage therapy against an acute respiratory pathogen. Cell Host Microbe 2017;22:38–47.e4, http://dx.doi.org/10.1016/j.chom.2017.06.018.

[132] Hemlata, Tiwari A. Applications of bioinformatics tools to combat the antibiotic resistance. Int Conf Soft Comput Tech Implementations, ICSCTI 2015 2016:96–8, http://dx.doi.org/10.1109/ICSCTI.2015.7489545.

[133] Sartini S, Permana AD, Mitra S, Tareq AM, Salim E, Ahmad I, et al. Current state and promising opportunities on pharmaceutical approaches in the treatment of polymicrobial diseases. Pathogens 2021;10:245, http://dx.doi.org/10.3390/pathogens10020245.

[134] Biswas S, Raoult D, Rolain JM. A bioinformatic approach to understanding antibiotic resistance in intracellular bacteria through whole genome analysis. Int J Antimicrob Agents 2008;32:207–20, http://dx.doi.org/10.1016/j.ijantimicag.2008.03.017.

[135] Gwinn M, Maccannell D, Armstrong GL. Next-generation sequencing of infectious pathogens. JAMA - J Am Med Assoc 2019;321:893–4, http://dx.doi.org/10.1001/jama.2018.21669.

[136] Blundell TL, Sibanda BL, Montalavão RW, Brewerton S, Chelliah V, Worth CL, et al. Structural biology and bioinformatics in drug design: opportunities and challenges for target identification and lead discovery. Philos Trans R Soc Br 2005;10:1–11.

[137] Zhang X, Payne M, Lan R. In silico identification of serovar-specific genes for salmonella serotyping. Front Microbiol 2019;10, http://dx.doi.org/10.3389/fmicb.2019.00835.

[138] Zhou F, Xu Y. cBar: a computer program to distinguish plasmid-derived from chromosome-derived sequence fragments in metagenomics data. Bioinformatics 2010;26:2051–2, http://dx.doi.org/10.1093/bioinformatics/btq299.

[139] Lanza VF, de Toro M, Garcillán-Barcia MP, Mora A, Blanco J, Coque TM, et al. Plasmid flux in *Escherichia coli* ST131 sublineages, analyzed by plasmid constellation network (PLACNET), a new method for plasmid reconstruction from whole genome sequences. PLoS Genet 2014;10, http://dx.doi.org/10.1371/journal.pgen.1004766.

[140] Antipov D, Hartwick N, Shen M, Raiko M, Lapidus A, Pevzner PA. PlasmidSPAdes: assembling plasmids from whole genome sequencing data. Bioinformatics 2016;32:3380–7, http://dx.doi.org/10.1093/bioinformatics/btw493.

[141] Emran TB, Nasir Uddin MM, Rahman A, Uddin Z, Islam M. Phytochemical, antimicrobial, cytotoxic, analgesic and anti-inflammatory properties of *Azadirachta indica*: a therapeutic study. J Bioanal Biomed 2015;S12:1–7, http://dx.doi.org/10.4172/1948-593X.S12-007.

[142] Jesus TF, Ribeiro-Gonçalves B, Silva DN, Bortolaia V, Ramirez M, Carriço JA. Plasmid ATLAS: plasmid visual analytics and identification in high-throughput sequencing data. Nucleic Acids Res 2019;47:D188–94, http://dx.doi.org/10.1093/nar/gky1073.

