Molecular mechanisms of multidrug resistance in clinically relevant enteropathogenic bacteria (Review)

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Received June 27 2022; Accepted September 21, 2022

DOI: 10.3892/etm.2022.11689

Abstract. Multidrug resistant (MDR) enteropathogenic bacteria are a growing problem within the clinical environment due to their acquired tolerance to a wide range of antibiotics, thus causing severe illnesses and a tremendous economic impact in the healthcare sector. Due to its difficult treatment, knowledge and understanding of the molecular mechanisms that confer this resistance are needed. The aim of the present review is to describe the mechanisms of antibiotic resistance from a genomic perspective observed in bacteria, including naturally acquired resistance. The present review also discusses common pharmacological and alternative treatments used in cases of infection caused by MDR bacteria, thus covering necessary information for the development of novel antimicrobials and adjuvant molecules inhibiting bacterial proliferation.

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Key words: enteropathogenic bacteria, multidrug resistance, gastrointestinal disease, horizontal gene transfer, therapeutic research

1. Introduction

Gastrointestinal diseases are the most frequent cause for medical consultation and one of the leading causes of death worldwide (1,2). In America, 77 million individuals get sick annually due to food poisoning and, according to the World Health Organization (WHO), one in ten individuals get sick each year for the same reason worldwide. As a result, ~420,000 individuals die on a yearly basis, of which ~30% are children under five years of age. It must be noted that diarrheal diseases correspond to more than half of the cases of gastrointestinal illnesses, for which ~95% of cases can be associated with *Campylobacter spp., Escherichia coli* and non-typhoidal *Salmonella spp.* (3).

In 2017, the WHO published a list of drug resistant bacteria for which there is a growing need to develop new antibiotics as even the current most effective of them, such as carbapenems and cephalosporins, are now ineffective. This list is divided into three categories (critical, high, and medium priority) based on how urgently these antibiotics are needed. The critical priority group includes multidrug resistant (MDR) bacteria that are especially dangerous for vulnerable individuals, or individuals under specialized care, due to the high risk of infection, complications, disease severity and mortality (4). Some of the bacteria included in this group are: *Acinetobacter spp., Pseudomonas spp., Klebsiella spp., Escherichia coli, Serratia spp. and Proteus spp.* (4), all of which have different infection pathways in the host (5).

Different molecular mechanisms of bacterial resistance to antibiotics have been described so far. Due to the importance of this phenomenon in public health, the present review gathers engaging and relevant information concerning the most common enteropathogenic bacteria in clinical practice and describes the molecular mechanisms for the acquisition or *de novo* development of antibiotic resistance, thus seeking to enlighten the reader in this regard and provide a greater understanding of this process.

Thorough research was conducted in the writing of the present manuscript, primarily employing informatic tools such as PubMed (https: //pubmed.ncbi.nlm.nih.gov/), Scopus (https: //www.scopus.com/home.uri), Scielo (https: //scielo.org/), Medigraphic (https: //www.medigraphic.com/newMedi/) and Science Direct (https: //www.sciencedirect.com/). The terms used in this search included: Enterobacteria, multidrug resistance, enteropathogenic bacteria, multidrug-resistant bacteria, bacterial drug resistance, horizontal gene transfer and gastrointestinal diseases. Inclusion criteria included English language and full-length articles. Exclusion criteria: Publications from 2012 to 2022 were prioritized. Older publications were also reviewed and introduced in the present study if deemed relevant. A total of 99 research and review articles were used in the present study (Fig. 1).

2. Multidrug resistant enteropathogenic bacteria

The family Enterobacteriaceae includes several genus and species of both gram-negative and -positive bacilli (for example *Enterococcus spp*.), a number of which are present in water, soil, plants and the intestinal microbiota of humans and animals; however, their diversity is often dictated by geographical area (6) and often develop as opportunistic pathogens causing severe infections in humans (Table I) (7).

These bacteria are associated with 10-20% cases of infectious diarrhea in children worldwide (8,9). The majority of patients affected by these bacteria only require an hydroelectrolytic imbalance intervention, caused by dehydration or antibiotics treatment, the latter of which diminishes the duration of the disease, reduces its transmission and prevents complications (10). In some cases, it is possible that severe infections can be caused by multidrug-resistant enterobacteria or by enterotoxin producing bacteria, and for this reason special epidemiological surveillance is necessary (10,11). A report made in 2017 revealed that antibiotic resistance in Latin America was as high as 45%, followed by Europe with 39%, the US with 8%, and Canada with 5% (12).

In 2019 Levin-Reisman *et al* (13) described the different phenotypic traits enabling bacteria to acquire resistance to antibacterial agents, such as tolerance, persistence and resistance. Tolerance is the ability of a bacterial population to survive and grow under toxic conditions, such as high concentrations of antibiotics, thus prolonging treatment duration; notably, this acquired resistance may or may not be inherited to daughter cells (13-15). Persistence is the ability of bacteria to survive a specific drug concentration, prolonging the duration of treatment unless corrected (13). These persistent bacteria can withstand antibiotic treatment without affecting the drugs' minimum inhibitory concentration (MIC), presenting a biphasic death curve because the majority of the bacterial population dies, with only a small subpopulation persisting for a longer time (13-15). Resistance is the ability to grow in the presence of environmental stress or high concentrations of antibiotics, regardless of the treatment's duration, due to the increased MIC required to effectively destroy the microorganism (13-16).

