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MOLECULAR MECHANISMS AND GENETIC DETERMINANTS OF RESISTANCE TO ANTIBACTERIAL DRUGS IN MICROORGANISMS
(review)

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Abstract

The emergence of antibiotic resistance is a serious public health problem, since antibiotic-resistant bacteria that develops in conditions of agro-industrial enterprises can easily transmit to humans through products and raw materials of animal origin and contaminate the environment with agricultural waste. Several reviews cover the problem (C. Manyi-Loh et al., 2018; A.N. Panin et al., 2017). A significant number of publications describe the mechanisms of antibiotic resistance, including modification of the target affected by the drug; the acquisition of metabolic pathways alternative to those inhibited by an antimicrobial agent; overproduction of the target enzyme; enzymatic inactivation and active efflux of the antibiotic (it's excretion outside the microbial cell). These mechanisms can be natural for some microorganisms or acquired from other microorganisms (M.F. Varela et al., 2021; W.C. Reygaert, 2018; A.L. Bisekenova et al., 2015). Understanding these mechanisms will allow us to choose the best treatment option for each specific infectious disease and develop antimicrobial drugs that prevent the spread of resistant microorganisms. The most clinically significant antibiotic resistance genes are usually located on different mobile genetic elements (MGE) that can move intracellularly (between the bacterial chromosome and plasmids) or intercellularly (within the same species or between different species or genera) (C.O. Vrancianu et al., 2020). Among the three main mechanisms involved in horizontal gene transfer, transformation of antibiotic resistance genes between bacterial species happens rarely. However, conjugation with the participation of mobile genetic elements, such as transposons and plasmids, is the most effective and important method of spreading antibiotic resistance (J.M. Bello-López et al., 2019). The purpose of this review is to describe antibiotic resistance genes distinctive for the microbiota of farm animals under the conditions of the agro-industrial complexes, as well as the mechanisms of the formation of antibacterial resistance to antimicrobial drugs used in veterinary medicine. In addition, this report covers the direct localization of the genetic determinants of antibiotic resistance, outlines the main measures to control antibiotic resistance, which include i) reducing the use of antibiotics due to improving animals' welfare and living conditions and ii) monitoring and supervision of the spread of antibiotic-resistant bacteria.

Keywords: antibiotic resistance, livestock sector, mechanisms of resistance, antibiotic drugs, mobile genetic elements, genetic determinants, microorganisms

The spread of antibiotic-resistant bacteria is a growing problem worldwide [1]. With the discovery of penicillin in 1928, many life-threatening or even fatal diseases became curable, which brought obvious benefits to specialists in the field of veterinary medicine and animal science. Since the 1960s, however, antibiotics have been widely used as growth promoters in farm animals [2]. In 2017, the World Health Organization (WHO) published a list of bacteria that require the

development of new antibiotics. According to the WHO, antimicrobial resistance causes 25,000 deaths per year in the European Union (EU) and 700,000 worldwide. With the current unfavorable trend, by 2050 antibiotic-resistant bacteria may cause more deaths than cancer [1, 3].

The purpose of this review was to describe the genes for antibiotic resistance of bacteria that persist in the agro-industrial complex and are characteristic of the microbiota of farm animals, as well as the mechanisms for the formation of antibacterial resistance to antimicrobial drugs used in veterinary medicine.

The uncontrolled use of antibiotics leads to the accumulation of low sub-inhibitory concentrations in the tissues and intestines of treated animals and in the environment, which promotes the selection of antibiotic-resistant bacteria, enhances their growth, the mutation process occurs and the introduction of de novo mutations (4). In addition, the presence of antibiotics can stimulate biofilm formation and horizontal gene transfer (HGT) in some bacteria. For example, transfer of resistance to azithromycin, ciprofloxacin or tigecycline has been observed in *Enterococcus faecalis* and *Pseudomonas aeruginosa* [5].