[143] Orlek A, Stoesser N, Anjum MF, Doumith M, Ellington MJ, Peto T, et al. Plasmid classification in an era of whole-genome sequencing: application in studies of antibiotic resistance epidemiology. Front Microbiol 2017;8, http://dx.doi.org/10.3389/fmicb.2017.00182.
[144] Hall JPJ, Brockhurst MA, Harrison E. Sampling the mobile gene pool: innovation via horizontal gene transfer in bacteria. Philos Trans R Soc B Biol Sci 2017;372, http://dx.doi.org/10.1098/rstb.2016.0424.
[145] Shintani M, Sanchez ZK, Kimbara K. Genomics of microbial plasmids: classification and identification based on replication and transfer systems and host taxonomy. Front Microbiol 2015;6, http://dx.doi.org/10.3389/fmicb.2015.00242.
[146] Frost LS, Leplae R, Summers AO, Toussaint A. Mobile genetic elements: the agents of open source evolution. Nat Rev Microbiol 2005;3:722–32, http://dx.doi.org/10.1038/nrmicro1235.
[147] Carattoli A, Zankari E, Garciá-Fernández A, Larsen MV, Lund O, Villa L, et al. In silico detection and typing of plasmids using plasmidfinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother 2014;58:3895–903, http://dx.doi.org/10.1128/AAC.02412-14.
[148] O'Leary NA, Wright MW, Brister JR, Ciufo S, Haddad D, McVeigh R, et al. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res 2016;44:D733–45, http://dx.doi.org/10.1093/nar/gkv1189.
[149] Kudirkiene E, Andoh LA, Ahmed S, Herrero-Fresno A, Dalsgaard A, Obiri-Danso K, et al. The use of a combined bioinformatics approach to locate antibiotic resistance genes on plasmids from whole genome sequences of *Salmonella enterica* serovars from humans in Ghana. Front Microbiol 2018;9, http://dx.doi.org/10.3389/fmicb.2018.01010.
[150] Moradigaravand D, Palm M, Farewell A, Mustonen V, Warringer J, Parts L. Prediction of antibiotic resistance in *Escherichia coli* from large-scale pan-genome data. PLoS Comput Biol 2018;14, http://dx.doi.org/10.1371/journal.pcbi.1006258.
[151] Rozwandowicz M, Brouwer MSM, Fischer J, Wagenaar JA, Gonzalez-Zorn B, Guerra B, et al. Plasmids carrying antimicrobial resistance genes in *Enterobacteriaceae*. J Antimicrob Chemother 2018;73:1121–37, http://dx.doi.org/10.1093/jac/dkx488.
[152] Hendriksen RS, Bortolaia V, Tate H, Tyson GH, Aarestrup FM, McDermott PF. Using genomics to track global antimicrobial resistance. Front Public Health Serv Syst Res 2019;7, http://dx.doi.org/10.3389/fpubh.2019.00242.
[153] Munk P, Andersen VD, de Knegt L, Jensen MS, Knudsen BE, Lukjancenko O, et al. A sampling and metagenomic sequencing-based methodology for monitoring antimicrobial resistance in swine herds. J Antimicrob Chemother 2017;72:385–92, http://dx.doi.org/10.1093/jac/dkw415.
[154] Munk P, Knudsen BE, Lukjacenko O, Duarte ASR, Van Gompel L, Luiken REC, et al. Abundance and diversity of the faecal resistome in slaughter pigs and broilers in nine European countries. Nat Microbiol 2018;3:898–908, http://dx.doi.org/10.1038/s41564-018-0192-9.
[155] Van Gompel L, Luiken REC, Sarrazin S, Munk P, Knudsen BE, Hansen RB, et al. The antimicrobial resistome in relation to antimicrobial use and biosecurity in pig farming, a metagenome-wide association study in nine European countries. J Antimicrob Chemother 2019;74:865–76, http://dx.doi.org/10.1093/jac/dky518.
[156] Alcock BP, Raphenya AR, Lau TTY, Tsang KK, Bouchard M, Edalatmand A, et al. CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. Nucleic Acids Res 2020;48:D517–25, http://dx.doi.org/10.1093/nar/gkz935.
[157] Nainu F, Permana AD, Djide NJN, Anjani QK, Utami RN, Rumata NR, et al. Pharmaceutical approaches on antimicrobial resistance: prospects and challenge. Antibiotics 2021;10:981, http://dx.doi.org/10.3390/antibiotics10080981.
[158] Tiwari R, Dhama K. Antibiotic resistance: a frightening health dilemma. Am J Pharmacol Toxicol 2014;9:174–6, http://dx.doi.org/10.3844/ajptsp.2014.174.176.
[159] Ghannad MS, Mohammadi A. Bacteriophage: time to re-evaluate the potential of phage therapy as a promising agent to control multidrug-resistant bacteria. Iran J Basic Med Sci 2012;15:693–701, http://dx.doi.org/10.22038/ijbms.2012.4840.
[160] Hasan ME, Shahriar A, Shams F, Nath AK, Emran TB. Correlation between biofilm formation and antimicrobial susceptibility pattern toward extended spectrum β-lactamase (ESBL)-and non-ESBL-producing uropathogenic bacteria. J Basic Clin Physiol Pharmacol 2021;32:1–15, http://dx.doi.org/10.1515/jbcpp-2019-0296.
