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Tracking Antimicrobial Resistance in Environmental Waters: Risks, Routes, and Regulatory Needs

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Abstract

Background: Antimicrobial resistance (AMR) in gram-negative bacteria represents a growing global health threat, increasingly fueled by environmental factors. Wastewater, agricultural runoff, aquaculture, and pharmaceutical discharge contribute to the release of antibiotic residues, resistant bacteria, and resistance genes into ecosystems. **Objective:** This literature review explores the environmental persistence and dissemination of antibiotic resistance, focusing on the mechanisms by which multidrug-resistant (MDR) genes are maintained and transferred—particularly through water sources—and highlights emerging mitigation strategies and regulatory challenges. **Key Findings:** Gram-negative bacteria such as *Salmonella enterica* and ESKAPE pathogens exhibit intrinsic and acquired resistance mechanisms, including efflux pumps and mobile genetic elements. Environmental reservoirs, especially wastewater treatment plants, facilitate horizontal gene transfer between pathogenic and non-pathogenic bacteria. Agricultural practices, antibiotic overuse, and inadequate waste management further exacerbate the spread. While ozone treatment, membrane bioreactor systems, bacteriophage therapy, and efflux pump inhibitors show promise in reducing AMR load, widespread implementation remains limited. Policy efforts—at federal, state, and international levels—lack consistency and enforcement, particularly regarding environmental discharge and regulation of antibiotic residues. **Conclusion:** Environmental AMR, particularly via water systems, poses a substantial public health risk. A coordinated One Health approach, incorporating policy reform, technological advancements, and cross-sector collaboration, is essential to address AMR at its environmental source and mitigate its global spread.

Keywords

Antimicrobial Resistance, Gram-Negative Bacteria, Wastewater Treatment,

Multidrug Resistance, Environmental Health, *Salmonella enterica*, Horizontal Gene Transfer

1. Introduction

1.1. The Importance of Antibiotic Resistance in Gram Negative Bacteria

Antimicrobial resistance (AMR) occurs when bacteria and other organisms acquire resistance mechanisms, [1] rendering antibiotics ineffective and making infections more difficult to treat [1]. AMR is a growing concern in clinical applications, with several pathogens developing resistance to multiple classes of antimicrobials. While previous reviews have focused on the mechanism of antibiotic resistance and its spread, especially in healthcare settings [2]-[5], less research has been conducted on the maintenance of antibiotic resistance in the environmental settings.

Compared to gram-positive bacteria, gram-negative bacteria demonstrate greater resistance to antibiotics and chemotherapeutic agents [6]. This can be attributed to their outer membrane, which limits the penetration of hydrophilic solutes, as well as the low fluidity of the lipopolysaccharide leaflet [6]. Additionally, these bacteria possess multiple drug efflux pumps that further contribute to their resistance capability [6].

A paper by Nikaido *et al.* (1998) [6] explains the function of these efflux pumps. Spanning both the inner and outer membranes of gram-negative bacteria, these pumps are powered by the proton-motive force, allowing them to expel a wide range of substances, including detergents, dyes, and antibiotics. They can also eliminate compounds such as beta-lactams, which typically have difficulty crossing the cytoplasmic membrane. Overexpression of these efflux pumps can significantly increase the minimum inhibitory concentrations (MICs) of antibiotics, contributing to the high resistance observed in gram-negative bacteria.

This literature review aims to explore and attempts to answer how we can prevent pathogenic bacteria from passing multidrug resistant (MDR) genes to non-pathogenic bacteria in the environment by analyzing current information on gram-negative AMR in water sources.

1.2. Environmental Antibiotic Resistance

The environment can become contaminated with antibiotics due to human and animal waste, pesticide use, pharmaceutical manufacturing wastage, and aquaculture [7]. This is a major problem since these factors contribute to the spread of resistance among human populations [7].