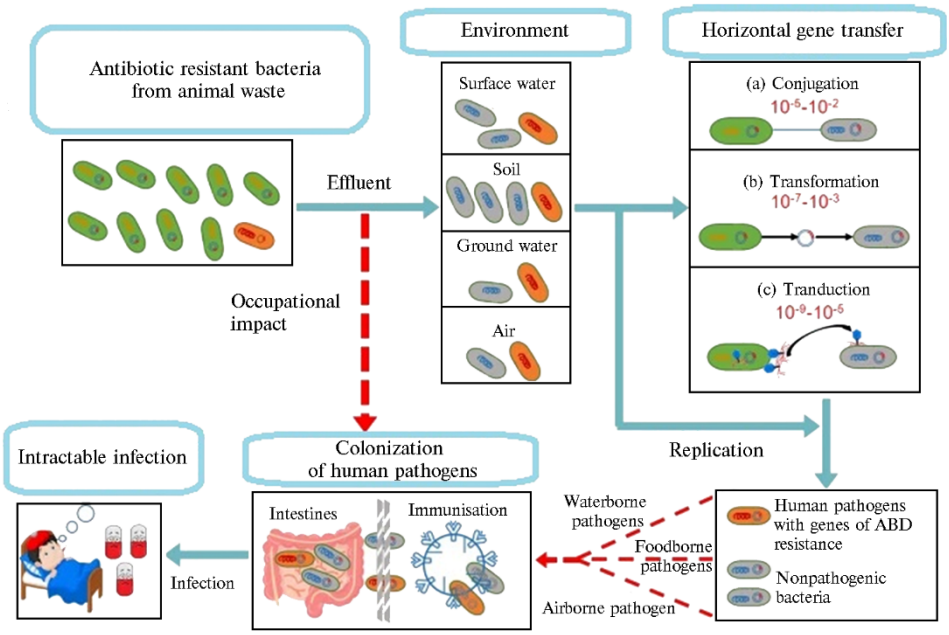
The main transfer of antibiotic resistance genes occurs through HGT, that is, through the exchange of transposable genetic elements (TGEs), such as plasmids or transposons encoding antibiotic resistance genes, between bacterial species, even if they are not closely related (6). Some countries have officially restricted the use of antibiotics in animal husbandry to therapeutic use only (eg the EU in 2006 under legislation 1831/2003/EC). However, antibiotics are still overused in areas with high livestock intensity: the US, Russia, India, China and South Africa [7]. In the US, antimicrobial treatment of animals used for food production accounts for approximately 80% of the total annual volume. However, the vast majority of antibiotics are essential drugs used to treat common infections or are required for surgery, organ transplantation or chemotherapy in humans [8]. In the Russian Federation, from March 1, 2022, an order came into force on the approval of the list of antibacterial drugs intended for the treatment of infectious and parasitic diseases of animals caused by pathogenic and opportunistic microorganisms, in respect of which restrictions are imposed on the use for therapeutic purposes, including for treatment of farm animals. This list divides antibacterials into group A (prohibited for all animals; prohibited for food producing animals), group B (second choice drugs) and group C (first choice drugs) [9].

There are a number of mechanisms that contribute to the development of resistance of a bacterial cell to one or more antimicrobial drugs: a decrease in the accumulation of an antimicrobial drug inside the cell through a decrease in wall permeability and/or active efflux (removal) of the antimicrobial drug from the bacterial cell; enzymatic modification or degradation (inactivation) of the antimicrobial agent; acquisition of alternative metabolic pathways to existing ones (formation of a metabolic shunt); modification or protection of the antimicrobial target; overproduction of the target enzyme [11-13].

Reducing the accumulation of antimicrobial drug in the bacterial cell by reducing the permeability of the cell wall and active efflux. Efflux pumps are protein carriers localized in the cytoplasmic membrane of all cell types that require a source of chemical energy to perform their function. Some of them are primary active transporters, using the hydrolysis of adenosine triphosphate as an energy source, while others are secondary active transporters (uniporters, symporters, or antiporters), in which transport is associated with an electrochemical potential difference created by the removal of hydrogen or sodium ions outside the cell [14]. The change in permeability that

occurs in the outer membranes of bacterial cells causes a decrease in the entry of the antibiotic into the cell; at the same time, efflux pumps are activated, and the rest of the drug is excreted outside the cell. These resistance mechanisms correlate with each other and always have an cumulative effect on drugs [15]. Cell permeability undergoes changes through the acquisition of mutations in porins (protein channels that pass through the cell membrane). These mutations include loss of the porin, alteration in the size or conductivity of the porin channel, or lower porin expression [16]. As for efflux pumps, some of them are constantly expressed, mediating the innate resistance of bacteria, while others are expressed under the influence of an inducer. In addition, overexpression of efflux pumps may contribute to higher resistance. Overexpression can be transient and occur in the presence of an effector (phenotypic resistance) or be permanent when mutants are selected for regulatory elements of efflux pump expression (acquired or secondary resistance) [17].

In reviews by S. Hernando-Amado et al. [18] and W.C. Reygaert [19] efflux pumps are grouped into five main structural superfamilies — resistance-nodulation-division (RND), small multidrug resistance (SMR), multidrug and toxin extrusion (MATE), major facilitator superfamily (MFS) and ATP-binding cassette (ABC) [18, 19]. This classification is based on three criteria: amino acid sequence identity, substrate specificity, and the source of energy needed to drive efflux [20]. A.E. Ebbensgaard et al. [21] also mention the family of proteobacterial antimicrobial compound efflux (PACE). While RND and PACE are unique to Gram-negative bacteria, SMR, MATE, MFS and ABC are found in both Gram-positive and Gram-negative members of the microbial community [21].



Possible pathways for the transfer of antibiotic resistance genes from animal waste to human pathogens. Bacteria containing antibiotic resistance genes move through drainage, treated wastewater, and solid waste from livestock enterprises to various host environments. Horizontal gene transfer between antibacterial drug-resistant bacteria (ARB) and autochthonous microflora occurs through three main mechanisms: conjugation (involving plasmids), transformation (involving free DNA) and transduction (involving bacteriophages) with indicated frequencies based on data literature. Pathogenic bacteria are able to penetrate into the human body through the alimentary route, as well as as a result of occupational exposure. The bacteria then multiply in the body (especially in the gut), causing endogenous or exogenous infections [10].

ABC transporters are functionally diverse and mediate ATP-dependent

import or export of solutes. ABC transporters contain transmembrane domains (TMDs) that are able to recognize substrates and transfer these substances through the membrane through conformational switches, and nucleotide binding domains (NBDs) that bind and hydrolyze ATP, controlling the transport cycle [22, 23]. The mechanism for the transfer of substances of this family operates on the principle of variable access, such a structure has three states: open inward, closed or open outward to move substrates through the membrane.