[161] Embleton ML, Nair SP, Heywood W, Menon DC, Cookson BD, Wilson M. Development of a novel targeting system for lethal photosensitization of antibiotic-resistant strains of *Staphylococcus aureus*. Antimicrob Agents Chemother 2005;49:3690–6, http://dx.doi.org/10.1128/AAC.49.9.3690-3696.2005.
[162] Larocca D, Brug MA, Jensen-pergakes K, Ravey E, Gonzalez A, Baird A. Evolving phage vectors for cell targeted gene delivery. Curr Pharm Biotechnol 2005;3:45–57, http://dx.doi.org/10.2174/1389201023378490.
[163] Young R, Gill JJ. Phage therapy redux—what is to be done? Science (80-) 2015;350:1163–4, http://dx.doi.org/10.1126/science.aad6791.
[164] Bertozzi Silva J, Storms Z, Sauvageau D. Host receptors for bacteriophage adsorption. FEMS Microbiol Lett 2016;363, http://dx.doi.org/10.1093/femsle/fnw002.
[165] Lin DM, Koskella B, Lin HC. Phage therapy: an alternative to antibiotics in the age of multi-drug resistance. World J Gastrointest Pharmacol Ther 2017;8:162, http://dx.doi.org/10.4292/wjgpt.v8.i3.162.
[166] Kollberg H. Avian antibodies (IgY)—a new weapon against antibiotic resistance. Clin Microbiol Open Access 2017;06, http://dx.doi.org/10.4172/2327-5073.1000304.
[167] Gordillo Altamirano FL, Barr JJ. Phage therapy in the postantibiotic era. Clin Microbiol Rev 2019;32, http://dx.doi.org/10.1128/CMR.00066-18.
[168] Ghosh C, Sarkar P, Issa R, Haldar J. Alternatives to conventional antibiotics in the era of antimicrobial resistance. Trends Microbiol 2019;27:323–38, http://dx.doi.org/10.1016/j.tim.2018.12.010.
[169] Kaufmann SHE, Flesch IEA. Cytokines in antibacterial resistance: possible applications for immunomodulation. Lung 1990;168:1025–32, http://dx.doi.org/10.1007/BF02718240.
[170] Eleraky NE, Allam A, Hassan SB, Omar MM. Nanomedicine fight against antibacterial resistance: an overview of the recent pharmaceutical innovations. Pharmaceutics 2020;12, http://dx.doi.org/10.3390/pharmaceutics12020142.
[171] Lombardo D, Kiselev MA, Caccamo MT. Smart nanoparticles for drug delivery application: development of versatile nanocarrier platforms in biotechnology and nanomedicine. J Nanomater 2019;2019, http://dx.doi.org/10.1155/2019/3702518.
[172] Abed N, Couvreur P. Nanocarriers for antibiotics: a promising solution to treat intracellular bacterial infections. Int J Antimicrob Agents 2014;43:485–96, http://dx.doi.org/10.1016/j.ijantimicag.2014.02.009.
[173] Terreni M, Taccani M, Pregnolato M. New antibiotics for multidrug-resistant bacterial strains: latest research developments and future perspectives. Molecules 2021;26, http://dx.doi.org/10.3390/molecules26092671.
[174] Ouwehand AC, Forssten S, Hibberd AA, Lyra A, Stahl B. Probiotic approach to prevent antibiotic resistance. Ann Med 2016;48:246–55, http://dx.doi.org/10.3109/07853890.2016.1161232.
[175] Vadhana P, Singh BR, Bharadwaj M. Emergence of herbal antimicrobial drug resistance in clinical bacterial isolates. Pharm Anal Acta 2015;6, http://dx.doi.org/10.4172/2153-2435.1000434.
[176] Gelband H, Laxminarayan R. Tackling antimicrobial resistance at global and local scales. Trends Microbiol 2015;23:524–6, http://dx.doi.org/10.1016/j.tim.2015.06.005.
[177] Laxminarayan R, Gelband H. A global subsidy: key to affordable drugs for malaria? Health Aff 2009;28:949–61, http://dx.doi.org/10.1377/hlthaff.28.4.949.
[178] Arrow KJ, Panosian C, Gelband H. Saving lives, buying time: economics of malaria drugs in an age of resistance. Inst Med Comm Econ Antimalar Drugs 2004:125.
[179] Freeman A, Kwarteng A, Febir LG, Amenga-Etego S, Owusu-Agyei S, Asante KP. Two years post affordable medicines facility for malaria program: availability and prices of anti-malarial drugs in central Ghana. J Pharm Policy Pract 2017;10, http://dx.doi.org/10.1186/s40545-017-0103-0.
[180] Rahman MA, bin Imran T, Islam S. Antioxidative, antimicrobial and cytotoxic effects of the phenolics of *Leea indica* leaf extract. Saudi J Biol Sci 2013;20:213–25, http://dx.doi.org/10.1016/j.sjbs.2012.11.007.
[181] Nguyen KV, Do NTT, Chandna A, Nguyen TV, Pham CV, Doan PM, et al. Antibiotic use and resistance in emerging economies: a situation analysis for Viet Nam. BMC Public Health 2013;13:1158.
[182] Shahriar A, Alo M, Hossain MF, Emran TB, Uddin MZ, Paul A. Prevalence of multi-drug resistance traits in probiotic bacterial species from fermented milk products in Bangladesh. Microbiol Res Int 2019;29:1–10, http://dx.doi.org/10.9734/mrji/2019/v29i230161.