Firstly, human waste contains traces of antibiotics [7]. Unfortunately, wastewater treatment plants (WWTPs) are unable to remove all antibiotic residues and resistant genes [7]. This is especially seen in wastewater from hospitals where pa-

tients are prescribed antibiotics and antifungals [7]. A study by Berglund *et al.* (2023) investigated the emergence of mobile antibiotic resistance genes (ARGs) in wastewater environments [8]. They analyzed 22 bacterial species and determined the taxonomic origins of mobile ARGs based on previous research. The results showed that these species originated from wastewaters and human stool. The researchers focused on Mobilizing Insertion Sequence Elements (MISE) and their presence in the genome of the origin species. They examined complete genomes and metagenomic data to determine the environments where MISE are abundant. The findings revealed that MISE are present in untreated hospital effluent, wastewater treatment plant influent, and poultry feces [8]. Additionally, the study demonstrated that origin species carrying ARGs are more commonly observed in wastewaters and contaminated water/sediments, which may be linked to human feces [8]. The authors suggest that the mobilization of ARGs in human and animal microbiota occurs as a response to the selective pressure of antibiotics, given the connection between these species and infections [8].

Secondly, antibiotics used in livestock production, ultimately lead to contamination of soil and water sources [7]. The use of untreated animal manure as fertilizer can also contribute to the spread of resistant bacteria in the environment [7]. In the US, over 25 million pounds of antimicrobials are used annually in food-animal production, with approximately 13.5 million pounds of antibiotics being excreted in animal fecal waste each year [9]. These waste products containing antibiotics can enter the environment through various pathways such as manure, soil, runoff, or by being discharged as wastewater effluent into surface water and sediment [9]. In a paper by Williams *et al.* (2016) where the characterization of AMR among aquatic environments is reviewed [10], they determined that surface water from which drinking water originates, is the discharge point for wastewater as well as runoff from animal farms [10]. This causes the spread of AMR between humans and animals [10]. In this article, studies discussing AMR in surface and recreational waters, municipal wastewater, healthcare-associated wastewater, airplane wastewater, industrial wastewater, farm animal wastewater, and aquaculture were reviewed. It also explored treatment technologies such as disinfection, activated sludge digestion, sludge composting, swine vermicomposting, fertilization, ionic liquid, and drinking water treatment as potential ways to prevent dissemination of AMR into the environment.

Additionally, the use of pesticides in agriculture to mitigate crop diseases leads to the contamination of soil and water, also leading to resistance in the environment [7]. Human health can be affected when these pesticides are similar to antibiotics used in human medicine [7]. A paper titled "The source, fate and prospect of antibiotic resistance genes in soil: A review" highlights the impact of ARGs on public health in soil environments specifically. Soil is a reservoir and propagation hotspot for ARGs [11]. Factors like microbiome composition, soil physicochemical properties (pH and moisture), antibiotics, heavy metals, polycyclic aromatic hydrocarbons, and pesticides, were discussed in relation to their impact on ARGs.

Among these factors, microbial community structure, mobile genetic elements, pH, and heavy metals were identified as having a significant influence on ARG profiles [11].

Aquaculture operations frequently use antibiotics, leading to contamination of water sources [7]. A study by Doğan and Önalan (2023) [12] aimed to determine the variations of antimicrobial resistance genes (specifically *tet* and *str* genes that confer resistance to tetracycline and streptomycin respectively) in freshwater fish species. Antimicrobial resistance is a concerning issue in public health, and understanding the presence and diversity of resistance genes in different environments, such as freshwater ecosystems, can provide insights into potential sources and pathways of resistance transmission [12]. By analyzing these genes in freshwater fish species, the researchers sought to contribute to our understanding of antimicrobial resistance dynamics in aquatic environments [12]. The researchers sampled 70 fish belonging to seven different species. They were collected from two regions, one region with no fish farming activities and another region with fish farming activities. They found that two species, C. *carpio* and C. *regium* had higher *tet* and *str* gene expression compared to a control group which had no antibiotic use [12]. In other species, a lower but significant expression level was seen [12]. The researchers concluded that even in the absence of antibiotic use, the fish recirculating water system can cause the spread of antibiotic resistance genes [12]. Other factors such as pH and heavy metals can also contribute [12]. Ultimately, the ecosystem serves as a connection between humans, animals, and the environment [13]. AMR transmission and the mixing of mobile genetic elements are thus able to spread to hosts [13]. A paper by Samreen *et al.* (2021) [13] describes how biocides and heavy metals contribute to the development and dissemination of AMR. These metals have resistance mechanisms similar to antibiotics such as reduced membrane permeability, metal alteration, efflux of metals, target site modification, and metal sequestration. Biocidal products exert microbiostatic or microbicidal effects against various microorganisms.