The acquisition of antibiotic or antimicrobial resistance is a natural selection process of bacteria and thus considered as part of their evolutionary path. In this regard, the indiscriminate use of antibiotics exerts a high selective pressure on them, which results in genomic changes that translate into multidrug resistance, as seen with greater frequency in developing countries (15,17).

Depending on the number or type of antimicrobials, resistant bacteria can be classified as MDR, which occurs when clinically relevant microorganisms have developed resistance to three or more classes of commonly used antibiotics and/or antimicrobials (18,19); extensively drug-resistant (XDR), microorganisms resistant to at least one agent of all antimicrobial classes; and pandrug-resistant, which includes microorganisms resistant to all agents in all antimicrobial classes (20). Most of these multidrug-resistant bacteria are typically gram-negative enterobacteria representing an important therapeutic challenge in the treatment of life-threatening infections (12,15).

3. Molecular mechanisms of multidrug resistance

Antibiotic resistance can be permanently maintained once it has been fixed in the genome or it can be just temporary if the selective pressure is absent causing non-resistant bacteria to proliferate instead. Drug resistance often appears due to the acquisition of exogenous DNA or through genomic DNA mutations (21).

From an evolutionary perspective, bacteria have several advantages over other organisms because they have short replication time, large populations and capacity for horizontal gene transfer, which enables bacteria to adopt, use, propagate and fix advantageous genetic information between strains and species, such as antibiotic resistance (22).

The limitation of both resources and nutrients within the environment is a decisive factor exerting great selective pressure on bacteria, forcing the stressed populations to adapt or die. As a result, the genetic variations providing a survival advantage become fixed in the bacterial population, thus taking another step in their evolution as a species (23,24).

The genetic evolution of bacteria mostly occurs due to recombination events allowing gene acquisition, segment duplication, fusion of homologous regions, functional domain exchange and gene deletion (24,25). Acquisition of exogenous genomic material occurs via horizontal gene transfer (HGT), which enables bacteria to absorb and incorporate genetic material of diverse origin, thus giving rise to different genotypes between populations of the same species. Further, HGT events can also confer pathogenicity factors related to virulence, symbiosis, resistance and metabolism, among others (24,25).

Three major mechanisms of HGT have been described until 2019: i) Natural transformation (26); ii) conjugation (27); and iii) transduction (28). However, Soler and Forterre (25) proposed a fourth mechanism called vesiduction in 2020 (Fig. 2).

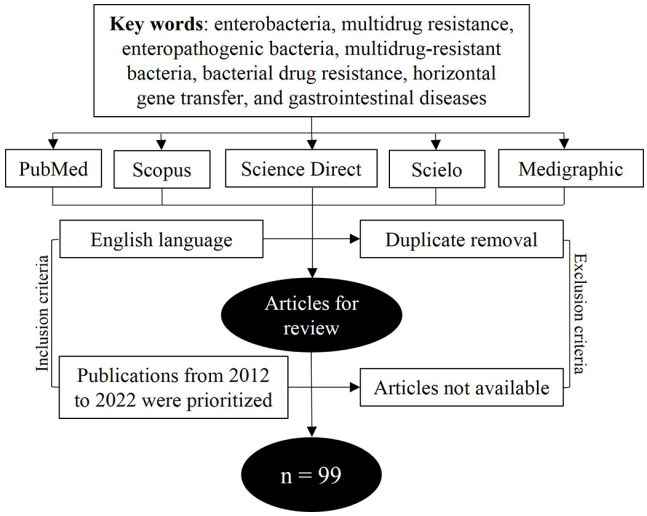

Figure 1. Flow chart of the bibliographic search.

Natural transformation. This phenomenon represents the active transfer of genes from extracellular free DNA from lysed bacteria to a living, competent bacterium that captures and incorporates it into its genome through DNA recombination. This process contributes to genetic variability, shapes evolution and, in the case of pathogenic bacteria, it is responsible for their adaptation to host cells, fosters the spread of antibiotics resistance, promotes antigenic variation and leads to the acquisition of new virulence factors (29-31). In addition, natural transformation also promotes DNA exchange between taxonomically distant bacteria (29-31).

In 2018, Ellison *et al* (31) demonstrated the ability of bacteria to capture and introduce free DNA molecules through surface appendages known as competing pili. Using *Vibrio cholerae* as a model, type IV competing pili were demonstrated to be able to capture and bind double-stranded DNA from the extracellular space. Once bound to DNA, the pili retracts and mobilizes the captured DNA molecule towards the cell surface, where it is finally internalized.

Conjugation. This process explains the exchange of genetic material from donor bacteria with an adjacent recipient through a sexual pilus or physical contact, requiring the formation of a pore between both bacteria while connected as a mating pair. The exchanged genetic elements can usually provide resistance to drugs, antiseptics and/or disinfectants (30,32).