The MFS Group is the largest and most diverse conveyor family. It includes uniporters (provide the movement of substrates through the lipid bilayer in one direction along the concentration gradient, regardless of other molecules), symporters (transport of matter and ions in one direction outside the cell) and antiporters (movement of ions and substances in opposite directions). Most members of this family function as separate monomeric units. They are 400 to 600 amino acid residues in length and have 12 or 14 transmembrane helices organized as two domains, each consisting of six helical bundles. The variable access switching mechanism for MFS proteins has two states: open to the inside of the cell or open to the outside during the transport cycle.

Other proteins of the MATE family use H^+ and/or Na^+ transmembrane gradients for transport. All currently known structures of MATE transporters are fixed in the external state (open to the outside). Probably, the state of "open inside the cell" is achieved due to the transfer of lipids with the help of flippase to the cytoplasmic side of the membrane [24].

RND-type efflux systems consist of three components: integral membrane protein (IMP), periplasmic membrane fusion protein (MFP), and outer membrane protein (OMP). The H^+ transmembrane electrochemical potential drives the drug efflux associated with RND. All RND transporters have a rather atypical structure for a group of secondary transporters. Most transport systems of the RND superfamily consist of large polypeptide chains containing 12 transmembrane domains. The large periplasmic domain is involved in substrate recognition and forms a cavity that can accept multiple drugs at the same time [25].

Proteins of the SMR family consist of only four transmembrane helices, but function as homodimers or heterodimers. The first three transmembrane helices form an active cavity for substrate binding, while the fourth helix is mainly involved in dimerization. During transport, two states of the pump alternate: inward-facing or outward-facing through a conformational exchange of two protomers [24].

Transport proteins of the PACE family form resistance to a number of biocides used as disinfectants and antiseptics. The range of functions and transport mechanisms operating in these proteins are not well understood. PACE proteins are known to have several conserved amino acid motifs that likely play a role in substrate transport. PACE proteins also have a conserved region between the N- and C-terminal amino acids. They probably evolved as a result of duplication of an ancestral protein consisting of two transmembrane helices [26].

These mechanisms of formation of bacterial resistance have been identified for tetracyclines, macrolides, quinolones and amphenicols. Approximately 30 tetracycline resistance genes, such as *tet* (A, B, C, D, E, G, H, J, K), encode a tetracycline-specific efflux pump that both Gram-positive and Gram-negative bacteria have and is usually encoded by transposons. and integrons. In addition, several *mef* genes encode an efflux pump specific for macrolides, which reduces their intracellular concentration. Unlike the *erm* genes, the *mef* genes can only protect against macrolides, resulting in an M phenotype. There is evidence that although the efflux mechanism mediating quinolone resistance is sensitive to reserpine, this phenotype is more of a multidrug resistance phenotype established

by non-specific efflux [11]. The *cml* and *flo* genes encode specific efflux pumps found mainly in Gram-negative bacteria.

Enzymatic modification or degradation (inactivation) of an antimicrobial agent. Bacteria are able to synthesize enzymes that chemically modify the antibiotic target by adding additional chemical groups. For example, the mph genes are mostly found in Gram-negative bacteria and mediate macrolide inactivation, which limits their clinical relevance. The vat genes, which also code for enzyme inactivation, have been found in *Enterococcus* spp. and *Staphylococcus* spp. [11]. The second type of enzyme chemically modifies the antibiotic itself, which prevents the antibiotic from binding to its target site. This mechanism of antibiotic resistance can be provided by enzymes that modify aminoglycosides, in particular N-acetyltransferases, which add an additional acetyl group (CH3CO−) to aminoglycoside antibiotics, such as kanamycin. Binding to the ribosome is disrupted and bacteria become resistant [27]. Most of these enzymes have a narrow spectrum of activity. For example, ANT(2″)-I can only inactivate gentamicin, tobramycin, and kanamycin. Bifunctional phosphotransferases and/or acetyltransferases found in Gram-positive cocci inactivate most aminoglycosides. The genes for these enzymes are often found in plasmids and transposons and can be mobilized as gene cassettes between integrons. In addition, some of the 1000 different β-lactamases known to date are only able to hydrolyze a few substrates, while others can also inactivate third-generation cephalosporins (extended-spectrum β-lactamases, ESBLs) and β-lactamase inhibitors, such as clavulanic acid. acid. β-Lactamases are widely dispersed in bacterial groups and can be encoded by chromosomal or plasmid genes. A recently discovered modified aminoglycoside resistance enzyme AAC(6′)-lb-cr is able to inactivate ciprofloxacin. The enzyme is quite common in clinical practice in isolates of intestinal bacteria with reduced sensitivity to ciprofloxacin. Bacteria producing chloramphenicol acetyltransferase, which is encoded by multiple cat genes and has the ability to inactivate chloramphenicol, become resistant to the antibiotic. The *cat* genes have been found in both Gram-positive and Gram-negative bacteria [11]. Table 1 shows the main types of enzymes that modify antimicrobials.

