Antibiotic resistance genes in bacteria: Occurrence, spread, and control

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Abstract
The production and use of antibiotics are becoming increasingly common worldwide, and the problem of antibiotic resistance is increasing alarmingly. Drug-resistant infections threaten human life and health and impose a heavy burden on the global economy. The origin and molecular basis of bacterial resistance is the presence of antibiotic resistance genes (ARGs). Investigations on ARGs mostly focus on the environments in which antibiotics are frequently used, such as hospitals and farms. This literature review summarizes the current knowledge of the occurrence of antibiotic-resistant bacteria in non-clinical environments, such as air, aircraft wastewater, migratory bird feces, and sea areas in-depth, which have rarely been involved in previous studies. Furthermore, the mechanism of action of plasmid and phage during horizontal gene transfer was analyzed, and the transmission mechanism of ARGs was summarized. This review highlights the new mechanisms that enhance antibiotic resistance and the evolutionary background of multidrug resistance; in addition, some promising points for controlling or reducing the occurrence and spread of antimicrobial resistance are also proposed.

KEYWORDS
antibiotic resistance genes, antimicrobial resistance, drug-resistant bacteria, horizontal gene transfer, multidrug resistance

1 | INTRODUCTION

Antibiotics were hailed as the greatest discovery of modern medicine in the 20th century and have played an important role in controlling infectious diseases in humans [1]. Antibiotics are commonly administered to treat infections in humans and animals and have been used widely as an animal growth promoter, irrespective of whether a bacterial infection has been detected [2]. It was reported that global antibiotic consumption had increased by 35% in the first decade of the 21st century [3]. According to statistics, the use of antibiotics in China was about 162,000 tons in 2013, of which, animal consumption accounted for 52% [4]. This resulted in the accumulation of antibiotic residues and antibiotic-resistant bacteria in the animal’s gut,
which were excreted in the animal's feces [5]. Antibiotics are now probably used in almost all regions of the world to increase productivity and economic efficiency. According to the National Institute of Animal Health, about 104–110 million livestock, 7.5–8.6 billion chickens, 60–92 million pigs, and 275–292 million turkeys are fed various levels of antibiotics [6,7]. Thus, the pollution of antibiotics is not only regional but also global. Various types of antibiotic resistance genes (ARGs) are often found in livestock manure around the world. Infections caused by antibiotic-resistant bacteria are a major threat to global public health. In the European Union alone, antibiotic-resistant infections are estimated to cause more than 30,000 deaths annually, with Italy and Greece having the highest number of cases [8]. In low- and middle-income countries in Asia, Africa, and South America, multidrug-resistant infections have higher morbidity and mortality rates [9–11]. The number of deaths from antibiotic-resistant infections worldwide is expected to rise from 700,000 in 2014 to 10 million annually by 2050, with the cumulative cost of healthcare and reduced productivity reaching $100 trillion [12].

The presence of ARGs is the root cause of bacterial resistance. Pathogenic bacteria acquire ARGs through plasmid exchange at the gene level and develop strong resistance to antibiotics. ARG-carrying plasmids, integrons (In), and transposons (Tn) in bacteria can undergo horizontal gene transfer (HGT) among strains of the same species and different species. Even after the death of resistant strains, exposed DNA carrying ARGs exist in the environment for a long time under the protection of deoxynucleotide enzymes [13,14]. The abuse of antibiotics and the ARG pollution induced by antibiotics has attracted extensive attention from scholars around the world, but the reported studies have mostly focused on the pollution of one or several typical antibiotics or ARGs; detailed systematic research on bacteria resistant to multiple antibiotics and diversity of ARGs is lacking.

This paper reviews the progress of research on the emergence, transmission, and control of antibiotic resistance. It also includes the classification and distribution of ARGs, their occurrence and release in animal farms, their environmental transmission, and their potential contact routes with human pathogens. In addition, the efficiency of the removal of ARGs by different treatment processes is also evaluated. Finally, in this paper, we highlight key research needs and provide suggestions to reduce the risk of transmission of ARGs from livestock waste, thereby reducing health risks in society.

2 MECHANISM OF ANTIBIOTIC RESISTANCE

2.1 Bacterial adaption to antibiotics

The development of antibiotic resistance is a natural phenomenon that is encoded by the ARGs of microorganisms and is the product of billions of years of evolution. Bacteria in the environment already carry ARGs that aid in the development of resistance to newly approved antibiotics long before the first clinical use of the antibiotics [15]. Studies of bacteria from permafrost samples have shown that they had developed resistance in the absence of human activity [16,17]. Bacteria can develop intrinsic resistance to certain antibiotics. The intrinsic resistance of a bacterium to a particular antibiotic is due to its inherent structural and/or functional properties that provide resistance to the action of that antibiotic. Some antibiotics can be effectively eliminated by efflux after they have entered the cells through porin, whereas some antibiotics are unable to pass through the outer membrane, and hence, cannot reach their target sites (Figure 1). The intrinsic resistance of a strain is due to the lack of sensitive targets for a particular antibiotic. For example, the biocide triclosan cannot inhibit the growth of the gram-negative Pseudomonas spp., because the fabI-insensitive allele carries another enoyl-ACP reductase enzyme, which is the target of the antibiotic in the sensitive species [18]. Moreover, the proportion of anionic phospholipids in the plasma membrane of Gram-negative bacteria is lower than that in Gram-positive bacteria, which reduces the efficiency of Ca2+-mediated daptomycin insertion into its plasma membrane [19]. Thus, liposidomycin is effective against Gram-positive bacteria but not against Gram-negative bacteria.

In addition to having intrinsic resistance, bacteria can also acquire resistance to antibiotics through different mechanisms. First, the intracellular concentration of antibiotics is minimized due to poor bacterial osmosis or antibiotic efflux [20]. Second, bacteria can modify antibiotic targets through genetic mutations or posttranslational modifications of the target protein [21]. Third, bacteria can inactivate antibiotics by hydrolyzing or modifying them [22]. Reduced expression of porin proteins in Pseudomonas spp. and Acinetobacter spp. significantly promotes resistance to new drugs such as carbapenems and cephalosporins, usually mediated through enzymatic degradation [23–25]. For instance, in the absence of carbapenems, Enterobacteriaceae might show clinically relevant carbapenem resistance if mutations reduce the production of porin proteins or if mutant
alleles of porin proteins are present. The selection pressure exerted by these antibiotics is conducive to the emergence of porin gene mutations and the emergence of genes that regulate the expression of porins [24]. The bacterial efflux pump actively transports many antibiotics to the extracellular environment, rendering them ineffective. This is the main reason for the inherent resistance of Gram-negative bacteria to many drugs that can be used to treat Gram-positive bacterial infections. When the efflux pump is overexpressed, it may also cause resistance to previously sensitive antibiotics. A large amount of information from bacterial genomics indicates that there are multiple efflux systems in bacteria, and multiple-specific efflux pump combinations can cause multidrug resistance (MDR) and significantly increase bacterial resistance to multiple antibiotics. In Pseudomonas aeruginosa, the MexXY pump system has been recognized as one of the major determinants of aminoglycoside resistance [26]. The expression of bacterial multidrug efflux systems is usually controlled by transcriptional regulators, which inhibit or activate the transcription of multidrug efflux genes [27,28]. In multidrug-resistant bacteria, the expression of high levels of efflux genes is often due to mutations in the regulatory network that controls efflux pump expression. These mutations might be present in local repressors, global transcription factors, or intergenic sites that alter the expression of pump genes or their regulators [29–31]. For example, a single-base pair mutation was detected in the consensus −10 sequence upstream of the mtrC gene in Neisseria gonorrhoeae, which resulted in a new and more active promoter that led to the constitutive overexpression of efflux pumps, and thus, imparted MDR [32]. Most antibiotics function by binding to the target protein with high affinity and specificity, thus inhibiting the normal activity and function of the target. Changes in the structure of the target protein in microorganisms can effectively prevent the binding of antibiotics without affecting the normal function of the target, resulting in drug resistance. During infection, however, there are usually a large number of different types of pathogens, and the selection pressure of the antibiotics forces microbes to code for mutations in the genes of antibiotic targets [33], which causes microbes to gain antibiotic resistance; the strains with such mutations multiply faster than those without the ARGs. For example, the clinical application of linezolid resulted in a copy mutation in Streptococcus pneumoniae and Staphylococcus aureus resistance genes that were recombined at high frequency between homologous alleles, rapidly producing a population that favored carrying the mutant allele [34,35]. In addition, modifying targets to gain antibiotic resistance is an effective strategy and does not require a mutation in the gene encoding the target molecule. In recent years, it has been found that target protection is the clinically relevant mechanism of several important antibiotic resistance. For example, chloramphenicol-florfenicol resistance (cfr) methyltransferase specifically methylates A2503 in 23S rRNA, providing resistance to a variety of drugs that have targets near this site, including phenicols, pleuromutilins, streptogramins, lincosamides, and oxazolidinones [36].