1.3. Antibiotic Resistance in Specific Bacterial Species

Antibiotic resistance in bacterial species occurs through various mechanisms, including genetic mutations, horizontal gene transfer, and selective pressure from the overuse and misuse of antibiotics. When bacteria are exposed to antibiotics, those with mutations that confer resistance to the drug are more likely to survive and propagate, passing on these resistant traits to their progeny. Horizontal gene transfer allows bacteria to acquire resistance genes from other bacteria, further spreading resistance across species. The global issue of antibiotic resistance is exacerbated by the extensive and often inappropriate use of antibiotics in both human healthcare and agriculture, creating an environment in which resistance can thrive [14]. Additionally, the overprescription of antibiotics for viral infections, lack of proper diagnostics, and inadequate infection control measures contribute to the accelerating spread of resistance [15]. As bacteria evolve to resist multiple

classes of antibiotics, previously treatable infections become increasingly difficult to manage, leading to higher mortality rates, prolonged hospital stays, and greater healthcare costs. The World Health Organization (WHO) has classified antibiotic resistance as one of the most urgent global health threats, as it undermines the effectiveness of life-saving antibiotics and limits treatment options for common infections, such as pneumonia, tuberculosis, and urinary tract infections [16]. The interconnected nature of global travel, trade, and food supply chains further facilitates the spread of resistant bacteria across borders, making this a problem that requires urgent global cooperation and action.

1.4. WHO-Identified Priority Pathogens

The World Health Organization (WHO) has highlighted several critical pathogens that pose an immediate threat due to their increasing resistance to available treatments. Among these, ***Escherichia coli***, ***Klebsiella pneumoniae***, ***Staphylococcus aureus***, and ***Pseudomonas aeruginosa*** are notable examples. Recent studies have shown that ***E. coli*** is particularly concerning due to its growing resistance to fluoroquinolones and third-generation cephalosporins, often driven by the acquisition of extended-spectrum beta-lactamases (ESBLs) and carbapenemases [17]. Similarly, ***K. pneumoniae*** has seen an alarming rise in carbapenem resistance, linked to the spread of New Delhi metallo-beta-lactamase (NDM)-producing strains [18]. ***S. aureus***, including methicillin-resistant strains (MRSA), remains a significant hospital-acquired pathogen, with a troubling trend toward multidrug resistance [19]. ***P. aeruginosa***, notorious for its environmental resilience, has shown increasing resistance to beta-lactams, aminoglycosides, and fluoroquinolones, complicating treatment options [20]. These species, among others, exemplify the broader issue of ABR, which threatens to undermine the effectiveness of antibiotics, complicating the treatment of infections and increasing morbidity and mortality worldwide. Recent literature continues to underscore the urgency of global surveillance and the need for innovative solutions to combat this growing resistance crisis.

1.5. Focus on *Salmonella enterica*

This review will narrow its focus to a specific bacterial genus, *Salmonella*, which continues to be of concern in both environmental and clinical contexts. There are physiological, biochemical, environmental, and molecular factors that allow for its resistance [21]. *S. enterica* is a gram-negative bacterium that is passed through contaminated food and water between humans and animals [22]. Salmonellosis causes gastroenteritis, septicemia, and enteric fever. Enteric fevers are caused by *S. enterica* serovars Typhi and Paratyphi [22], which are human-restricted. The epidemiology of typhoid and other enteric fevers involve person-to-person transmission because these organisms do not have a significant animal reservoir [23]. Unlike Typhi and Paratyphi, nontyphoidal Salmonellae (NTS) are not human-restricted [22]. The *S. enterica* serotypes Typhimurium and Enteritidis have res-