1. Main classes of enzymes that modify antimicrobials (27)

Classes	Enzymes	Inactivated antibiotics
Hydrolases	β-Lactamases (penicillinases, cephalosporinases, carbapenemases)	Penicillins, cephalosporins, carbapenems and monobactams
	Esterases	Macrolides
	Epoxide hydrolases	Phosphomycin
Transferases	Acetyltransferases	Aminoglycosides, fenicols, quinolones, streptogramin A
	Phosphotransferases	Macrolides, rifamycins, phosfomycin, aminoglycosides
	Nucleotidyltransferase	Aminoglycosides, lincosamides
	Glycosyltransferase	Macrolides, rifamycins
	ADP-ribosyl transferases	Rifamycins
	Glutathione-S-transferase	Phosfomycin
Redox enzymes	Monooxygenases	Tetracyclines, rifamycins
	Liase	Streptogramin B

Acquisition of alternative metabolic pathways (formation of a metabolic shunt). This mechanism of resistance is quite specific, most often associated with the acquisition of new genes by bacteria, which make it possible to produce an alternative target (usually an enzyme) that is resistant to the action of an antibiotic. Bacteria also synthesize the original target, which is sensitive to antibiotics. An alternative target mediates the development of resistance in bacteria, taking on the role of the original target, i.e., a metabolic shunt is formed [28, 29]. A striking example of such a mechanism of resistance to quinolone

antibiotics is the imitation of a target molecule by a protein of the MfpA family of pentapeptide repeats. It was noted that when MfpA was expressed on the pGADIV plasmid in *Mycobacterium smegmatis* or *Mycobacterium bovis*, the minimum inhibitory concentration (MIC) for all fluoroquinolones increased 2-8-fold, and when mfpA was eliminated from the chromosome of *M. smegmatis*, the MIC decreased by 2-4 times (30). The MfpA protein mimics the structure of DNA and interacts with DNA gyrase or topoisomerase IV, thereby protecting them from the inhibitory effect of quinolones that bind to these target enzymes [31].

Another class of antibiotics, glycopeptides, bind the terminal residues of D-alanyl-D-alanine to the cell wall of pentapeptide precursors, blocking the next stages of cell wall synthesis (trans-glycosylation and transpeptidation). The van genes alter peptidoglycan synthesis pathways such that D-alanyl-D-lactate or D-alanyl-D-serine is formed instead of D-alanyl-D-alanine. Clusters of van genes (five or more genes) are required to achieve glycopeptide resistance, so the entire cluster must be moved horizontally, probably by conjugation. Some *van* genes, apparently derived from vancomycin-producing organisms, were passed on to members of the genus Streptomyces and then to Gram-positive cocci [32, 33].

The action of β-lactam antibiotics is based on the suppression of several enzymes responsible for the synthesis of bacterial cell walls (penicillin-binding proteins, PBP). The acquisition of alternative enzymes promotes the development of cell resistance to many or all β-lactams. Thus, *Staphylococcus aureus* becomes resistant to most β-lactam antibiotics, in particular to penicillin. Methicillin-resistant *Staphylococcus aureus* (MRSA) acquires resistance to β-lactam antibiotics by obtaining an additional copy of penicillin-binding protein 2a (PBP2a), which serves as a target for β-lactam antibiotics and retains its functionality in their presence [33]. Based on B.A. Wall et al. (11) and D.M. Boothe (33) reports, it is known that altered PBPs underlie penicillin resistance in Streptococcus pneumoniae acquired through transformation, accumulation of repeated point mutations, or through recombination between PBP genes in related streptococcal species. Organisms without a cell wall, such as *Mycoplasma*, are inherently resistant to β-lactams and to all antimicrobials that act to inhibit or interfere with cell wall synthesis of target bacteria [34]. The phenotypic form of resistance can occur in the presence of spheroplasts (incomplete cell wall) or protoplasts (no cell wall). These L-forms require a hyperosmotic environment (eg, renal medulla) to survive or they will be lysed [33].

Modification or protection of the antimicrobial target. One of the common mechanisms used by bacteria to acquire resistance to antibacterial drugs is to change or protect the antibiotic target. As bacteria grow and reproduce, they copy their genome. Sometimes errors occur in the DNA sequence during the copying process (for example, adenine is replaced by cytosine). By themselves, such events are rare, but large population sizes greatly increase their frequency. If one of these mutations occurs at the location of the gene encoding the protein that is the target of the antibiotic, then the latter can no longer bind to the target. There is a selection of bacteria resistant to the action of antimicrobial agents. This is the general mechanism of penicillin resistance in Streptococcus pneumoniae when mutations in penicillin-binding proteins (PBPs) are acquired.

P. Valderrama-Carmona et al. [35] reported about 16S rRNA methylases which modify the nucleic acid molecule, which alters the structure of the ribosome to prevent binding of aminoglycosides, in the intestinal bacteria *Pseudomonas* spp. and Gram-positive cocci [35]. Ribosomal mutations can also make ribosomes insensitive to aminoglycosides. The erm gene (erythromycin ribosomal methylase gene family) provides resistance to macrolide antibiotics such as erythromycin. Methylation occurs in the portion of the ribosome that is targeted by

erythromycin, erythromycin loses its ability to bind to the target, and bacteria can continue to grow in the presence of the antibiotic [27]. This modification protects the ribosome from other chemically unrelated antimicrobials such as lincosamides and streptogramins. The so-called macrolide-lincosamide-streptogramin B (MLSB) phenotype is a clear example of cross-resistance. The erm genes are often localized on transposable genetic elements; *erm*(B) and *tet*(M) are located in Tn1545, the streptococcal conjugative transposon [36]. Sulfonamides, in turn, are able to inhibit the enzyme dihydropteroate synthetase (DHPS), and trimethoprim can inhobit dihydrofolate reductase (DHFR).