**FIGURE 1** The mechanism of bacterial resistance. A, B, C, D, and E are different antibiotics; ABP, antibiotic binding protein. Antibiotic A can enter the cell via a membrane-spanning porin protein, reach its target, and play an antibacterial role; bacteria develop resistance by altering the structure of the antibiotic binding proteins so that they cannot bind to antibiotics. Bacteria bind to targets of antibiotic B by producing chemical groups, so that antibiotic B does not work. After antibiotic C enters the cell, it is discharged out of the cell through the AcrAB–TolC efflux pump, resulting in antibiotic resistance. Bacteria produce enzymes that hydrolyze antibiotic D and develop resistance. Antibiotic E cannot cross the outer membrane, and hence, is unable to access the target ABP.
2.2 | Bacteria develop resistance by altering antibiotics

Besides preventing antibiotics from entering cells or changing themselves, bacteria can develop resistance by destroying or modifying antibiotics. Bacteria can inactivate antibiotics by hydrolyzing them (Figure 1). Enzyme-catalyzed modification of antibiotics is the main mechanism of antibiotic resistance. After many years of research, thousands of enzymes have been found that can degrade and modify different kinds of antibiotics, including β-lactams, aminoglycosides, phenicols, and macrolides [37]. Some enzymes break down different antibiotics in the same class; for example, the β-lactam antibiotics, such as penicillins, cephalosporins, carbapenems, and monobasins, can be hydrolyzed by various β-lactamases [37–39]. Antibiotic-resistant bacteria can inactivate antibiotics through the transfer of chemical groups (Figure 1). Bacteria produce enzymes that add chemical groups to the susceptible sites of antibiotic molecules, preventing them from binding to target proteins and rendering them ineffective. This leads to the development of resistance to some antibiotics. A variety of different chemical groups (including acyl, phosphates, and nucleotides) can be transferred by enzymes to the susceptible sites; these enzymes comprise a large and diverse family of antibiotic-resistant enzymes [40]. Because aminoglycosides are large molecules with many exposed hydroxyl and amide groups, they are particularly susceptible to modifications, thus leading to high levels of resistance to the modified antibiotics. There are three main types of aminoglycoside modifying enzymes, which include acetyltransferases, phosphotransferases, and nucleotidyltransferases. A study found a new genomic island in Campylobacter spp. (isolated from broilers) that encodes six aminoglycoside modifiers, including members of all the three classes of enzymes that confer resistance to several aminoglycoside antibiotics [41].

2.3 | Other mechanisms of bacterial resistance

Although the majority of clinically important antibiotic resistance mechanisms have been well-characterized, there are still some key gaps in our knowledge. One such gap concerns the mechanism by which the ABC-F proteins mediate resistance to a broad array of clinically important antibiotic classes that target protein synthesis in Gram-positive pathogens. Sharkey et al. [42] found that the antibiotic-resistance ABC-F proteins mediate ribosomal resistance, and these proteins replace bound antibiotics in ribosomes, providing resistance through ribosomal protection. In Mycobacterium tuberculosis, mutations in RV2887 lead to the upregulation of RV0560c expression. RV0560C is an S-adenosyl-L-methionine-dependent methyltransferase, which can be inactivated by the N-methylation modification of the pyridine benzimidazole 14 [43]. In addition, bacteria living in biofilms often get low levels of oxygen and nutrients. Bacteria have metabolic adaptability to survive in stressful environments, which lead to the generation of drug resistance [44]. Filamentous phages promote resistance by “isolating” antibiotics by forming liquid crystal structures [45]. A recent study found that bacteria do not have to acquire ARGs to become resistant. DNA integration occurs in the natural transformation process of bacteria. Exogenous transformed DNA (tDNA) can be effectively integrated into the bacterial genome in the form of single-stranded DNA after ingestion by bacteria. The resulting heterologous double-stranded bodies are separated after chromosome replication, and the integrated tDNA products are rapidly expressed before bacterial division. This allows only one of the daughters to inherit the integrated DNA, but the other also inherits its protein products. This mechanism can promote the phenotypic inheritance of antibiotic resistance in Vibrio cholerae and S. pneumoniae. Perhaps most surprisingly, this nongenetic inheritance of antibiotic resistance is long-lasting and can confer resistance to untransformed cells across multiple generations [46].

2.4 | Mechanisms of MDR

“Resistance” denotes the inherent resistance of drug-resistant bacteria and is the phenotypic expression of pre-existing resistance genes. When resistance to an antimicrobial compound is a phenotypic expression of a resistance-encoding gene following HGT [47], it is called “acquired” resistance [48]. MDR can arise via two genetic mechanisms: either by de novo mutation (e.g., point mutations, insertions, deletions, or duplications) or by bacterial recombination, which is the transfer of DNA from one bacterium to another by means other than vertical transmission. The evolution of MDR was inhibited by the initial diversity in single-antibiotic resistance in bacterial populations. Contrastingly, recombination promoted the evolution of MDR in polycultures [49]. MDR evolved more rapidly in polycultures by recombination with bacteria resistant to any other treatment, suggesting that evolution through recombination of existing diversity was more important than through novel mutations. In the absence of recombination, standing diversity inhibits the evolution of novel traits such as MDR that rely on bringing together
different traits already present in the population. Recently, in multidrug-resistant clinical isolates, such as pneumococci, it was found that if both recombinant and non-recombinant bacteria exist, the recombinant population might evolve MDR and result in the extinction of the single drug-resistant non-recombinant genotype [50]. Not only is the use of novel antimicrobial molecules likely to foster the evolution of de novo resistance mechanisms, but it can also favor the emergence of pathogens possessing yet unidentified combinations of resistance genes [51]. However, in combination therapy, gens possessing yet unidentified combinations of mechanisms, but it can also favor the emergence of pathogen.

Not only is the use of novel antimicrobial molecules likely to foster the evolution of MDR and result in the extinction of pneumococci, it was found that if both recombinant and non-recombinant bacteria exist, the recombinant genotype [50].

JIAN ET AL.

The widespread application of antibiotics in medical care and animal husbandry creates selection pressure on bacteria in the animal’s digestive system to acquire and maintain ARGs. This causes an increase in the relative abundance of resistant strains. Different ARGs of bacteria can develop resistance to different types of antibiotics. Resistance genes can be divided into the following categories based on the class of antibiotics they grant resistance to which include tetracyclines (tet), sulfonamides (sul), β-lactams (bla), macrolides (erm), aminoglycosides (aac), fluoroquinolone (fca), colistin (mcr), vancomycin (van), and multidrug (mdr) [56].