ervoirs in farm animals, thus consumption of contaminated and raw meat is the main mode of NTS transmission [23]. Nontyphoidal salmonellosis is similar to gastroenteritis as symptoms may involve nausea and diarrhea [22]. *Salmonella* can also cause chronic conditions such as Reiter's syndrome, a form of arthritis [22]. Salmonellae have a lipopolysaccharide coat and possess the ability to invade cells and replicate intracellularly [23]. These bacteria are found in the ileum and colon and they invade the intestinal epithelium by binding to receptors on the epithelial cell surface, leading to an acute inflammatory response [23]. In a recent study by Robertson *et al.* (2023) [24], Illumina sequencing data was used to provide a current characterization of plasmids and their AMR associations within the *Salmonella* species. About 16% of *Salmonella* cases in the US exhibit resistance to at least one essential antibiotic [24]. Bacteria can acquire antibiotic resistance through mutations or mobile genetic elements (MGEs) that carry genes that confer this resistance [24]. Plasmids are an example of MGEs which allow for horizontal transfer of AMR genes [24]. Although mostly circular, linear plasmids are found in some gram negative bacteria like *Salmonella* [24]. Linear plasmids are found to confer advantages such as enhanced nuclear translocation [25]. The authors describe replicon and relaxase typing as efficient methods for plasmid classification which give us information on AMR and virulence [24]. The results of this study were that *Salmonella* virulence plasmids were largely inherited vertically and that plasmids identified in this bacterium have the ability to replicate in other pathogens and be transferred between commensal and pathogenic bacteria [24]. Vertical transfer is when the plasmid is transported from the mother to daughter cells during cell division, whereas horizontal transfer occurs through conjugation [26]. In vertical transfer, cells divide and produce copies of themselves, leading to more cells over time [26]. This mode of transfer also ensures that resistance traits are inherited by subsequent generations [26].

1.6. Environmental Presence of ESKAPE Pathogens

Another group of pathogenic bacteria, the "ESKAPE" pathogens include *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species). These pathogens can escape bactericidal activity of antibiotics, making them a great threat to human health [27]. **Figure 1** highlights their mechanisms of antibiotic resistance. In a review paper by Marutescu *et al.* (2023), it was found that WWTPs provided these bacteria with additional virulence determinants [28]. Of these pathogens, those that are mostly found in the environment are *P. aeruginosa* and enterococci.

P. aeruginosa, an aerobic gram-negative bacterium is found in the environment and causes nosocomial infections [27]. It demonstrates both intrinsic and acquired mechanisms of resistance including efflux pumps, decreased outer membrane permeability, and lack of proteins that allow diffusion of antibiotics across the outer membrane [27]. Due to this bacterium being adapted to low osmolarity environments, it is often found in water sources. 95% of hospital wastewater sam-

ples were positive for multidrug-resistant *P. aeruginosa* in this study. In a paper by Gervasoni *et al.* (2023), this bacterium's ability to form biofilms was discussed. Due to this, they are found in a variety of aquatic environments [29].

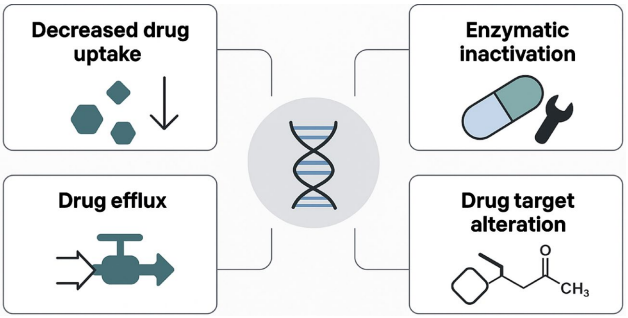

Figure 1. Mechanisms of antibiotic resistance in ESKAPE pathogens. Adapted from: [28].