Of particular importance is the *sul/I* gene encoding DHPS. It serves as part of the conserved region of class 1 integrons. By acquiring (via horizontal transfer) genes for DHPS enzymes and/or DHFR variants that are not inhibited by these drugs, bacteria become resistant [11]. Mutations in the *gyr* and/or *par* genes encoding DNA gyrase and topoisomerase IV, respectively, allow these enzymes to complete the three-step process of DNA supercoiling in the presence of quinolones [37]. A single mutation can make a bacterial cell resistant to nalidixic acid, but two or more mutations are needed to achieve resistance to fluoroquinolones (e.g., ciprofloxacin and enrofloxacin). Although these mutations are recessive in nature (with presumably limited capacity for horizontal mobilization), transmission through transformation has been reported in streptococci because the newly acquired gene replaced the wild-type gene through recombination.

The *cfr* gene encodes RNA methyltransferase, as a result of which the ribosome is modified, preventing the binding of florfenicol to it, which leads to the emergence of resistance. Currently, the use of chloramphenicol in medicine is limited, and florfenicol is used only in veterinary medicine. However, *cfr* genes are of public health importance because the produced methylase also protects bacterial ribosomes from the action of linezolid, a class of oxazolidinones considered as a "last resort" against infections associated with *Staphylococcus aureus* and resistant enterococci in humans. The *cfr* genes have been found worldwide in clinical isolates resistant to linezolid.

Approximately 10 genes, including the tetracycline resistance *tet* genes (M, O, Q, S, T), encode proteins that interact with ribosomes, protecting them from binding to tetracyclines. In addition to enteric bacteria, tet genes, especially *tet*(M), are commonly found together with macrolide resistance genes in the same transposon in Gram-positive cocci, and can also be found in anaerobes.

The *qnr* gene group encodes a protein that protects enzymes of the topoisomerase group from the action of quinolones. These genes were first mentioned as a unique plasmid-mediated quinolone resistance mechanism found in enteric bacteria. Later, they were found in the chromosomes of many other organisms, along with related mdp genes of a similar nature. They code for low resistance to quinolones, often below the breakpoints for development of complete resistance in the clinical setting [11].

Overproduction of the target enzyme. Bacteria can also oversynthesize antibiotic targets, i.e. the concentration of the target protein exceeds the concentration of the antibiotic itself [38]. Therefore, the target protein is sufficient to continue its role in the cell in the presence of antibiotics. This is due to the mechanism of resistance to trimethoprim in *Escherichia coli* and *Haemophilus influenzae*. Trimethoprim is usually used with sulfamethoxazole (a combination known as co-trimoxazole, or SXT). Overexpression is sometimes found in association with mutations that reduce the ability of an antibiotic to bind to its target. Mutants overexpressing DHPS and/or DHFR are able to overcome the inhibitory ability of antifolate drugs at therapeutic concentrations and become resistant [32].

The role of mobile genetic elements in the spread of antibiotic resistance

genes. Researchers have yet to determine the involvement of transposable genetic elements (TGEs) in the spread of antibiotic resistance [39].

Plasmids are involved in the acquisition of resistance to most classes of antibiotics, including β-lactams, aminoglycosides, tetracyclines, sulfonamides, trimethoprims, macrolides, polymyxins, and quinolones, mainly in Gram-negative bacteria [28, 40]. Multidrug resistance plasmids are usually conjugative, capable of initiating not only their own transfer but also the transfer of other plasmids, and have mechanisms to control their cellular copy number and/or ability to replicate. Plasmids ensure the transfer of antibiotic resistance genes through various mechanisms such as active separation systems, random segregation, or post-segregation killing. S. Nolivos et al. [41] found that tetracycline-resistant *Escherichia coli* strains transferred their resistance to more than 70% of initially tetracycline-susceptible *Escherichia coli* strains in as little as 3 h by conjugative transfer of a transmissible plasmid, the *E. coli* fertility factor (F), carrying an insertion in the Tn10 transposon. In addition to conjugative plasmids, there are mobilizable plasmids that are smaller and do not self-transport, but can transport DNA to a specific host in the presence of conjugative plasmids. This transfer occurs both vertically and horizontally [39].

Insertion sequences (IS) are the smallest (0.7-2.5 kb) and simplest mobile genetic elements (MGEs) found in bacteria. They are flanked by short, mostly inverted, repeats that sometimes generate direct target repeats (DR) during integration into the target DNA [42]. Currently, more than 4500 ISs are described in the specialized database ISFinder (http://www-is.biotoul.fr) [43]. The role of IS in antibiotic resistance has been emphasized many times, especially in studies looking at resistance to colistin and carbapenem [39). Unlike complex transposons, which exist only as a single copy in a specific replicon, ISs can be present in multiple copies, thereby contributing to the accumulation of antibiotic resistance genes (ARGs) [32].