The top 10 ARGs reported from hospitals were multidrug, glycopeptide, and β-lactam ARGs (meca, vanA, vanB, and bla), while sulfonamide and tetracycline ARGs (sul and tet) were the most common ARGs from farms, wastewater treatment plants (WWTPs), water, and soil. In six types of habitats (farms, cities, WWTPs, water, soil, and air), the first 50 subtypes of ARGs included eight antibiotic families: β-lactam (bla), sulfanilamide (sul), tetracycline (tet), aminoglycoside (aad), multidrug (mec), amphenicol (flo), trimethoprim (dfr), and glycopeptide (van). Among them, mecA (multidrug) was the most common in the world, followed by β-lactam (bla), glycopeptides (vanA, vanB), sulfonamide (sul1, sul2), and tetracycline (tetM). The top 10 ARGs reported from Asia included blaNDM-1, blaCTX-M-15, mecA, blatem-1, sul1, vanA, blaKPC-2, sul2, blaCTX-M-14, and blaOXA-48 (responsible for β-lactam, multidrug, sulfonamide, and glycopeptide antibiotics). Therefore, the dissemination of ARGs is a global problem without borders or boundaries.
3.1 Livestock farms

Since most antibiotics used in livestock are for non-therapeutic purposes, such as growth promotion and disease prevention, low and sublethal concentrations of antibiotics are consistently present in the gastrointestinal environment of livestock, which promotes the production of ARGs. Although livestock accounts for more than half of all the antibiotics used globally, only 5%–15% of the publications from 2000 to 2020 on antibiotic-resistant genes investigated the contribution of livestock to potential risks. Although the number of relevant studies on livestock has been growing, it still does not exceed 17% of all the studies published on antibiotic resistance, as of 2020. Therefore, it is necessary to promote research in this area to reduce the risk of antibiotic resistance.

ARGs are found extensively in the wastes of industries associated with animal husbandry, and the content of resistance genes in these industries is much higher than that in hospitals, soil, groundwater, and surface water (Table 1). The higher concentration of antibiotic residues in the livestock farm waste than in human waste might be due to the continuous use of antibiotics to promote animal growth and prevent diseases [59]. This, in turn, imposes a greater selection pressure on the bacteria in livestock farms for the evolution of resistance genes. In different types of livestock and poultry farms, the ARG level in chicken manure was found to be higher than that in pig manure.

| Drug class | Genes | Matrices | Locations | Refs |
|------------|-------|----------|-----------|------|
| β-Lactams  | blaTEM, mecA, blaCTX-M-1,9 | Pigs, poultry, and cattle fecal wastes | Spain | [68] |
|            | blaCTX-M-25, blaTEM-116,229 | WWTPs | Argentina | [69] |
|            | blaCTX-M-9, mecA | Urban sewage and river water | Barcelona | [70] |
|            | blaCTX-M-15,13,109,114, blaTEM-116 | Human fecal samples | Spain | [71] |
|            | blaTEM, blaCTX-M, blaOXA-48-like, blaNDM | WWTPs | Washington State | [72] |
|            | blaTEM, blaCTX-M, blaPSE, blaCMY-2 | Urban surface water | South Korea | [73] |
|            | blaTEM, blaCTX-M, blaOXA-48-like, blaNDM | WWTPs | Washington State | [72] |
|            | blaTEM, blaCTX-M, blaNDM | Meat, pork, beef- and chicken-minced meat, ham, mortadella | Spain | [74] |
|            | blaCTX-M | River water | China | [75] |
| Quinolones | qnrA, qnrS | Human fecal samples | Spain | [76] |
|            | aac(6’)-Iu, IJe, Jy, qnrA3 | Urban surface water | South Korea | [73] |
|            | aac(6’)-Ib-cr, qnrS | Chicken feces | China | [77] |
|            | qnrA, qnrS | Meat, pork, beef- and chicken-minced meat, ham, mortadella | Spain | [74] |
|            | aac(6’)-Ib-cr | River water | China | [75] |
| Glycopeptides | VanY | Urban surface water | South Korea | [73] |
| Sulfanilamide | dfrB2 | Urban surface water | Antarctic and Mediterranean | [73] |
|              | sul1 | Seawater | | [71] |
|              | sul1, sul2 | Chicken feces | China | [77] |
|              | sul1 | Meat, pork, beef- and chicken-minced meat, ham, mortadella | Spain | [74] |
| Tetracyclines | tetW | Seawater | Antarctic and Mediterranean | [71] |
|              | tetM | Chicken feces | China | [77] |
| Macrolides   | ermB, ermF | Chicken feces | China | [77] |
|              | ermF | River water | China | [75] |

Abbreviation: WWTP, wastewater treatment plant.
and cattle farms, while the ARG pollution in layer manure and sow manure was higher than that in broiler manure and piglet/fattening pig manure, respectively [60,61]. Cheng et al. [62] investigated the abundance and diversity of tetracycline and sulfonamide resistance genes in chicken manure from small- and medium-sized chicken farms in Hangzhou, Eastern China. The results showed that the relative abundance of various ARGs in chicken manure from medium-sized chicken farms was higher than that from small chicken farms. Moreover, the relative abundance of tetQ was the highest in these farms, followed by the abundance of tetM and sul2. Osman et al. [63] showed that blaOXA-1, blaMOX-like, blaCTX-like, blaSHV, and blaFOX were ubiquitous in hatcheries. Tetracycline antibiotics are commonly used to treat diseases and promote growth in pig farming [4,63]. Therefore, tetracycline resistance genes are ubiquitous in pig feces, among which, the genes for the protection of ribosomal proteins are the most abundant. Cheng et al. [64] showed that the abundance of ribosomal protective protein genes (tetQ, tetW, and tetO) in pig manure was higher than that of effenter pump protein genes (tetA, tetB, tetC, tetG, and tetL). In addition, Ma et al. [65] showed that high levels of tetracycline, multidrug, erythromycin, and aminoglycoside resistance genes were found in samples of human, chicken, and pig feces. The content of ARGs in adult chicken feces was significantly higher than that in other feces. Wang et al. [66] identified 330 ARGs in 18 samples of human, chicken, and pig feces, which were resistant to 21 types of antibiotics. Among them, the abundance of resistance genes for tetracycline, macrolide–lincosamide–streptogramin B, aminoglycosides, lincosamides, and β-lactams were higher than that of the other ARGs; the abundance of tetracycline-ARG was the highest in all the samples. Metatranscriptomic analysis revealed that 49.4%, 66.5%, and 56.6% of ARGs were expressed in human, chicken, and pig samples, respectively; the ARGs were mainly for tetracycline, aminoglycosides, and β-lactam. In general, the abundance of ARGs in cattle and fish feces was found to be lower than that in pig and chicken feces, and the absolute abundance of ARGs in pig wastewater was about two to three orders of magnitude higher than that in fish ponds [60]. The diversity of ARGs in waste from chicken and pig farms was also higher than that from cattle, sheep farms, and fish ponds, possibly due to the more extensive use of antibiotics in chicken and pig farms for the prevention of diseases and promotion of growth [67]. Eastern China has the highest proportion of antibiotic use and an estimated total annual excretion of 5400 tons, with 37% of excretion from pigs, which is higher than those from humans and other animals [4]. Qian et al. [61] analyzed the relative abundance of ARGs in feces of different livestock and poultry and found that the number of bacterial species and total abundance of ARGs in cow dung was lower than those in chicken and pig farms. Among all the ARGs found, β-lactam resistance genes had the highest degree of pollution.