Enterococci are gram-positive cocci which can survive in hostile environments. *E. faecalis* and *E. faecium* cause most of the enterococcal infections [27]. This bacterial species is becoming increasingly resistant due to various reasons including the large use of broad-spectrum antibiotics in hospitals [27]. Broad spectrum antibiotics include penicillins and cephalosporins which increase normal gram-negative intestinal microbiota, thereby promoting intestinal colonization of *E. faecium* [27]. A genomic analysis by Shobo *et al.* (2023) provided information on the spread of *E. faecalis* in hospitals as well as its acquisition of resistant genes is such settings [30]. The researchers found that this specie was responsible for over 80% of hospital cases and can acquire additional resistance through the transfer of plasmids, prophages, insertion sequences, and other mobile genetic elements [30]. Another study by Paul B. Tchounwou (2015) isolated *Enterococcus* species in hospital wastewater and effluent of WWTPs instead of looking at the internal hospital environment [31]. He determined that 62 of the 66 isolates belonged to the *Enterococcus* genus, with majority being *E. faecalis* [31]. In these isolated species, he found a high number of genotypic virulence factors including Zn-metalloendopeptidase (*gelE*), collagen binding protein (*ace*) and endocarditic antigen (*efaA*) [31].

2. Antibiotic Leakage into the Environment

2.1. How and Where Does the Antibiotic Leakage Occur

MDR species of *Salmonella* and other pathogens have been identified in environmental water sources. These waterways are a potential reservoir for MDR. There are several potential sources of these resistant bacteria including commercial agriculture practices, water waste treatment plants, medical center waste disposal, and intrinsic antibiotic resistance already present in bacterial populations. These environmental waterways not only offer a reservoir for MDR bacteria but may also potentially present opportunities for pathogenic bacteria to transfer their antibiotic resistance genes to nonpathogenic populations.

Antibiotics enter the environment through feces, urine, manure, and waste or unused antibiotics from drug production, medical systems, and households [32]. These antibiotics can contaminate the soil, surface water, and groundwater through leaching or runoff [32]. Groundwater, which serves as our primary source of freshwater, has been found to contain pharmaceuticals and personal care products [33]. As a result, antibiotics accumulate in groundwater and pose ecological and human health risks. Continuous release of antibiotics into groundwater leads to selection of ARBs and ARGs [33]. A global review titled "Antibiotics and antibiotic resistant genes in groundwater" aims to understand the transport of antibiotics and ARGs, analyzes contamination profiles, and identifies key drivers of ARBs [33].

The decomposition of solid waste in landfills contributes to the evolution of the antibiotic resistome [32]. Consequently, antibiotics and antibiotic resistomes are discharged into the environment or wastewater treatment plants (WWTPs) for further treatment, making landfills and leachates important reservoirs of antibiotics [32]. Zhang *et al.* found that landfills are hotspots of AR transmission and the wide distribution of antibiotics, antibiotic resistant bacteria (ARBs), and ARGs in refuse and leachates furthers AR transmission risk [32]. Antibiotic residues, bacterial communities, MGEs, heavy metals, organic matter, and physicochemical properties all play a role in the spread of AR [32]. Wastewater treatment is the largest reservoir and hurdle to clear. The presence of carbon sources, electron acceptors, particles onto which bacteria can adsorb, as well as a stable pH and temperature allow for a diversity of microorganisms to live in wastewater [34]. ARGs from the environment, humans, and animals are brought together here [34]. WWTPs play a role in maintaining and spreading ARGs and ARBs [35]. Antibiotic residues in wastewater treatment plants can shape the microbial community and facilitate the horizontal transfer of ARGs [36]. The occurrence of ARGs and ARB in WWTPs has been widely reported, and some studies have detected ARGs conferring resistance to all categories of antibiotics [36].

2.2. Water Sources as Reservoirs

There are many potential sources of both antibiotic leakage and MDR bacteria that could be contributing to the maintenance of resistance bacterial populations in the environment. These include commercial agriculture practices, wastewater treatment plants, medical center waste disposal, and intrinsic antibiotic resistance [37]. Some examples of intrinsic antibiotic resistance include lack of a target site, efflux pumps, high neutralizing capacity, little drug concentration, and response to stress [38]. The greatest amounts of MDR bacteria, as well as the most diversity in AMR was found to be in waterways with runoff from wastewater treatment plants [37]. Urban wastewater treatment plants offer an environment that is rich in organic nutrients, large amounts of bacteria, and strong selective pressures from pharmaceutical residues and pesticides, contributing to MDR and AMR [37].