The occurrence of a conjugation event, and thus of an effective DNA transfer, requires cell-cell contact between a donor and a recipient cell. There are two types of genetic elements than can be exchanged during this conjugation: i) Conjugative plasmids, found in free form within the intracellular space and with autonomous replication capacity; and ii) integrated-conjugative elements, or conjugative transposons, that can integrate into the genome of the recipient cell. Since these plasmids were part of the donor's genome prior to the exchange, the latter are rarely, if ever, found free in the cytoplasm and do not replicate autonomously (33,34). In the majority of bacteria, conjugation occurs by transferring single-stranded DNA molecules through the type IV secretion system contained in the conjugative element, which can also transfer DNA from bacteria to eukaryotes (33,34).

Transduction. Transduction is mediated by bacterial viruses called bacteriophages. When a phage infection culminates in bacterial lysis, some viral particles can encapsulate bacterial DNA fragments, thus producing transducer particles. Upon subsequent infection, the transducer particles inject bacterial DNA into the next bacterium host, which may acquire new genetic traits after adopting the exogenous DNA (35). Generalized transduction occurs when any of the bacterial genes maintains the same probability of being encapsulated in a transducing particle and transferred into a recipient. On the other hand, specialized transduction defines the transference of specific genes, such as those located next to the bacteriophage's DNA (30,36,37).

Vesiduction. Vesiduction was proposed in 2020 by Soler and Forterre (25). It involves the donation and/or acquisition of exogenous material from extracellular vesicles, a phenomenon that has been observed in all three domains of life. Vesicles are secreted through the cell membrane of Gram-positive bacteria and the outer cell membrane of Gram-negative bacteria. The precise mechanism is not yet fully understood, and it may be possible that it differs according to the composition of the cell wall and the proteins used in the construction of the vesicles (38,39). These vesicles can fulfill different physiological roles that are not mutually exclusive with genetic material transference, such as the transport of peptidoglycan hydrolases or toxins, and other effector proteins that may be involved in the elimination of concurrent microorganisms through competition or pathogenicity. Some vesicles can also transport intercellular communication molecules (39).

Regardless of their inherent differences, all of the previously mentioned mechanisms for genetic acquisition can be driven by RecA-dependent recombination, illegitimate recombination, transposition or integration (25).

4. Molecular mechanisms of multidrug resistance generation

There are different mechanisms of natural resistance that can appear through other pathways; however, these are usually induced by the presence or prolonged exposure of hazardous molecules (such as antibiotics) resulting in the proliferation of those populations with advantageous biological changes (40,41). The majority of these mechanisms are specifically developed by bacteria to generate resistance to antibiotics or antimicrobials and may involve the modification of existing genomic material through spontaneous mutations that might be punctual or massive. These resistant populations thrive thanks to the action of bactericidal molecules eliminating the cells lacking tolerance or resistance; in other words, the microorganisms are forced to evolve in order to survive (21). This selective pressure has become the standard in areas such as hospitals, biohazard waste disposal areas, pharmaceutical industry effluents, wastewater, manure treated soils, animal breeding and aquaculture areas (21).

Inherent (natural) resistance. Natural resistance to drugs, antibiotics or antimicrobials is a trait often shared between different species of microorganisms, which may be due to the same physiology or spontaneous genetic mutations regardless of previous exposure to these molecules (42,43). An example

Table I. Common enteropathogenic bacteria in clinical practice and their symptoms.

Bacteria	Antibiotic resistance	Pathology	Mechanism of action	(Refs.)
Klebsiella pneumoniae	Carbapenems, β-lactams, aminoglycosides, quinolones, tigecycline, polymyxin.	Acute Diarrheic Syndrome, urinary tract infections, cystitis, pneumonia, endocarditis, septicemia.	Adherence and biofilm formation by type 1 and type 3 pili.	(8,85,86)
Escherichia coli	Cephalosporins, fluoroquinolones.	Acute watery diarrhea, bloody diarrhea.	A/E and changes of the host apical enterocyte membrane. Activation of T3SS and formation of A/E.	(2,5,8,87)
Shigella spp.	Cephalosporins, ampicillin, co-trimoxazole, nalidixic acid.	Bloody diarrhea.	T3SS encoded on a large plasmid and transport of effector proteins.	(5,10,88,89)
Salmonella spp.	Quinolones, nalidixic acid.	Acute watery diarrhea, bloody diarrhea, enteric fever.	Activation of T3SS and transport of effector proteins.	(5,10,90)
Campylobacter spp.	Quinolones, tetracycline.	Enteric fever, acute watery diarrhea, bloody diarrhea.	Presence of flagella, high molecular weight plasmids, surface adhesins and chemotactic factors.	(10,90,91)
Vibrio cholerae	Ampicillin, nalidixic acid, co-trimoxazole.	Acute liquid diarrhea.	Biofilms formation and production of extended-spectrum β-lactamases.	(90,92,93)
Aeromonas spp.	Beta-lactams, tetracyclines, glycylcyclines, fluoroquinolones, aminoglycosides, sulfamethoxazole-trimethoprim.	Acute watery diarrhea, bloody diarrhea.	Travel by the blood to the first organ it finds where it produces the toxic enterotoxin aerolysin.	(2,94,95)
Yersinia enterocolitica	Nalidixic acid.	Enteric fever, bloody diarrhea.	Activation of T3SS and transport of effector proteins and/or apoptosis. Yersinia forms microcolonies and starts replication.	(5,10,90,96)
Staphylococcus aureus	Penicillin, methicillin.	Acute liquid diarrhea.	Inoculation into an open wound. adhesion and invasion of host epithelial cells by microbial surface components recognizing adhesive matrix molecules.	(2,90,97)
Enterococcus spp.	Vancomycin, Beta-lactams, glycopeptides, aminoglycosides, tetracyclines, quinolones, macrolides.	Sepsis, endocarditis, urinary tract infections.	When pathologic alterations are caused by either direct toxin activity or indirectly by bystander damage from the inflammatory response, enterococci are able to outpace host defenses, multiply at rates that are faster than clearance, and overwhelm the host.	(18,98,99)

A/E, attaching/effacing lesion; T3SS, Type III secretion system.