### 3.2 WWTPs and sewage

Apart from focusing on the animal husbandry industry, many studies on ARG have also focused on other environments, such as WWTPs (Table 1). A recent study investigated ARGs in aircraft sewage. The sewage from the aircraft tank showed high ARG diversity compared to conventional sewage, and many different ARGs were found in a small quantity of bacterial DNA. The β-lactamase gene blaCARB-4 was found to be relatively abundant in aircraft wastewater but had never been detected in any sample of municipal wastewater that was not discharged from airports. Moreover, the relative abundance of ARGs in the effluent carried by aircraft is higher than that in the ordinary untreated municipal effluent. Thus, contaminated water carried by aircraft can effectively contribute to the rapid and global spread of antibiotic resistance [78]. It was found that the taxonomic diversity of bacteria in human and animal samples was low, and the abundance of insecticide/metal resistance genes and mobile genetic elements (MGEs) was low, while the abundance of ARGs was high. Environments polluted with discharges from pharmaceutical production units and the Beijing smog had the highest relative abundance and diversity of ARGs, followed by wastewater/sludge; human and animal microbiomes showed intermediate figures, and ARGs were considerably lower in other external environments [79].

### 3.3 Air

Previous meta-analyses of the diversity of ARGs in metagenomes from different types of environments did not include air. The abundance and diversity of ARGs in the air are affected by the environment. Hence, their potential importance has largely gone unnoticed. Here, we describe air as a hotspot for the development of resistance and/or transmission of ARGs. There are many ARGs in the air around animal farms and sewage treatment plants; the former mainly comprises aminoglycoside macrolides and tetracycline ARGs, while the latter mainly comprises MDR and bacitracin ARGs [80]. Antibiotic resistance and detoxifying genes were widely found in the air of Beijing [81]. Among all the culturable bacterial strains, MDR strains accounted for 23.7% (18/76), 22.4% (13/58) in polluted weather, and 27.8% (5/18) in unpolluted weather. Most cultivable strains
were resistant to penicillin [82]. Compared to industrial and urban areas, the seasonal differences of airborne bacteria and ARGs in PM2.5 from rural areas were the most obvious; the total number of bacteria was significantly lower in winter, and specific ARGs were enriched (diluted in spring). The correlation between ARGs and the mobile genetic factor IntI1 decreased from rural areas to urban and industrial areas [83]. Li et al. [84] collected sufficient amounts of particulate matter in the atmosphere of 19 cities from 13 countries around the world and used high-throughput molecular biotechnology to find that the global atmosphere is being polluted by drug-resistant genes to varying degrees and some regions show a trend of increase in the atmospheric drug-resistance genes every year. The detected genes were used to express resistance to drugs, including quinolones, β-lactams, macrolides, tetracyclines, sulfonamides, aminoglycosides, and vancomycin. It was also found that the abundance and species distribution of ARGs in the atmosphere of different cities were significantly different, and the total relative abundance of ARGs was over two orders of magnitude, among which, the most prevalent ARG was β-lactam resistance gene blatem, whose relative abundance increased by 178% from 2004 to 2014 (taking the atmosphere of Xi’an as an example). These studies indicated that resistance genes in the drug-resistant gene pool might be transmitted through the air, providing important references for the comprehensive and effective control of infections transmitted by drug-resistant bacteria.

4 | TRANSMISSION OF RESISTANCE GENES

The use of antibiotics in animal husbandry accounts for approximately 50% of the total use of antibiotics. Therefore, the intestines and feces of livestock and poultry are the largest repositories of drug-resistant strains and ARGs and contribute to the large-scale spread of ARGs, which has been studied in detail by many researchers. The spread of drug-resistant strains occurs through the concerted activities of MGEs, able to move within or between DNA molecules, which include insertion sequences (IS), Tn, and gene cassettes/In, and those that can move between bacterial cells, such as plasmids and integrative conjugative elements. Together these elements play a central role in facilitating horizontal genetic exchange, and therefore, promote the acquisition and spread of resistance genes [85]. IS and Tn are discrete DNA segments that can move (with associated resistance genes) almost randomly to new locations in the same or different DNA molecules within a single cell. The IS elements affect antibiotic/xenobiotic resistance by directly inactivating uptake determinants. Examples include increased carbapenem resistance because of IS transposition into the porin coding oprD gene of numerous P. aeruginosa isolates and P. putida; the porin coding carO gene of A. baumannii; the porin-coding ompE36 gene of Enterobacter aerogenes; and the porin-coding ompK36 gene of Klebsiella pneumoniae [86]. The influence of the IS in antibiotic resistance phenotypes has been covered in recent reviews [85,86]. ARGs are transmitted through HGT, which is important for bacteria to survive and is one of the main driving mechanisms for the transfer of ARGs. The three main mechanisms of bacterial HGT are intracellular conjugation (mediated by plasmids and integrative conjugative elements), natural transformation (mediated by bacteriophages), and transduction (uptake of extracellular DNA) [87] (Figure 2).

4.1 | Resistance plasmids

Plasmids are important vehicles for carrying other MGEs and acquired AMR genes associated with these elements in both Gram-negative and Gram-positive bacteria, and they vary in size from less than a kilobase to several megabases [88]. In resistance plasmids, these accessory regions are typically made up of one or more resistance genes and associated mobile elements described above (IS, Tn, and/or In). Plasmid replication initiates at a defined region, the origin, triggered either by an RNA transcript or, more commonly, by the binding of an initiation protein (Rep), encoded by a rep gene on the plasmid, to the proximal iterated DNA repeat sequences termed as iterons. Three modes of plasmid replication have been described for circular plasmids: (i) rolling circle (RC) replication is commonly used by small plasmids in Gram-positive and, less commonly, Gram-negative bacteria. This mode of replication effectively limits plasmid size, so RC plasmids are usually cryptic or carry only a single resistance gene. (ii) Relying on an initiator-mediated localized melting of double-stranded DNA (dsDNA) at the origin to trigger replication based on RNA primers. (iii) IncQ plasmids use the third mode of replication, termed strand displacement, where both DNA strands are replicated continuously in opposite directions from the origin; these plasmids are also usually small [85]. Plasmid propagation is facilitated not only through vertical transmission via cell division but also horizontal transmission to other bacterial cells. Therefore, plasmids can promote the transmission of ARGs. The main characteristics and classification of known resistance plasmids are shown in Table 2.
Bacteriophages

Bacteriophages are viruses that infect bacteria and replicate inside them. Phages can transfer DNA from one bacterium to another through genetic transduction. Antagonistic coevolution (AC) between bacteria and phages is the reciprocal evolution of bacterial resistance and phage infectivity. AC between bacteria and bacteriophages plays a key role in driving and maintaining microbial diversity [91]. Bacteriophages are considered to be the most abundant agents of intercellular HGT. Phage transfer is the main way for bacteria to acquire virulence factors and antibiotic resistance and is often regarded as the primary driving force of microbial evolution. So far, there are two known mechanisms of genetic transduction, which include generalized transduction (GT) and specialized transduction (ST). GT is the process by which pac-type phages can package any bacterial DNA and transfer it to another bacterium, whereas ST is limited to the transfer of specific sets of genes. In a recent study, researchers identified a third model of genetic transduction, known as lateral transduction, which appears to be the most effective means of transferring large segments of bacterial chromosomes (hundreds of thousands of bases long) from one bacterium to another at extremely high frequencies [92]. The discovery of this efficient pattern of gene transfer could explain rapid bacterial evolution, such as the evolution of multidrug-resistant strains. For example, the phage element Φ HKU.vir, which carries the superantigen gene ssa and the Spec and DNase genes spd1, has led to the emergence of multiple resistance to Streptococcus pyogenes emm12 [93]. Phages can be excised either spontaneously or by inducing excision by activating bacterial SOS reactions. After induction, the phage enters a lytic cycle, leading to the generation of phage progeny and the lysis of host cells. During the lysis cycle, bacterial DNA (rather than phage DNA) may be packaged into a phage capsid to produce a transduction particle that, upon release from the (donor) host cell, can transfer the bacterial DNA to recipient cells by conjugation, transformation, and transduction. Conjugation: after direct contact between the two bacteria, plasmids exchange DNA between the bacteria to enable the recipient cells to obtain resistance genes. Transformation: resistance genes can be incorporated into the chromosomes or plasmids of the recipient cells by the process of lysis, when exposed DNA is released by one bacterium and absorbed by another. Transduction: resistance genes are transferred from one bacterium to another by phages and can be incorporated into the chromosomes of the recipient cells. Natural transformation occurs through direct uptake and integration of extracellular DNA. ARG, antibiotic resistance gene