In a study by Berglund *et al.*, it was found that wastewaters and environments

impacted by wastewater were hotspots for initial mobilization of resistant genes. Various metagenomes were analyzed and it was found that amongst them all, wastewater had the highest abundance of origin species and mobilizing elements [8]. Another study by Karkman *et al.* also discussed wastewater and its treatment plants as hotspots for selection and transfer of AMR. These wastewater systems act as reservoirs and sources of antibiotic resistance, as well as potential sites for horizontal gene transfer between different bacterial species [39].

Another paper focused on recreational waters being recipients and natural reservoirs for AMR bacteria and ARGs [9]. Untreated wastewater, medical waste, treated wastewater, biosolids, agriculture, aquaculture, and birds were also explored as contributing factors [9]. Firstly, there is a high concentration of pathogenic and nonpathogenic microbes and antibiotics in untreated wastewater [9]. Overflows of sewers can lead to wastewater entering surface waters. In the US, there are 2300 - 7500 sewer overflows each year [5]. Also, the presence of antibiotics and their residues in municipal wastewater influent can be attributed to pharmaceutical disposal, excretion from patients undergoing pharmaceutical treatments, and the use of antimicrobials for cleaning surfaces, hands, or clothing [9]. Additionally, the blending of raw sewage with pre-treated and/or raw medical waste in hospitals can create a hotspot for the transfer of antibiotic resistance genes [9]. Treated wastewater can be discharged to surface waters and also serve as a point source for AMR [9]. This paper cites multiple studies which discuss that wastewater treatment does not reduce the proportion of resistant bacteria. In the United States, municipal waste undergoes primary and secondary wastewater treatment processes, often followed by disinfection steps to eliminate pathogens before releasing the treated effluent into surface waters [9]. The frequency of disinfection may vary depending on the intended use of the receiving water bodies, with some wastewater treatment plants implementing seasonal disinfection rather than year-round [9]. These processes may only partially remove pharmaceuticals, including antibiotics [9]. Studies have detected various pharmaceutical compounds, including both human and veterinary antibiotics, in the effluent of treated wastewater, suggesting that antibiotic residues can persist even after treatment [9]. Biosolids refers to the sludge generated by sewage treatment which is used as soil amendment [9]. This may be a source of AMR as well. Additionally, over 60% of livestock and poultry receive antibiotics, making animal feeding operations a large source of antibiotics [9]. Lastly, fecal matter of wild birds, which is widespread in many regions, can be dispersed in the environment, potentially leading to direct contamination of surface waters or through surface runoff [9]. The potential contribution of wild birds and seagulls to the dissemination of ARBs and ARGs requires additional investigation, particularly considering their long-distance migration behavior [9].

To better illustrate the scale of antibiotic release and antimicrobial resistance across sectors, **Table 1** summarizes key quantitative data from studies included in this review, highlighting the burden of antibiotic release, ARG pervalence, and

resistance rates across environmental and clinical contexts.

Table 1. Comparative quantitative data on antimicrobial resistance (AMR), antibiotic residues, and ARG prevalence across environmental and clinical sources.

Source/ Environment	Region/ Setting	Parameter	Reported Value	Reference
Food-animal production	U.S.	Antimicrobials used annually	>25 million lb/yr	Williams *et al.* 2016 [10]
Animal fecal waste	U.S.	Antibiotics excreted	~13.5 million lb/yr	Williams *et al.* 2016 [10]
Sewer overflows	U.S.	Untreated wastewater overflows per year	2300 - 7500 events/yr	Nappier *et al.* 2020 [9]
Hospital wastewater (*P. aeruginosa*)	–	MDR *P. aeruginosa* prevalence in samples	95% positive	Gervasoni *et al.* 2023 [29]
Wastewater effluent (*Enterococcus*)	South Africa	*Enterococcus* isolates from effluent	62 of 66 isolates (≈94%)	Iweriebor *et al.* 2015 [31]
Hospital infections (*E. faecalis*)	South Africa	Share of hospital enterococcal infections	>80%	Shobo *et al.* 2023 [30]
Clinical burden—*Salmonella*	U.S.	Resistant to ≥1 essential antibiotic	~16% of cases	Robertson *et al.* 2023 [24]
Efflux-pump inhibitor (MDR *Salmonella*)	Laboratory isolates	Reduction in tetracycline MIC with EPIs	16 - 32 × decrease	Le *et al.* 2018 [60]