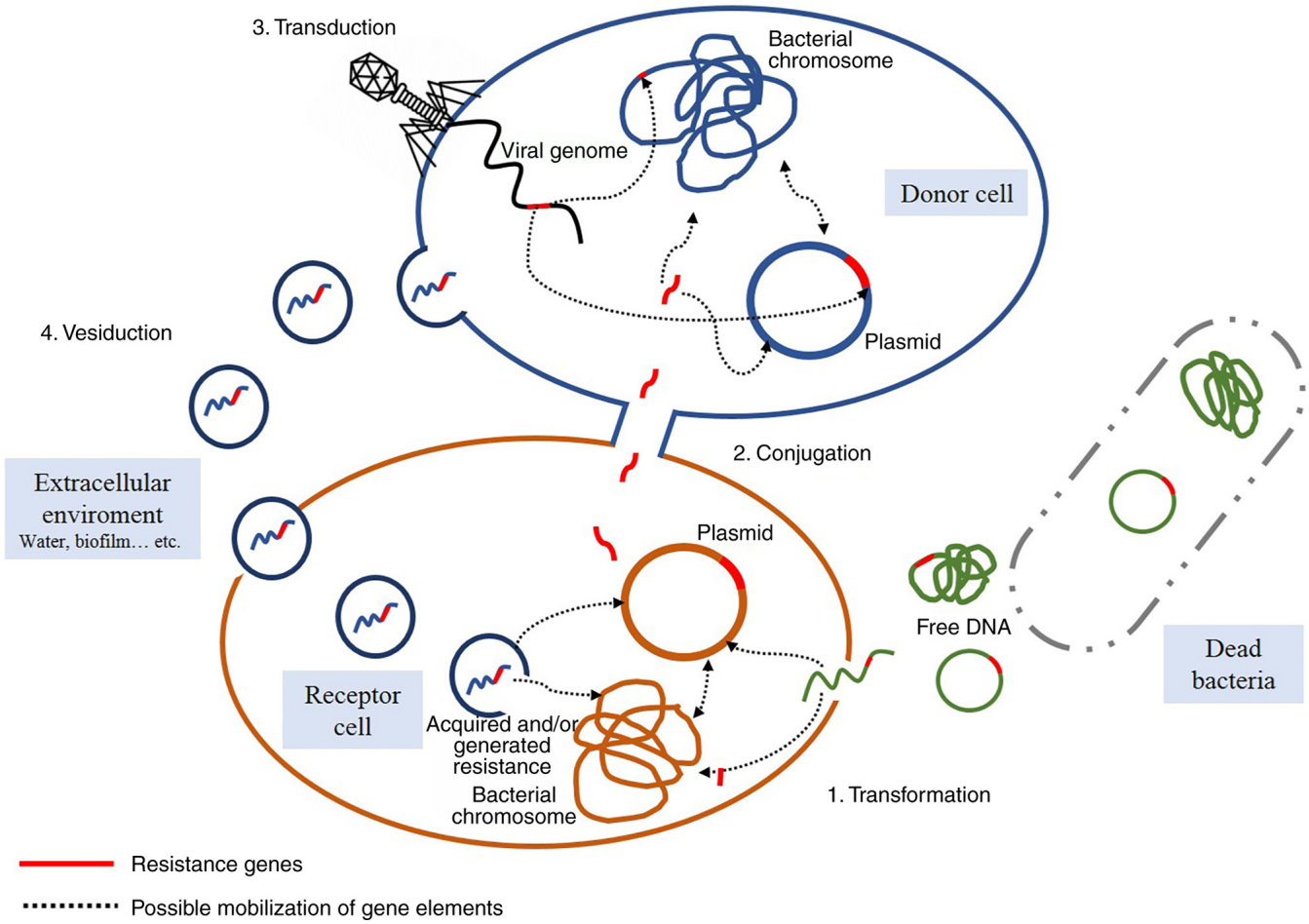

Figure 2. Molecular mechanisms of multidrug resistance acquired by horizontal gene transfer.

of intrinsic antibiotics resistance conferred by physiology can be seen in bacteria of the *Mycoplasma* genus, whose members are highly resistant to drugs targeting the cell wall, such as β-lactams and glycopeptides (44,45); although some antibiotics normally have difficulty crossing the outer membrane of Gram-negative bacteria. For example, vancomycin inhibits cell wall synthesis by targeting d-Ala-d-Ala peptide precursor units of Gram-positive bacteria, thus preventing the assembly of peptidoglycan layers and transpeptidation (46). By contrast, this antibiotic cannot affect Gram-negative bacteria since it is unable to cross the outer membrane, and thus kept from accessing the cell wall (46). Even though these events occur naturally in the environment, (47) it must be mentioned that the intrinsic resistance to antibiotics is not considered as a clinical problem because previously developed antibiotics do not target these bacteria.