FIGURE 2 Horizontal transfer of antibiotic resistance genes. Resistance genes are transferred from donor to recipient cells by conjugation, transformation, and transduction. Conjugation: after direct contact between the two bacteria, plasmids exchange DNA between the bacteria to enable the recipient cells to obtain resistance genes. Transformation: resistance genes can be incorporated into the chromosomes or plasmids of the recipient cells by the process of lysis, when exposed DNA is released by one bacterium and absorbed by another. Transduction: resistance genes are transferred from one bacterium to another by phages and can be incorporated into the chromosomes of the recipient cells. Natural transformation occurs through direct uptake and integration of extracellular DNA. ARG, antibiotic resistance gene
### Table 2: Main characteristics and classification of known resistance plasmids

| Plasmids          | Size     | ARGS                                                                 | Drug class                                                                 | Refs |
|-------------------|----------|----------------------------------------------------------------------|---------------------------------------------------------------------------|------|
| Enterobacteriaceae|          |                                                                      |                                                                           |      |
| IncF              | 45–200 kb| *blaCTX-M-1, 14, TEM-1, NDM-1, rmtB*                                  | Aminoglycosides, quinolones                                               | [89] |
| IncI or MOBp      | 50–250 kb| *blaCTX-M-1, 5, TEM-52, KPC-3, CMY-2, mcr-1, mcr-1.5, sul1, sul2, aad, strA, strB, aac(6)-I* | Aminoglycosides, tetracyclines, quinolones                                |      |
| IncK, IncB/O, IncZ | 80–150 kb| *blaCMY-2, CTX-M-1, 14, ACC-4, SCO-1, TEM-1, sul1, sul2, aad, strA, strB, aac(6)-I* | Sulfonamides, ampicillin, tetracycline, chloramphenicol                    |      |
| IncA/C            | 40–230 kb| *blaTEM, SHV, CTX-M, CMY, DHA, OXA, NDM, IMP, sul1, sul2, aphA1, aadA, aadB, strA, strB, aac(6)-I, tet(A), floR, catA1, dfrA* | Carbapenemases, sulfonamides, aminoglycosides, tetracyclines, chloramphenicol, trimethoprim |      |
| IncH              | 75–400 kb| *blaCTX-M-2, TEM-1, NDM-1, mcr-1, mcr-1.5, sul1, sul2*                | Trimethoprim, streptomycin, spectinomycin                                 |      |
| IncP              | 70–275 kb| *mcr-1, mcr-1.6, dfrA1, tet(A) and sul1*                             | β-Lactamases, sulfonamides, aminoglycosides, and tetracyclines            |      |
| IncL/M            | 50–80 kb | *blaOXA-28, CTX-M-1, 3, TEM-1, NDM-1, sul1, aacA4, tetA*              | β-Lactamases                                                             |      |
| IncN              | 30–70 kb | *blaCTX-M-1, VIM-1, tetA, tetR, strA, strB*                           | β-Lactamases, sulfonamides, quinolones, aminoglycosides, tetracyclines, and streptomycin |      |
| Colicinogenic plasmids | 6–40 kb | *blaCTX-M-17, CMY-31, 36, sul1, qnrS1, strA/B, tetA, mcr-4*            | β-Lactamases, tetracyclines, colistin                                     |      |
| IncX              | 30–50 kb | *blaKPC, NDM, mcr-1, mcr-2*                                          | β-Lactamases, colistin                                                    |      |
| IncQ1             |          | *tet (X4)*                                                            | Tigecycline, eravacycline                                                 | [90] |
| Pseudomonas aeruginosa |  |                                                                      |                                                                           |      |
| IncP-2            | 300–500 kb| *blaIMP-9, its variant blaIMP-45, or blaVIM-2*                       | Carbapenemase                                                             | [85] |
| A. baumannii      |          |                                                                      |                                                                           |      |
| pRAY-like         | ~6 kb    | *aadB gene cassette*                                                  | Gentamicin and tobramycin                                                |      |
| RepAcI6           | –        | *blaOXA-23, aphA6*                                                    | β-Lactamases, kanamycin and amikacin                                      |      |
| pNDM-BJ01         |          | *blaNDM-1*                                                            | β-Lactamases                                                             |      |
| Staphylococcus spp.|          |                                                                      |                                                                           |      |
| pT181             | <10 kb   | *tet(K)*                                                              | Tetracycline                                                             |      |
| pC194             |          | *cat*                                                                 | Chloramphenicol                                                          |      |
| pE194             |          | *erm(C)*                                                              | Erythromycin                                                             |      |
| Enterococcus spp. |          |                                                                      |                                                                           |      |
| RepA_N (pRUM-like)| –        | *Cat, aphA-3, erm(B), aadE, sat4, vanA*                              | Chloramphenicol, kanamycin/neomycin, streptomycin, streptothricin, vancomycin |      |

Abbreviation: ARGs, antibiotic resistance genes.
another (recipient) cell, a phenomenon known as GT (Figure 2) [94]. Phages enable their host to acquire ARGs from neighboring cells. A recent study found that the phages could aid S. aureus, which contains the prophage, to acquire genes that are beneficial for competition. After a phage infection kills a competitor bacteria, the bacterial DNA may be captured by viral transgenic particles that return to S. aureus, which contains the prophage and can transfer the genes to the host cell [95]. In addition to promoting the transfer of resistance genes, phages can also promote bacterial resistance. For example, filamentous phages promote the development of *Pseudomonas* resistance by “isolating” antibiotics by forming liquid crystal structures [45]. Moreover, many studies suggest that many ARG-carrying phages are present in the environment (Table 2), suggesting that bacteriophages can be environmental vectors for the horizontal transfer of ARGs.

Recently, the bacteriophage crAssphage was identified from human fecal metagenomes. Its genome (~97 kbp) is six times more abundant in publicly available metagenomes than all other known phages together; it comprises up to 90% and 22% of all reads in virus-like particle (VLP)-derived metagenomes and total community metagenomes, respectively, and it comprises 1.68% of all human fecal metagenomic sequencing reads in the public databases [96]. Karkman et al. [97] analyzed the relative abundance of resistance genes and the accompanying extent of fecal pollution in publicly available metagenomic data, using crAssphage sequences as a marker of human fecal contamination (crAssphage is exceptionally abundant in, and specific to, human feces). The results showed that the existence of resistance genes could largely be explained by fecal contamination, and there was no obvious indication of selection in the environment except for the high concentration of antibiotic contamination produced during the production process. Therefore, it is necessary to consider the level of fecal contamination to avoid making false assumptions about environmental selection for antibiotic resistance.