Transposons (Tn) are a category of MGEs carrying antibiotic resistance genes. Many Tn have the ability to move to different parts of the genome (both intra- and intermolecular), mediating ARG mobility [44]. Bacterial Tn can be divided into two types: composite (two IS elements flanking the central gene) and complex (containing the *tnpA* gene encoding transposase and the *tnpR* gene encoding resolvase). The predominant ARG-containing transposons are Tn5 thaat encodes neomycin and kanamycin resistance in *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, Tn10 that encodes tetracycline resistance, Tn9, Tn903, Tn1525, and Tn2350 [40].

Integrons are MGEs capable of accumulating gene cassettes, including ARGs, and distributing them through other mobile genetic elements. They are most commonly found in Gram-negative bacteria, but they are also present in Gram-positive bacteria. Integrons contain the integrase gene (*intI1*), an enzyme that performs site-specific recombination, which leads to the insertion of one or more gene cassettes into the integron platform. Integrons are divided into several classes (1st, 2nd, and 3rd) depending on the amino acid sequence of the IntI enzyme [36]. Class 1 integrons, which are commonly associated with plasmids, are most commonly found in hospital clinical isolates and have also been found in bacterial pathogens detected in food production (e.g., at livestock farms) [45].

Genomic islands are a category of integrative and conjugative elements capable of mediating their own excision. The study of multiple genomic islands has revealed several common and significant characteristics of these chromosomal regions. They are 10-200 kb DNA fragments that are inserted into tRNA genes [46]. Genomic islands contain repetitive recognition sequences and cryptic genes encoding factors that are involved in integration, insertion or transfer [32].

Integrative and conjugative elements (ICE) are responsible for the horizontal transfer of most resistance and virulence factors. ICEs are 18-600 kb in size and are similar to genomic islands in having an insertion at a specific site, associations with phage integrase genes, and flanking with inverted repeats. The excision and integration of ICE is accomplished by a recombinase, often referred to as an integrase. ICE-associated integrases are tyrosine or serine recombinases. Integrative and conjugative elements have the ability to mobilize neighboring sequences, including genomic islands or composite transposons carrying ARGs [39].

Table 2 shows the main types of genetic determinants of resistance to antibacterial drugs in microorganisms.

2. Examples of genetic determinants of antibiotic resistance in microorganisms

Resistance marker gene (bacteria - carriers of genes)	Gene localization	Encoded traits	Mechanism of antibiotic resistance	References
aac(6′)-Ib (*Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Escherichia coli*)	Gene cassettes are located in the first position in class 1 integrons and are associated with weak variants of the PC promoter (PCW or PCH1)	Aminoglycoside resistance (gentamicin)	Enzymatic modification of aminoglycoside antibiotics, namely O-phosphorylation, O-nucleotidylation and N-acetylation, is catalyzed by aminoglycoside phosphotransferases (APH), aminoglycoside nucleotide diltransferases (ANT) and aminoglycoside acetyltransferase (AAC)	[47-49]
aac(6′)-Ib-cr (*P. aeruginosa*, *E. cloacae*, *K. pneumoniae*, *E. coli*)		Resistance to aminoglycosides (gentamicin) and fluoroquinolones (ciproflox-cinofloxacin)	Enzymatic modification of aminoglycoside antibiotics, namely O-phosphorylation, O-nucleotidylation and N-acetylation, is catalyzed by aminoglycoside phosphotransferases (APH), aminoglycoside nucleotide diltransferases (ANT) and aminoglycoside acetyltransferase (AAC), gene mutations (targets); efflux pump	[47-49]
strB, или *aph(6)-Id*, *strA,* или *aph(3′′)-Ib* (*E. coli*, *K. pneumonia*, *Enterococcus faecium*, *P. aeruginosa*)	In non-conjugative plasmid RSF1010 of a wide range of hosts; in transposon Tn5393 with insertion sequence IS1133	Aminoglycoside resistance (streptomycin)	Enzymatic modification of antibiotics, catalyzing the modification of —OH or —NH2 groups in the core of 2-deoxystreptamine or sugar fragments	[47, 50, 51]
aphA, или *aph(3′)IIa* (*E. coli*, *S. aureus*)	In Tn5	Resistance to aminoglycosides (neomycin, kanamycin)	Enzymatic modification of antibiotics	[47, 48, 51]
bla$_{CMY-2}$ (*Citrobacter freundii*, *E. coli*, *K. pneumoniae*, *Salmonella enterica*, *Proteus mirabilis*)	In conjugative/non-conjugative plasmid IncA/C; insertion sequence ISEcp1	Resistance to β-lactams (ampicillin, cefoxitin, ceftriaxon, amoxicillin, ceftiofur)	Pproduction of β-lactamase class C. Resistance is due to formation of a stable acyl enzyme intermediate; due to the high affinity of the antibiotic and the enzyme, the antibiotic is "trapped" and does not reach the target	[47, 50, 52]
bla$_{TEM-1}$ (*K. pneumoniae*)	In pBR322 plasmid	Resistance to β-lactams (ampicillin)	Target modification	[47, 50, 53]
bla$_{CARB-3}$ (*P. aeruginosa*, *E. coli*)	Mobile gene cassettes are located in integrons 1st class	Resistance to β-lactams (ampicillin)	Target modification	[47, 50, 53]
bla$_{CTX-M}$ (*E. coli*, *K. pneumoniae*, *Proteus* spp., *Enterobacter* spp., *Citrobacter* spp., *Salmonella* spp., *P. aeruginosa*)	On plasmids of the IncF family	Resistance to β-lactams (1st generation: cephalosporins: cefazolin, cephalothin, cephalexin; 2nd generation: cefu-roxime, cefaclor; 3rd generation: cefo-taxime, ceftriaxone, ceftazidime, cefix-sim	Target modification	[50, 55]