Spontaneous mutations. Spontaneous mutations occur by random nucleotide changes that induce different effects; for example, amino acid sequence variations that may lead to altered phenotypes. These mutations can be caused by DNA replication errors or through the action of mutagenic agents. It must be noted that acquired mutations are often detrimental, so these are usually not inherited, are rarely widespread and often are just isolated events (21,48). However, when a mutation provides a biological advantage, this change can become fixed in the population through vertical gene transfer and become a dominant trait (21,48). The frequency of spontaneous mutations related to antibiotic resistance occur at a rate of $1x10^{-5}$ to $2x10^{-8}$ in members of the *Chlamydiaceae* and *Helicobacter pylori* (49,50). Though this would appear to be a rare event, in reality antibiotic resistance appears in bacterial populations within a relatively short period of time, accelerating further when exposed to a selective agent due to exponential growth rate and the number of cells generated per replication cycle (51). For example, the gastric pathogen *Helicobacter pylori* can have different mutations in the 23S rRNA, *gyrA* and *rpoB* genes, which are responsible for resistance to clarithromycin, ciprofloxacin and rifampicin, respectively (50). The capacity of *Chlamydia trachomatis* to resist antibiotics such as azithromycin, tetracycline and fluoroquinolone has also been attributed to spontaneous mutations (52). Although this mutation rate is not even across the board, there are bacterial subpopulations with a significant tendency to acquire and accumulate spontaneous mutations, which is why they often present a greater number of mutation events compared with what is commonly observed (21). These subpopulations are known as hypermutable and, although not all spontaneous mutations confer antibiotics resistance, this hypermutability is directly proportional with the increased resistance capacity (21).

Duplications. Gene duplications are often overlooked as the primary source of functional genomic diversity, originating new functions from a pre-existing gene. In addition, the generation of genetic copies derives into elements that can evolve independently due to inexistent selective pressure, further diversifying their functions (53). For example, the *Plasmodium falciparum* multidrug resistance protein transporter 1 gene plays an important role in the parasite's resistance to drugs due to the strong correlation between the number of copies and the resistance to artemisinin-based therapies, an anti-malaria drug used to reduce its mortality rate since the year 2000. By 2020, Calçada *et al* (54) reported the threat of resistance against this drug due to the appearance of new duplication events and the presence of single nucleotide polymorphisms in current strains.

5. Drugs and bacterial response

As aforementioned, the genetic elements leading to drug resistance can be spread between different microorganisms in different manner, from the horizontal (transformation, transduction, conjugation or vesiduction) to vertical gene transfer (from mother to daughter cells) of either intrinsic or extrinsically acquired genomic modifications, such as spontaneous mutations, duplications, insertions, deletions or transpositions. The mechanism of antibiotics resistance is highly dependent on the way the drug itself works against the bacterium, regardless of how this resistance was acquired, thus deriving in different survival pathways that may limit the absorption of drugs, modify the target molecules, directly inactivate the drug and/or secrete it into the microenvironment (Fig. 3) (55).

6. Mechanisms of antibiotics

Antibiotics with the capacity to inhibit or kill a wide range of bacteria are known as broad-spectrum antibiotics, whereas that those that only affect certain types of bacteria are known as narrow-spectrum antibiotics. Antibiotics typically target the structure or metabolic processes of bacteria, preventing their replication (56-58). In this regard, the most common mechanisms consist in the inhibition of cell wall synthesis, DNA replication or transcription, protein synthesis, metabolic pathways or directly through cell membrane degradation (41,57).

Cell wall synthesis inhibition. The majority of bacterial cells are surrounded by a rigid peptidoglycan layer consisting of long sugar polymers linked through peptide bonds. This structure is needed for survival as it protects the bacteria from osmotic pressure and other hostile conditions from the environment (56,57,59). Drugs such as penicillin and cephalosporins inhibit the formation of peptide bonds in the bacterial cell wall, thus effectively killing the microorganism (56). By contrast, glycopeptides inhibit bacterial growth by inhibiting peptidoglycan synthesis (56,57).

Cell membrane function inhibition. In comparison with gram-positive bacteria, gram-negatives have a greater resistance to antimicrobials due to the existence of an external cell membrane regulating both intracellular and extracellular substance flow (56,60). The drugs targeting this external cell membrane are specific for each microbial group because their function depends on the lipid content of such membrane; however, these drugs can sometimes be toxic, thus limiting their use (56,57). For example, Daptomycin can rupture the cell membrane due to depolarization, whereas that polymyxins bind to the lipid fraction of the membrane's lipopolysaccharide layer, thus causing its disintegration (57).

Nucleic acid synthesis inhibition. Nucleic acid synthesis is important for the survival of living beings, including bacteria. The cellular processes responsible for cell replication and bacterial conservation can be negatively affected due to the interruption of this process by drugs that block DNA replication or transcription (56,57). In this regard, antibiotics such as quinolones interfere with the functionality of the helicase enzyme preventing the function of unwinding DNA, effecting the process of DNA replication and repair. On the other hand, they can exert their action by inhibiting topoisomerase II and IV of bacteria, preventing the synthesis of RNA (61).