### 4.3 Gene transfer agents

Gene transfer agents (GTAs) are phage-like particles containing DNA produced by certain species of bacteria and archaea [98]. GTAs, first discovered in the 1970s, are small VLPs that can transfer random segments of the entire genome of host bacteria between cells [99]. The most remarkable is that no previous infection of the bacterial host by a transducing phage is necessary to generate GTAs. This is because the genes encoding GTA capsids are already present in the bacterial chromosome as a sort of “usable” capsid element to mobilize bacterial DNA [100]. GTAs are an unusual approach to HGT that appears to be a hybrid of phage transduction and natural transformation [101]. The genes being transferred may improve fitness or resilience but can also lead to AMR. The maintenance of the GTA is driven by the selection to increase the likelihood of GTA. It can be argued that this process is beneficial at the population level by facilitating adaptive evolution of the host-adaptation systems and thereby expansion of the host-range size [102]. GTAs have the potential to drive bacterial evolution and genome plasticity, including the spread of virulence and AMR genes. GTA dose, or multiplicity of infection, was linearly correlated with increased resistance to antibiotics. In the coral reef environment, the frequency of GTA-mediated kanamycin resistance genes was significantly higher than that of spontaneous resistance [103]. The well-understood GTA system is of the purple-sulfur marine bacterium *Rhodobacter capsulatus* [104]. Bárdy et al. [105] observed that the GTA structure of Erythrobacter capsulatum (RcGTA) resembles that of a tailed phage, with an oblate head shortened in the direction of the tail axis, which limits its packaging capacity to less than 4500 bp of linear dsDNA. The tail channel of RcGTA contains trimer proteins that open and contract within the RcGTA substrate to allow DNA to be ejected into the bacterial periplast. Fogg [106] identified and characterized a transcription factor (Rcc01865, renamed GafA) that binds directly to the RcGTA promoter. Overexpression of gafA in wild-type *R. capsulatus* SB1003 increased antibiotic gene transfer frequencies by 57-fold, compared to 94-fold for the stable hyperproducer phenotype. The GafA promoter is, in turn, bound by both the pleiotropic regulators CtrA and GtaR near the transcription start site. CtrA and GafA are both required for optimal RcGTA expression, packaging of DNA and release of infective particles. GafA is identified as the first direct activator of GTA expression to be reported for any species. *Dinoroseobacter shibae* has been shown to produce DsGTAs similar to RcGTAs particles [107]. In addition, other forms of GTA have been discovered. *Brachyspira hyodysenteriae* produces GTA-like particles, classified as the siphovirus-type, and referred to as VSH-1 [108]. These VSH-1 particles are known to mediate the transfer of a variety of markers between cells, including virulence genes and antibiotic resistance [109]. In addition, the PBSX phage-like particles are produced by *Bacillus* spp., Dd1 particles are produced by *Desulfovibrio desulfuricans*, and VTA particles are produced by archaeon *Methanococcus voltae*. They all participate in GHT [109].
4.4 Transmission mechanisms of resistance genes in the environment

Another important source of antibiotic resistance determinants is animal husbandry, which uses more antibiotics than what is prescribed to humans. Therefore, MGEs are indispensable for HGT and are often detected in livestock manure; thus, showing a strong positive correlation with ARGs. The chicken gut has more MGEs than the guts of humans and pigs [66]. MGE-mediated HGT occurs in the soil, aquatic, gut, and biofilm communities. A recent metagenomic survey also identified a family of conjugated Tn that spread wildly in the human gut microbiome. It has been demonstrated that some plasmids and plasmid families may be unique to the human gut microbiome. The MGEs considerably enrich the gut microbial ecosystem, and thus, make it a site for efficient genetic exchange, including the transfer of ARGs [110,111]. However, the genes exchanged are not limited to ARGs, but also include virulence genes, and genes of the core gut microbiome, such as those encoding bile acid hydrolases [112] or butyric acid metabolic enzymes [113]. Many factors in the intestinal tract and the external environment contribute to the enhancement of HGT. For example, the presence of insects, earthworms, protozoa, and fungi in the soil affects the conjugated plasmid in the soil, which in turn, affects HGT [114]. Ciliates in aquatic ecosystems and the rumen can increase the rate of binding transfer between *Escherichia coli* strains by two orders of magnitude, and the mechanism can reduce the accumulation of bacterial vehicles [115]. Biofilms on rivers, streams, hot springs, and human and animal teeth can also contribute to an increase in antibiotic resistance [116]. In addition, microplastics (MPs) in water are effective carriers of environmental microorganisms and antibiotic resistance bacteria (ARB), making it possible for ARGs to be continuously introduced into the aquaculture environment. The hydrophobic surface of plastics provides a shelter for microorganisms and promotes the formation of biofilms, which form on MPs and allow a strong interaction between microorganisms and the nutrient-rich environment. The formation of a biofilm protects the ARB by preventing the surface microorganisms of MPs from being exposed to ultraviolet (UV) light. MPs change the composition of ARGs in water and sediments, and in turn, MGEs promote the movement of ARGs. Thus, MPs are a hotspot for gene exchange, promoting the spread of ARGs in water and sediment [117]. All these mechanisms may contribute to the spread of antibiotic resistance in bacterial populations. However, the animal gut can be considered the most favorable environment for HGT. First, the host continues to provide nutrients to maintain the active metabolism of the gut microbiome. Second, the high density of the bacterial population increases interaction between bacterial cells, which is conducive to gene transfer. Third, the constant body temperature of an endotherm allows bacterial cells to operate efficiently. Finally, the diversity of intestinal microbiota may amplify HGT [118]. Moreover, antibiotic treatment or ingestion of food with antibiotic residues may substantially damage the structure of the intestinal flora and promote the integration of intestinal microorganisms and the proliferation of ARGs by eliminating the competition of ARB. For example, the selection pressure of residual antibiotics (e.g., >60 ng/kg in meat products) has been shown to alter the type and increase the abundance of ARGs in the human gut [119].

While studying ARGs, researchers focused on the spread of ARBs and ARGs that discharged into the environment from animal farms, medical institutions, pharmaceutical institutions, and sewage treatment plants. ARGs can be spread by HGT between ARBs [85] and can also be transferred from ARBs to nonresistant strains in the environment. The newly formed ARBs may eventually reach and infect humans in various ways [120,121]. Whole-genome sequencing identified the prevalence of *blaNDM* positive *E. coli* in farms, as well as in flies, dogs, and farmers, thus demonstrating that *blaNDM* can rapidly contaminate humans through dogs, flies, and wild birds. This provides direct evidence for the spread of carbapenem-resistant *E. coli* and environmental pollution [122]. ARBs and ARGs from organic fertilizers have been detected in crops grown on soil enriched with organic fertilizers [123]. Endophytic bacteria that possess ARGs can colonize plants and can persist throughout the vegetable growth stage [124]. Therefore, microbial ecosystems are not isolated, and there is a possibility of horizontal gene exchange among different microbial ecosystems (Figure 3).

Besides antibiotics, heavy metals such as Cu and Zn are also important additives in animal feed and play an important role in their healthy growth. When heavy metals are added in excess, they spread to the soil and other environments, causing heavy metal pollution. Heavy metals can impose selection stress on ARGs through coreistance, cross-resistance, and coregulation to maintain an abundance of ARGs [125,126]. The presence of heavy metals provides another coselective pressure for antibiotic resistance [127,128], thereby promoting the formation and propagation of ARGs in the soil. Lin et al. [129] evaluated the levels of heavy metals and the abundance of ARGs in the soil with heavy metal manure. They found that increasing the amount of heavy metal manure used increased the abundance of heavy metals and ARGs in the soil, indicating that heavy metals...
FIGURE 3  path of propagation of antibiotic resistance genes (ARGs). Antibiotic resistance bacteria (ARB) form when antibiotics enter livestock and poultry farms, hospitals, and communities. ARBs are colonized in the guts of humans and animals and excreted into the environment (e.g., through the application of fertilizers and wastewater to the land for irrigation). Moreover, wastewater treatment plants (WWTPs) and pharmaceutical plants also discharge significant levels of ARBs into the environment (e.g., through wastewater discharges and runoff). ARBs carry ARGs into the soil, air, surface water, and groundwater, where horizontal gene transfer occurs between ARBs and opportunistic pathogens, leading to the emergence of multidrug resistance in many pathogens. ARBs in the environment enter insects and protozoa, and then circulate between animals and humans through the food chain, promoting the transfer and spread of ARGs.