2.3. Agriculture and Commercial Food Farms

Use of antibiotics in agricultural practices is another concern. A paper by Jochum *et al.* (2021) [40] discussed commercial chicken farms housing bacteria that are broadly resistant to last resort antibiotics. Their study aimed to detect bacteria in poultry farms which was resistant to colistin, carbapenems, and beta lactams, potentiate virulence risk in *E. coli* isolates, and assess AMR transfer via conjugates [40]. The researchers collected cecal contents from laying hens raised in both conventional cage (CC) and cage-free (CF) farms at three different maturity stages [40]. These samples were then randomly tested for the presence of extended-spectrum *β*-lactamase (ESBL)-producing *Enterobacteriaceae*, carbapenem-resistant *Acinetobacter* (CRA), and colistin-resistant *Escherichia coli* (CRE) using selective CHROMagar™ media [40]. They found that AmpC beta-lactamase was transferable through a large plasmid in ESBL-producing *E. coli* from CC [40]. The plasmid was transferable to different hosts, demonstrating its ability to transfer between both virulent and non-virulent bacteria [40].

Microplastics (MPs) play a role in the spread of ARGs in the environment. Due to the vast use of plastics and their release into the environment due to industrial activities, bodies of water are prone to microplastic contamination [41]. MPs allow for microbial colonization and formation of biofilm, where horizontal gene

transfer can occur [41]. Also, widespread use of antibiotics leads to their release into the environment, mainly through wastewater [41]. Therefore, wastewater treatment plants, especially in hospital plants, serve as hotspots for the selection of ARGs and their diffusion in the environment [41]. Consequently, the presence of drug-resistant bacteria and antimicrobial resistance genes on MPs makes them capable of transporting and spreading these genes and harmful microorganisms [41]. This emerging issue of microplastic-associated antimicrobial resistance poses a threat to the environment and, subsequently, to human health [41]. MPs have been found in various sources, including water supplies and even food and drinking water, highlighting the extent of the problem [41]. One of the concerning aspects of MPs is their ability to act as carriers, adsorbing chemicals and microorganisms from their surroundings, facilitated by the formation of biofilms [41]. Additionally, MPs have been identified as potential vectors for the long-distance dissemination of antibiotic resistance [41]. This is particularly concerning in hospital wastewater, where higher concentrations of antibiotic resistance genes (ARGs) and antibiotics are often found compared to urban wastewater [41]. The presence of ARGs on MPs in this environment can contribute to the selection and horizontal transfer of resistance genes, further amplifying the spread of antibiotic resistance [41]. Addressing the presence of ARGs on MPs is a critical issue that requires effective management of plastic waste disposal and the implementation of appropriate wastewater treatment strategies. Particularly in hospital settings, where the risk of antibiotic resistance is higher, it is crucial to ensure proper management practices to mitigate the diffusion of antibiotic resistance and safeguard human health [41].

3. Spread of Resistance from Pathogenic to Non-Pathogenic Bacteria

Pathogenic bacteria cause disease and invade host cells whereas non-pathogenic bacteria do not. Non-pathogenic bacteria may however carry genes which increase a pathogen's ability to amplify and spread [42]. A paper by Dionisio *et al.* (2023) [42] discusses non-pathogenic bacteria and their involvement in the dissemination of virulence and resistance genes. The authors discuss their ability to possess virulent genes, drug resistance genes, as well as non-housekeeping genes that can enhance the success of pathogens during infection [42]. Non-pathogenic cells that lack these genes can aid in the transfer of such genes to pathogenic cells by amplifying their presence within microbiomes [42]. Additionally, physical contact networks involving humans contribute to the transmission of pathogens within our population [42]. These networks refer to physical contact between families, friends, and sexual relationships.