Metabolic inhibitors. Some drugs act against important metabolic processes for survival, such as the folic acid pathway, which is necessary to produce important precursors in DNA synthesis (57). In this case, sulphonamides and trimethoprim release similar substrates to those produced and used by the bacteria in its normal metabolism (56,57). Each of these drugs is responsible for inhibiting different stages of folic acid metabolism. For example, the sulphonamides competitively inhibit dihydropteroate synthase, binding to it with greater affinity compared with the substrate produced by the bacteria; while trimethoprim is responsible for inhibiting dihydrofolate reductase at a later stage of folic acid synthesis (56,57,59).

Protein synthesis inhibition. Proteins play a role in various cellular structures and physiological processes; therefore, their synthesis is fundamental for survival (56,57). For this reason, drugs that inhibit protein biosynthesis by targeting the 70S prokaryotic ribosome (30S and 50S ribosomal subunits) constitute the largest class of antibiotics (56,57,59).

30S subunit inhibitors. Antibiotics such as tetracycline, aminoglycosides and streptomycin target and inhibit the 30S ribosome, blocking the passage of aminoacyl-tRNA towards the ribosome (57,59).

50S subunit inhibitors. Antibiotics targeting the 50S ribosomal subunit can act in two different ways, by blocking protein translation (oxazolidinones) or by blocking the elongation phase of protein synthesis (for example, macrolides). However, the latter may be ineffective when the elongation phase has advanced significantly (57,59). Natural antibiotics such as aminoglycosides are considered as bactericidal, whereas macrolides, tetracyclines, chloramphenicol, streptogramins and spectinomycin are considered as bacteriostatic (57).

7. Mechanisms of drug resistance

Antibiotics outlet, secretion or efflux pumps. Some bacteria have exporter proteins on their cell membrane that can rapidly transport the antibiotics from the cytoplasmic membrane in

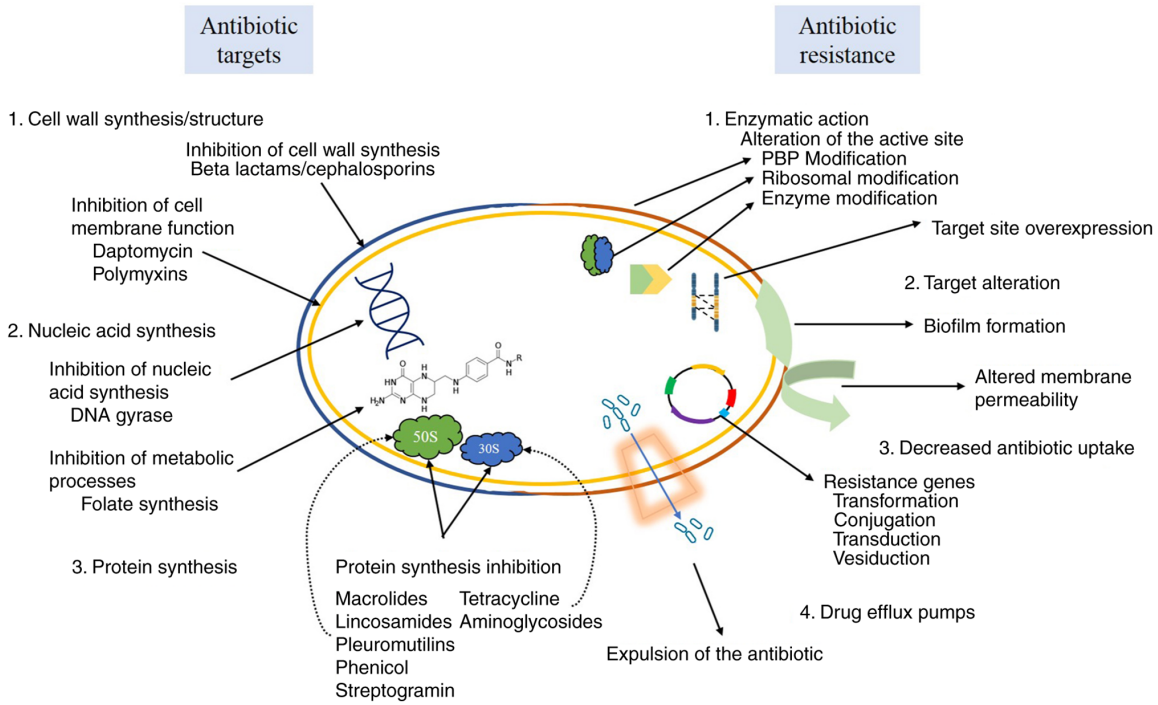

Figure 3. Antibiotics and bacterial resistance.

gram-positive bacteria and from the intermembrane space in gram-negative bacteria to the exterior of the cell without the help of energy-dependent efflux pumps, thus preventing the antibiotics from reaching their target (30,62,63). There are two groups of efflux pumps, some of them are specific whereas others can secrete diverse substances. These pumps are classified according to energy source and function; in this regard, the first group uses ATP as an energy source and functions through hydrolysis (63,64). By contrast, the second group uses the mobile force of protons as an energy source, enhancing secretion through the electrochemical potential of the membrane (63,64). A total of five families of efflux pumps have been described within the second group: i) The multidrug and toxic extrusion family; ii) the major facilitator super family; iii) the resistance nodulations cell division (RND) family; iv) the small MDR family; and v) the multidrug endosomal transporter family (62-64). These efflux pumps are widely distributed among gram-positive and -negative bacteria, except for the RND poly-selective superfamily, which is found gram-negative bacteria with very high frequency (62,63). These efflux pumps play a notable role in multidrug resistance due to their capacity to secrete a wide range of structurally unrelated drugs and molecules (62-64).