Contonued Table 2

AmpC (*E. coli*, *K. pneumoniae*, *Proteus* spp., *Enterobacter* spp., *Serratia* spp., *Citrobacter* spp., *Shigella* spp., *Salmonella* spp., *P. aeruginosa*)	Single nucleotide polymorphism, a gene conferring resistance to antibiotics; AmpC enzymes are encoded by chromosomal, plasmid genes and move between chromosomes and plasmids	Resistance to β-lactams (4th generation cephalosporins: cefepime)	Target modification	[50]
blaVIM-1 (*E. coli*, *K. pneumoniae*, *Proteus* spp., *Enterobacter* spp.)	In integrons of the 1st class, which are derivatives of Tn402 (also called Tn5090), a transposon characterized by the presence of a transposition module that includes a set of four genes (*tniR*/*tniC*, *tniQ*, *tniB* and *tniA*). Some of the Tn402 elements have been found in various Tn3-like transposons. In *Enterobacteriaceae* isolates, blaVIM-1 is part of the integrons located either in the In2-Tn402 element associated with Tn21 or in the Tn402 transposon, which is associated with the IncHI2 or IncI1 plasmid, respectively.	Resistance to β-lactams (carbapenems: meropenem, imipenem, doripenem)	Production of carbapenemases, enzymes capable of hydrolyzing almost all β-lactams, or through modifications to the outer cell membrane, in particular, by reducing the permeability of the cell membrane as a result of porin modification and/or production of an efflux pump	[50, 56, 57]
blaKPC-2 (*E. coli*, *Proteus* spp., *Enterobacter* spp., *P. aeruginosa*)	Located in the Tn3-related transpozone Tn4401, capable of high transposition frequency	Resistance to β-lactams (carbapenems: meropenem, imipenem, doripenem)	Production of β-lactamases (carbapenemases), presence of efflux pumps and mutations that alter the expression and/or function of porins and penicillin-binding proteins (PBPs)	[50, 58, 59]
tetA, *tetR*, *tetB*, *tetC*, *tetG* (*E. coli*, *K. pneumoniae*)	Located in the Tn10	Tetracycline resistance (tetracycline)	Efflux by tetracycline-specific pumps: the drug is actively pumped out of the bacterial cell unchanged	[47, 50, 60]
tetM (*Clostridium difficile*, *E. faecalis*, *E. faecium*, *S. aureus*, *E. coli*)	Located in the Tn916 и Tn1545		The ribosome can function at a high drug content inside the bacterial cell due to complex interactions with other bacterial proteins; proteins prevent tetracycline from binding to the ribosome and provide some degree of protection against it	[47, 50, 60]
sul1, *sul2*, *sul3* (*Enterococcus* spp., *C. freundii*, *E. coli*, *Klebsiella oxytoca*)	Located in small conjugative plasmids or large transimisive plasmids with multiple resistance in class 1 integrons	Resistance to sulfonamides (sulfanilamide)	Antibiotic target modification	[47, 50, 61]
floR, *cmlA* (*E. coli*, *K. pneumoniae*)	Mobile gene cassettes are located in integrons 1st class	Resistance to phenicols (chloramphenicol)	Active efflux: resistance to antibiotics due to the transport of antibiotics outside the cell	(47, 49, 62)
cat1 (*C. freundii*, *E. coli*, *Proteus vulgaris*)	Located in the Tn9	Resistance to fenicolas (chloramphenicol, florfenicol)	Enzymatic inactivation of an antibiotic causing drug resistance	[47, 50]
cfr (*E. faecalis*, *E. coli*, *S. aureus*)	Located in the plasmids pEF-01, pEC-01, pSCFS3	Resistance to oxazolidinones, streptogramin, lincosamide, fenicols	Mutational change or enzymatic modification of an antibiotic target leading to antibiotic resistance	[47, 63, 64]
fosA1 (*Serratia marcescens*)	Licated in the Tn2961	Fosfomycin resistance	Enzymatic inactivation of an antibiotic causing drug resistance	[47, 50, 65]

Contonued Table 2

mphA (*C. freundii*, *E. coli*)	Insertion sequence IS 26 in transposon Tn6242 in integrals of the 1st class	Resistance to macrolides (azithromycin, erythromycin)	Enzymatic inactivation of an antibiotic causing drug resistance	[47, 50, 56]
ErmB (*E. faecium*, *S. aureus*)	In a transposon Tn1546 located on plasmid pMCCL2	Macrolide resistance (erythromycin)	Mutational change or enzymatic modification of an antibiotic target leading to antibiotic resistance	[50, 67]
arr2 (*P. aeruginosa*, *C. freundii*, *E. coli*, *K. pneumoniae*)	Gene cassette localized in class 1 integron — In53, located on a composite trans-poson or plasmid	Resistance to rifampicin	Enzymatic inactivation of an antibiotic causing drug resistance	[47, 50, 68]
VanA (*E. faecium*, *E. faecalis*)	In transposon Tn5281 on plasmid pBEM10; in transposon Tn1546 on plasmid pIP816	Glycolepid resistance (teicoplanin, vancomycin)	Mutational change or enzymatic modification of an antibiotic target leading to antibiotic resistance	[47, 69]
GyrA, *ParC* (*Streptococcus* spp., *P. aeruginosa*, *Enterobacteriaceae*, *K. pneumoniae*)	Localization not clear	Resistance to fluoroquinolones (ciprofloxacin, ofloxacin, levofloxacin, cefixime)	Mutational change or enzymatic modification of the antibiotic target leading to antibiotic resistance; active efflux: resistance to antibiotics due to the transport of antibiotics outside the cell	[50, 70]