TABLE 3  List of priority pathogens for research and development of new antibiotics (according to WHO)

| Priority tiers | Pathogens                        | Antibiotic-resistant                                      |
|----------------|----------------------------------|-----------------------------------------------------------|
| Critical       | *Acinetobacter baumannii*        | Carbapenem-resistant                                      |
|                | *Pseudomonas aeruginosa*         | Carbapenem-resistant                                      |
|                | *Enterobacteriaceae*              | Carbapenem-resistant, third-generation cephalosporin-resistant |
| High           | *Enterococcus faecium*            | Vancomycin-resistant                                      |
|                | *Staphylococcus aureus*           | Methicillin-resistant, vancomycin-intermediate, and resistant |
|                | *Helicobacter pylori*             | Clarithromycin-resistant                                  |
|                | *Campylobacter spp.*              | Fluoroquinolone-resistant                                 |
|                | *Salmonella spp.*                 | Fluoroquinolone-resistant                                 |
|                | *Neisseria gonorrhoeae*           | Third-generation cephalosporin-resistant, fluoroquinolone-resistant |
| Medium         | *Streptococcus pneumoniae*        | Penicillin-nonsusceptible                                 |
|                | *Haemophilus influenzae*          | Ampicillin-resistant                                      |
|                | *Shigella spp.*                   | Fluoroquinolone-resistant                                 |

*Enterobacteriaceae include: *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter spp.*, *Serratia spp.*, *Proteus spp.*, *Providencia spp.*, and *Morganella spp.*
in livestock manure could promote the deposition of ARGs in the soil.

5 | CONTROL MEASURES AND EFFECTS OF RESISTANCE GENES IN DRUG-RESISTANT BACTERIA

The rapid emergence of drug-resistant bacteria is occurring worldwide, and the antibiotic resistance crisis has been blamed on the overuse and misuse of these drugs, as well as a lack of new drug development in the pharmaceutical industry due to reduced economic incentives and challenging regulatory requirements [130]. The WHO has a global priority list of antibiotic-resistant bacteria, grouping the pathogens according to the species and the type of resistance, and then grouping the results in three priority tiers: critical, high, and medium (Table 3). Mycobacteria (including M. tuberculosis, the cause of human tuberculosis), was not included in this list as it is well-known globally that new methods of treatment are urgently needed for tuberculosis [131]. More than 2.8 million antibiotic-resistant infections occur in the United States annually, leading to the death of more than 35,000 people. A variety of bacteria and fungi are listed in the U.S. AR Threat Report 2019 (Table 4) [132]. O’Connor et al. [133] found that 74% of antibiotic prescriptions were made in primary care. Many of these were for inappropriate treatment of acute respiratory tract infections. Primary care providers are highly influenced to prescribe by the expectations of the patients for antibiotics, clinical uncertainty, and workload-induced time pressures. Such a situation increases the misuse of antibiotics and accelerates the development of drug-resistant bacteria. Targeted and appropriate multidimensional educational interventions aimed at primary healthcare providers, mass media education campaigns aimed at health professionals and the public, and the use of delayed prescribing can reduce such inappropriate prescribing to regulate the use of antibiotics and slow down the emergence and development of drug-resistant bacteria.

To reduce the transmission of ARGs to the environment from livestock and poultry breeding farms, hospitals, and sewage treatment plants, people turned their attention to biological treatment technology. The main treatment methods of livestock and poultry manure include aerobic composting, anaerobic fermentation, biological treatment, UV disinfection, and chlorination. Manure carrying ARGs and antibiotic residues can adversely affect the environment when applied directly into the soil [134]. Aerobic composting is one of the main approaches for the harmless treatment of livestock manure and sludge, as well as fertilizer utilization. It is a self-heating aerobic fertilizer management technique for the decomposition of organic matter and is important for reducing ARGs in livestock manure. Selvam et al. [135] proved that aerobic composting could remove tetracycline resistance genes (tetQ, tetW, tetC, tetG, tetZ, and tetY), sulfanilamide resistance genes (sul1, sul2, dfrA1, and dfrA7), and fluoroquinolone resistance gene (gyrA) from livestock and poultry manure, suggesting that aerobic composting is efficient in removing resistance genes. Anaerobic fermentation is another

| Bacteria                                      | Hospitalized patients | Deaths | Healthcare costs | Year   |
|-----------------------------------------------|-----------------------|--------|------------------|--------|
| Carbapenem-resistant Acinetobacter spp.       | 8500                  | 700    | $281M            | 2017   |
| Drug-resistant Clostridoides difficile        | 223,900               | 12,800 | $1B              | 2017   |
| Carbapenem-resistant Enterobacteriaceae      | 13,100                | 1100   | $130M            | 2017   |
| Drug-resistant Neisseria gonorrhoeae         | 550,000               | 1.14M new infections | $133.4M | Each year|
| ESBL-producing Enterobacteriaceae            | 197,400               | 9100   | $1.2B            | 2017   |
| Vancomycin-resistant Enterococcus spp.       | 54,500                | 5400   | $539M            | 2017   |
| Drug-resistant nontyphoidal Salmonella spp.  | 212,500               | 70     |                  | Each year|
| Drug-resistant Salmonella Typhi              | 4100                  | <5     |                  | Each year|
| Drug-resistant Shigella spp.                 | 77,000                | <5     |                  | Each year|
| Methicillin-resistant Staphylococcus aureus  | 323,700               | 10,600 | $1.7B            | 2017   |
| Drug-resistant Streptococcus pneumoniae      | 900,000               | 3600   |                  | 2014   |
| Erythromycin-resistant Group A streptococci | 5400                  | 450    |                  | 2017   |
| Clindamycin-resistant Group B streptococci   | 13,000                | 720    |                  | 2016   |
effective way to treat organic solid wastes such as livestock manure and sludge and also has a reduction effect on ARGs. Sun et al. [136] studied the removal efficiency of solid-state anaerobic digestion (SAD) and conventional liquid AD on ARGs and MGEs (such as plasmid, integrin, and transposase) under different conditions. They found that compared to conventional liquid AD treatment, SAD treatment significantly reduced the abundance of six ARGs, including tetC, sul2, ermQ, ermX, qnrA, and aac(6’)-ib-cr. These results suggest that SAD is an effective technique for the removal of MGE and to reduce the risk of transmission and abundance of ARGs. Optimizing parameters for AD, such as temperature and solid retention time, can change microbial community structure and improve the biodegradation ability of organic pollutants in feces. For example, AD can significantly improve the removal of fecal pollutants after high-temperature pretreatment [137]. Besides aerobic composting and anaerobic fermentation, studies have largely focused on the effects of water treatment processes to reduce ARGs in potable water sewage and sludge. In the artificial treatment system, the abundance of tetracycline resistance genes tetA and tetB can be significantly decreased in the primary sedimentation tank of municipal sewage treatment plants [138]. UV disinfection reduced the abundance of mecA by 1log, but could not effectively remove vanA [139]. Although disinfection treatment can reduce the total amount of resistant bacteria, it may also increase the proportion of resistant bacteria [140]. Table 5 summarizes recent studies on the reduction of ARGs in livestock manure and sludge using biological treatment.

In addition, phage therapy also has broad prospects in the prevention of the production of resistance genes and the treatment of drug-resistant bacterial infection. The therapeutic use of bacteriophages is well-suited to be part of the multidimensional strategies to combat antibiotic resistance. Although phage therapy was first implemented almost a century ago, it was paused after the successful introduction of antibiotics. Phage therapy is recommended for patients who are allergic to antibiotics. Phages target specific host bacteria and do not kill other beneficial microbiota. When the bacterial host is killed, it ceases to function; phages also have no adverse effects on mammalian cells. Moreover, phages mutate along with the host bacteria when they develop resistance [149]. Therefore, phage therapy needs to be included in our protocol for treating antibiotic-resistant pathogens to prevent the widespread production and spread of ARGs, and the sooner this is implemented, the better it will be for the environment, in general, and humans, in particular. However, the development of bacteriophage-based antimicrobial therapies has been limited by the development of bacteriophage-resistant bacteria through receptor mutations. Yehl et al. [150] used natural evolution and structural modeling to identify the host-range-determining region (HRDR) in the T3 phage tail-filament protein. A more extensive phage library was constructed by mutating HRDR, and only the HI ring mutated phages in HRDR could inhibit the development of bacterial resistance. Mutated HRDR alters the host range of phages, and the modified phages can inhibit bacterial growth for long periods in vitro by preventing the emergence of drug resistance. Therefore, the ability to discover and optimize novel antimicrobial agents that can achieve long-term suppression of bacterial resistance has the potential to significantly improve the treatment of AMR. In another study, researchers engineered the highly mobile staphylococcal pathogenicity islands to create a gene transfer system containing the CRISPR system, which forms phage-like particles that can either be inserted into the target bacteria and then cut using CRISPR, or inhibit specific disease-causing genes in a targeted way. It can effectively kill S. aureus and Listeria in vitro and inhibit hemolysis and, in turn, can effectively control the infection of S. aureus in mice and prevent bacteria from developing resistance [151].