Permeability alterations in the outer cell membrane. The majority of antibiotics penetrate the bacterial membrane and target diverse intracellular processes; therefore, the concentration of antibiotics within the cell can be affected by alterations in the lipid bilayer of the membrane, modifying either the cell's diameter or number of porins (30,65). The bacterial cell envelope provides a selective barrier allowing the exchange of nutrients and signaling molecules with the microenvironment. This envelope is formed by the cell wall and the plasma membrane, and, in Gram-negatives, it provides an additional function as a physical barrier that reduces the permeability of a number of drugs (53,65). Notably, this envelope can also be targeted by antibiotics (53,65). The outer membrane of gram-negative bacteria is populated by proteins called porins, which determine its permeability and allow the entry of hydrophilic compounds into the cell. The absence or low number of porins can also prevent the entry of antimicrobial molecules, thus hindering their action in the cytoplasm and/or cell envelope (62,63).

Active site alterations. Bacteria have the ability to form metabolic substances that compete with antimicrobial drugs for the active site, preventing it from binding due to loss of affinity (30,63). There are two types of modifications in this regard as follows.

Penicillin-Binding-Protein (PBP) modification. Observed in Gram-positive bacteria, this effect is caused by variations in the peptidoglycan gene, which modify the antimicrobial binding site in the cell wall (30,62).

Ribosomal modification. The *ermA* and *ermB* genes can modify the ribosome's active site through methylation. These modifications occur in the 30S and 50S subunits of the 70S ribosome, affecting the target site of drugs such as aminoglycosides, macrolides, tetracyclines and lincosamides (30,66).

Enzymatic modification or inactivation of antibiotics. This is the most common mechanism of resistance observed in bacteria. It is achieved through the expression of enzymes with the capacity to modify the active component of the antibiotics, thus reducing their effectiveness. Three mechanisms have been reported so far: i) Redox reactions; ii) group transfer; and iii) enzymatic hydrolysis. The latter is the primary mechanism of resistance, with the clearest example being the hydrolysis of the beta-lactam ring of antibiotics. The enzymes

of gram-negative bacteria typically originate from a plasmid or have a transposon origin with constitutive and periplasmic expression. By contrast, this resistance is solely provided by a plasmid in gram-positive bacteria, which can be inducible and/or extracellular (40,65).

Biofilm formation. Biofilms are structured aggregations of bacterial cells enclosed in a self-synthesized extracellular matrix composed of different macromolecules such as proteins, nucleic acids and polysaccharides (63,67). Biofilms bacterial production protects them from ultraviolet light, dehydration, immune system or certain antibiotics. There are three important steps in biofilm formation: i) Adhesion, in this phase bacteria can attach to any give surface; ii) growth and maturation, occurs when bacteria secrete an exopolysaccharides matrix and mature from microcolonies to multi-layered cell clusters; and iii) shedding, which can be either active (initiated by the bacteria) or passive (caused by external factors) (30,62). Amongst the most common pathogens that develop biofilms are *S. aureus, P. aeruginosa, A. baumannii and K. pneumonia* (30,62).

Target site overexpression. This mechanism has been described in clinical isolates of mycobacteria with promoter duplications. This often results in the overexpression of genes that may include mutations affecting the target site of antibiotics or antimicrobials (30). In this regard, Martinez *et al* (68) describe the presence of plasmids in *E. coli* that provide resistance to amoxicillin-clavulanate as a result of the hyperproduction of plasmid-determined TEM-1 P-lactamase. TEM-1 β-lactamase is a known determinant of resistance to antibiotics, such as penicillin, cephalosporins and their derivatives, including second, third and fourth generation cephalosporins, monobactams and β-lactamase inhibitors. This enzyme inactivates the aforementioned compounds by hydrolyzing their lactam rings (69,70).

8. Treatment against multidrug-resistant bacteria

Some of the first-line drugs used in the treatment of serious infections caused by *Enterobacteriaceae* include penicillin, cephalosporins, carbapenems, fluoroquinolones, monobactams and, occasionally, aminoglycosides. However, bacterial resistance against these drugs is rapidly becoming widespread, thus making difficult these treatment (20,71). In some cases, second-line drugs are more effective against enterobacteria, as would be the case with polymyxins, tigecycline, aminoglycosides and fosfomycin (72). Pathogenic bacteria have evolved different strategies to overcome the host's response by avoiding highly competitive environments. For example, the mucosal barrier can be breached by mucinases, such as the Pic enzyme from *Shigella* and enteroaggregative from *Escherichia coli* (EAEC). Notably, the *Pic* gene can be found in a 'pathogenicity island' flanked by insert-like EAEC elements that have been acquired through horizontal gene transfer (24).