Control and containment of the spread of antibiotic resistance in animal husbandry. The spread of antimicrobial resistance among human and animal pathogens poses a huge threat to global public health. The use of antimicrobials in human and veterinary medicine, especially the use of large amounts of antibacterial agents in animal husbandry to promote animal growth, reinforces the unfortunate trend of the emergence and spread of antimicrobial-resistant bacteria [71, 72], which exacerbates the need for the rational use of antibiotics.

The World Health Organization (WHO) classifies fluoroquinolones, 3rd and 4th generation cephalosporins, macrolides, glycopeptides, and polymyxins as primary and critical antibacterial agents for human and veterinary use [73]. However, penicillin antibiotics, macrolides, and fluoroquinolones are used only in humans, while tetracyclines, penicillins, and sulfonamides are used only in animals.

First of all, reserve antibiotics in animal husbandry (erythromycin, oleandomycin, chloramphenicol, neomycin, monomycin, kanamycin, gentamicin, vancomycin, ciprofloxacin) should be used with caution [74]. The use of avoparcin as a feed additive has led to the emergence of bacteria resistant to vancomycin, an antibiotic of reserve for combating life-threatening infections caused by Gram-positive bacteria [75]. Recently, the veterinary service of molecular genetics (Servei Veterinari de Genиtica Molecular, SVGM) of the Universitat Autтnoma de Barcelona (UAB) analyzed and sequenced faecal samples from the owner of the farm and the animals kept there (cattle, pigs). The results showed that the isolated culture of *E. coli* in the studied samples from calves and pigs, as well as from the farmer, carried genes for resistance to colistin, the antibiotic of last resort in medicine. The experts concluded that the *mcr-1* resistance gene was transferred from animals to humans through horizontal transfer and plasmid exchange between *E. coli*, since calves and pigs received the antibiotic for preventive and therapeutic purposes, and the farm owner never received therapy with this drug [76].

One of the main challenges is to reduce the use of antibiotics in livestock production by improving the quality of life and animal welfare. In this regard, it is recommended that good practices for keeping and handling animals in livestock establishments and when transporting animals are recommended; improving

animal welfare (e.g., providing an optimal microclimate, quality water, adequate ventilation and space allocation) at all stages, including production, transport and slaughter; using locally adapted breeds that are more resistant to disease and stress, or animals selected for disease resistance (resistant animals will require fewer antimicrobial treatments); compliance with veterinary and sanitary, sanitary and hygienic rules, biosafety measures at agribusiness enterprises to prevent the use of medicines; adherence to strict disease control measures (eg vaccinations); the use of feed ingredients/additives that increase the efficiency of feed conversion to eliminate the use of antibiotics as growth stimulants (feed enzymes, competitive probiotics, prebiotics, acidifiers, plant extracts, nutraceuticals, essential oils, yeast, etc.); avoiding food ingredients with anti-nutritional properties (such as lectins and protease inhibitors); application of modern methods of waste disposal. Particular attention needs to be paid to primary production (specific supply chains) and the planning of practical actions that can be taken to reduce the need for antimicrobials and control the spread of antimicrobial-resistant organisms in the environment [11, 77].

In addition, it is necessary to organize and conduct monitoring and surveillance of the spread of antibiotic-resistant bacteria, including the assessment and identification of trends and sources of antimicrobial resistance in bacteria; discovery of new mechanisms of antimicrobial resistance; providing data necessary for the analysis of risks in relation to animal and human health; providing a basis for practical advice on animal and human health; providing information for monitoring antimicrobial prescribing in agricultural organizations and judicious use of recommendations; evaluation and determination of the effectiveness of measures to combat antibiotic resistance [78-80]. Because antibiotic resistance occurs as part of an irreversible process, it can be slowed down but not stopped. Therefore, there will always be a need to develop new antibiotics and diagnostic tests to combat the development of resistance [81].

Thus, various mechanisms of antibiotic resistance and ways of acquiring them by bacteria significantly complicate the process of selecting effective antibiotic therapy both in agro-industrial organizations and in medical institutions. The mechanisms of acquired and natural antibiotic resistance are inherently complex and vary from species to species, from strain to strain of microorganisms. Basically, intraspecific and interspecific acquisition of antibiotic resistance genes is carried out through horizontal transfer (conjugation, transformation, transduction). Key measures to combat antibiotic resistance include reducing the use of antibiotics by improving the quality of life and animal welfare; organizing and conducting monitoring and supervision of the spread of antibiotic-resistant bacteria; development of new antibiotics and test systems for diagnosing antibiotic resistance in bacteria.

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