The coproduction of synergistic antibiotics, or hybrid antibiotics with enhanced bioactivity, also plays an important role in combating bacterial resistance [152]. An example of a biosynthetic supercluster is cephalomycin-clavulanate biosynthetic gene clusters (BGC) in Streptomyces clavuligerus. The cephamycin and clavulanate operons are coregulated by the CcaR gene in the cephamycin BGC. Overexpression of CcaR, an antibiotic regulatory protein in Streptomyces, leads to increased production of cephalomycin and clavulanate. Coproduction of cephalomycin with clavulanate (two β-lactams) is beneficial. Cephalomycin is a cephalosporin antibiotic that inhibits bacterial penicillin-binding proteins and is sensitive to certain types of β-lactamases. Clavulanic acid is an effective inhibitor of many β-lactamases and can save β-lactam antibiotic activity against competing microorganisms that express β-lactamases [153]. A more recent example of a biosynthetic supercluster encoding coproduction of sulfazecin and bulgecin was discovered in Paraburkholderia acidophila ATCC 31363 and Burkholderia ubonensis ATCC 31433 [154]. The bactericidal effects of sulfazecin and bulgecin are synergistic and bulgecin has been shown to potentiate the antibacterial activity of clinical β-lactam antibiotics, including third-generation cephalosporin ceftazidime and meropenem. The biosynthetic pathway linking two different antibiotic parts in a single organism is called a “hybrid antibiotic” [152]. Hybrid antibiotics offer a potential advantage if one component faces
| Samples       | Treatment | ARGs                                      | Removal efficiency                                                                 | Refs |
|---------------|-----------|-------------------------------------------|------------------------------------------------------------------------------------|------|
| Poultry manure| COM       | mphA, aacC                                | Decreased by up to 1348-fold                                                      | [61] |
|               | COM       | cfxA, strA, strB, tetO, and tetQ          | Declined more than 100-fold                                                       | [61] |
|               | COM       | fexA, cfr, cmlA, floR, etA, tetB, tetL, tetM, tetW, tetQ, tetO, tetX | The average removal rate of ARGs was 0.86 log                                  | [141]|
|               | AD        | tetA, tetB, tetO, tetX, tetM, tetQ, tetW, sul1, sul2, floR, cmlA, intI1 | Removed at a rate of 34%–58%                                                      | [142]|
|               | BIO       | tetA, tetB, tetO, tetX, tetM, tetQ, tetW, sul1, sul2, floR, cmlA, intI1 | Removed at a rate of 87%–95% with activated carbon and microwave pretreatment | [142]|
| Swine manure  | COM       | cfxA, strA, strB, tetO, and tetQ          | Declined more than 100-fold                                                       | [61] |
|               | COM       | sul1, sul2, dfrA1, dfrA7, tetQ, tetW, tetC, tetG, tetZ, gyrA, parC       | Below the test line after 42 days of treatment                                    | [135]|
|               | AD        | tet, erm                                  | 1–2 logs decrease after 40 days of treatment                                      | [143]|
| Cattle manure | COM       | mphA, aacC                                | Increased up to 686-fold                                                          | [61] |
|               | COM       | cfxA, strA, strB, tetO, and tetQ          | Declined more than 100-fold                                                       | [61] |
|               | AD        | tetC, tetM, tetQ, tetW, tetX, sul1, sul2, gyrA, intII, intI2              | 20°C 5/10 ARGs reduced, 35°C 6/10 ARGs reduced, 55°C 8/10 ARGs reduced             | [138]|
|               | AD        | ermA, aphA2, blaTEM-1                     | Abundance decreases after anaerobic fermentation                                    | [144]|
| Swine wastewater| BIO     | tet, foa, sul, van, bla, aac              | 1.2 log decrease                                                                  | [145]|
| Wastewater    | AD        | sul1, sulII, ermB, ermF, tetO, tetG tetC  | 0.5–1.2 logs decreased increased                                                  | [146]|
|               | C         | ereA, ermB                                | 87% of ereA and 40% of ermB removed at 15 mg Cl 2 min/L                           | [147]|
|               | UE        | erm, tet                                  | 5 ml/cm²erm 3.0 ± 0.1 log decreased, tet 1.9 ± 0.1 log decreased; 10 ml/cm² not detected | [148]|

Abbreviations: AD, anaerobic digestion; ARGs, antibiotic resistance gene; BIO, biological treatment process; C, chlorination; COM, composting; UE, ultraviolet exposure.
modification by an inactivated enzyme, as the second antibiotic component may maintain functional activity. It is necessary to mine microbial BGC for hybrid antibiotics to complement current synthetic strategies to synthesize multifunctional hybrid antibiotics that overcome existing resistance mechanisms.

6 | SUMMARY AND CONCLUSIONS

The widespread application of antibiotics for global medical treatment and in the breeding industry has led to the accumulation of residues of antibiotics and drug-resistant bacteria in the intestinal tract of animals, which are excreted along with feces. The pollution of ARGs is not only regional but also global. The infection caused by antibiotic-resistant bacteria is a major threat to global public health. Under the selection pressure of antibiotics, antibiotic-sensitive strains can adapt to antibiotics through gene mutation and inactivate antibiotics by destroying and changing their structure; thus, developing drug resistance. These processes are regulated by ARGs, which are the main cause of drug resistance in antibiotic-resistant strains. The application of antibiotics leads to the colonization of antibiotic-resistant strains in human and animal intestines, where the ARGs spread via HGT in the intestinal microorganisms. This leads to the transfer of ARGs to opportunistic pathogens and the emergence of MDR genes in many such pathogens. ARBs carry resistance genes into the environment through animal excreta and then into insects and protozoa through the food chain. Eventually, they circulate between animals and humans, promoting the transfer and transmission of ARGs. The scope of research on bacterial resistance and resistance genes involves different environments such as air, aircraft sewage, migratory bird feces, and the sea (rarely involved before), which can provide a better understanding of the generation and the mechanism of transmission of drug resistance genes, as well as, help to control and reduce the transmission of AMR.

In recent years, extensive and detailed studies on the generation, transmission, and evolution of bacterial resistance mechanisms have contributed to the generation and transmission of AMR. Many physical and chemical methods have a certain subtractive effect on the resistance genes of drug-resistant bacteria. However, this cannot fundamentally solve the problem of antibiotic resistance. We summarize several promising points for controlling bacterial resistance: (i) the therapeutic use of bacteriophages is well-suited to be part of the multidimensional strategies to combat antibiotic resistance, (ii) the use of vaccines and phages to destroy specific antibiotic-resistant bacteria is promising for the control of antibiotic resistance, (iii) the coproduction of synergistic antibiotics, or hybrid antibiotics with enhanced bioactivity, also plays an important role in combating bacterial resistance, and (iv) society should spread knowledge and awareness of antibiotic use and resistance among the masses to take action against antibiotic resistance globally. However, national surveys on AMR almost always face some difficulties and limitations [155]. Countries around the world should develop methods and tools to enhance and monitor knowledge and awareness of antibiotic use and resistance in the general population for efficiently planning and managing drug resistance.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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