Empirical treatment with antibiotics. As a first line decision, empirical therapy becomes essential in the treatment of serious infections caused by bacteria. However, the emergence of bacterial resistance complicates its implementation, thus causing a serious dilemma between the selection of a broad-spectrum drug, which could induce greater drug resistance, or a narrow-spectrum drug, which could be completely ineffective (71,73). Regardless of its potential shortcomings, the latter could supply important information on the pathogen's susceptibility to certain antimicrobials (71,73). Several factors must be evaluated during the selection of antibiotics treatment, including susceptibility, risk of developing resistance, potential side effects, comorbidities, local epidemiology and clinical severity (10,71,74).

Combination antibiotic therapy, The combined therapy of antibiotics enables the synergistic effect of one or more drugs, potentially increasing the probability of an effective treatment and lowering the risk of bacterial resistance. However, the results of drug synergy tests observed *in vitro* do not always translate well into a clinical setting (71,75).

Alternative treatments. Alternative treatments can also be implemented in addition to antibiotics therapy if their contribution proves safe for the patient and does not enable the development of bacterial resistance, for example, phage therapy or competing microorganisms (76). There are some reports demonstrating the benefits of these treatments against multidrug-resistant pathogens, even suggesting they could be used as replacements for common drugs (76).

Phage therapy. Bacteriophages are bacteria-specific viruses that can infect bacteria through the binding of specific receptors on the cell's surface and injecting their genetic material. Once infected, the phage can go through a lysogenic cycle, where the phage's genome is integrated in the bacterial chromosome as an endogenous prophage, spreading horizontally during cell division. The virus can remain latent for prolonged periods of time during this cycle; however, environmental or cellular stress factors can re-activate the phage and induce its lytic cycle, in which the viral genome is no longer integrated in the bacterial chromosome and goes into a massive replication event, finally causing cell death after the phage's lytic proteins hydrolyze the cell wall. These liberated phage particles can then infect other bacteria and start the lytic cycle again (76-78). It must be mentioned that the infective capacity of bacteriophages is constrained to particular bacterial species, thus resulting in a reduced spectrum. Although this could be considered a shortcoming, it could also be considered as a positive aspect since they are unable to affect the intestinal microbiota or the host (76,78).

Probiotics, prebiotics and synbiotics. Probiotics are live microorganisms that play a beneficial role if administered in adequate amounts, regardless of those present in the essential diet or naturally in the intestinal microbiota of the host (79-81). On the other hand, prebiotics are non-digestible compounds (non-starch polysaccharides and non-digestible oligosaccharides) present in the daily diet and which help stimulate the growth or activity of the intestinal microbiota, favoring the development of beneficial microorganisms (79,80,82). Finally, synbiotics are a composition of the previous two and which are often found in the form of pharmaceutical or food preparations containing one or more probiotic organisms and prebiotic compounds in

order to provide a synergistic effect on the prebiotics, enhancing the development, activity and nutritional properties of the probiotics. The inclusion of synbiotics increases the density of probiotics and their health benefits (80,82). The probiotics used in clinical treatments are mainly composed of Gram-positive strains such as *Lactobacillus* that are resistant to the human digestive process. The administration of these microorganisms improves the epithelial barrier function, promotes the growth of beneficial bacteria, the proliferation of epithelial cells in the host (by upregulation of cell growth and downregulation of apoptosis), prevents the adhesion and colonization of pathogenic microorganisms and toxins, improves lactose digestion, produces antimicrobial peptides, regulates the immune response and improves the ability to regulate pH (76,83). Regarding the functional foods that seem to exert the best prebiotic effect, fructooligosaccharides, galacto-oligosaccharides and xylose-oligosaccharide, inulin and lactulose, can be mentioned. Some extracted from sources such as chicory and yacon roots, are reported (84). These can be used in symbiotic formulations with *Lactobacilli, Bifidobacteria spp, S. boulardii* and *B. coagulans,* among other probiotic agents (84).

9. Conclusions

Several clinically relevant bacterial strains are now resistant to multiple drugs. To counteract this phenomenon, novel compounds with the capacity to kill and/or prevent their proliferation are constantly being developed. However, the epidemiology and resistance of these strains varies widely according to geographical region. Therefore, alternative treatments are also being sought to enhance the effectiveness of antibiotics to reduce bacterial proliferation and prevent further spread of antibiotics resistance.

Acknowledgements

Thanks to Dr Daniel Díaz (Department of Pharmacology, Faculty of Medicine in Hradec Králové; Charles University, Prague, Czech Republic) for his kind assistance in proofreading and editing this manuscript.

Funding

No funding was received.

Availability of data and materials

Not applicable.

Authors' contributions

JAGV, KJGL and MASS contributed to the study design, and performed the literature review and data collection. JAGV, KJGL, ETAC and MASS contributed to the selection the relevant literature and critical interpretation. JAGV, KJGL, ETAC, MASS, JAMC, MPLE and NB drafted and improved the manuscript. JAGV, KJGL, ETAC, MASS, JAMC, MPLE and NB critically read and modified the manuscript. All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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