A paper by José L. Martínez (2012) [43] discusses bottlenecks modulating the transfer, spread, and stability of antibiotic resistance genes. Since human pathogens acquire resistance genes via horizontal gene transfer originating in non-pathogenic bacteria, it is important to consider how natural, non-clinical ecosystems

contribute to the evolution of resistance [43]. The author analyzed factors such as founder effects, ecological connectivity, fitness costs, and second-order selection [43]. The founder effect refers to a situation where the first transferred gene becomes dominant [43]. When multiple resistance genes with similar profiles are present, one gene typically prevails in human bacterial pathogens [43]. This outcome can be influenced by fitness costs and chance. For example, the TEM-1 beta-lactamase became predominant among *Enterobacteriaceae* family members after its acquisition and rapid spread [43]. Although many beta-lactamases exist, TEM-1 prevailed due to strong selective pressure from antibiotics [43]. However, the introduction of new antibiotics or inhibitors can lead to the evolution of different beta-lactamases [43]. Ecological connectivity is related to both the spatial arrangement of microorganisms and their ability to transfer genetic material [43]. In natural environments, there is a low concentration of antibiotics unlike human-related environments like WWTPs [43]. In environments like WWTPs, human pathogens and non-pathogenic bacteria coexist, so there is a higher chance for the transfer of resistance genes to bacterial pathogens [43]. Also, some microorganisms form genetic exchange communities which share features like plasmids or transposons [43]. Therefore, if a mobile genetic element carrying a resistance gene enters a genetic exchange community, it can cause the gene to become fixed in bacterial pathogen populations [43]. The spread in certain bacteria is however limited by fitness costs because some resistant bacteria are outcompeted by susceptible ones [43]. Fitness costs may be compensated by mutations that allow resistance to persist [43]. Lastly, resistance genes can be maintained in habitats without strong antibiotic pressure through second-order selection [43]. This refers to when selection for one antibiotic can select for the entire array of resistance genes on a specific genetic element [43]. Cross-selection is another process where selection for one compound results in resistance to another, such as multidrug efflux pumps conferring resistance to antibiotics, biocides, and heavy metals [43]. Plasmids may also encode stability systems that increase the chances of resistance gene maintenance [43].

Plasmids allow for the possibility of rapid horizontal transfer of resistance among bacterial populations [44]. This is highlighted in a paper titled "Dissemination of plasmid-encoded AmpC β-lactamases in antimicrobial resistant *Salmonella* serotypes originating from humans, pigs and the swine environment". MDR *Salmonella* carries resistance genes on either their chromosome or mobile genetic elements [44]. Commercial agriculture offers a reservoir for MDR genes that could potentially be exchanged through horizontal transfer [44]. In this study *Salmonella* strains were selected from humans, pigs and the farm environment to determine if the exchange of material is possible [44]. MDR plasmids were found in all isolates. Those taken from pigs and their environment had the highest frequency of MDR [44]. All three sites contained similar resistance profiles. Class I and II integrons were also found and could be important to the transfer of MDR genes [44]. Similar plasmid profiles were also associated with serotypes that con-

tained the same MDR pattern on all samples from different sites [44].

Multiple drug resistance (MDR) in *Enterobacteriaceae*, particularly *Salmonella* and *Escherichia coli*, is a major public health concern due to plasmid-mediated gene transfer. We analyzed *Salmonella enterica* clinical isolates for plasmid-borne MDR transfer to *E. coli* and assessed the fitness impact of carrying an Inc A/C plasmid. Our findings show that Inc A/C plasmids provide a fitness advantage in macrophages without imposing significant costs under stress conditions. These results highlight the role of plasmids in enhancing pathogenicity and underscore the need to better understand MDR dissemination mechanisms [45].

In another review paper titled "Plasmids, a Molecular Cornerstone of Antimicrobial Resistance in the One Health Era", plasmid-mediated AMR and its mechanisms is discussed in detail. Plasmids not only spread ARGs between bacteria within the same microbiome, but also between microbiomes, and habitats, as well as between animals, humans, and the environment as shown in **Figure 2** [46].

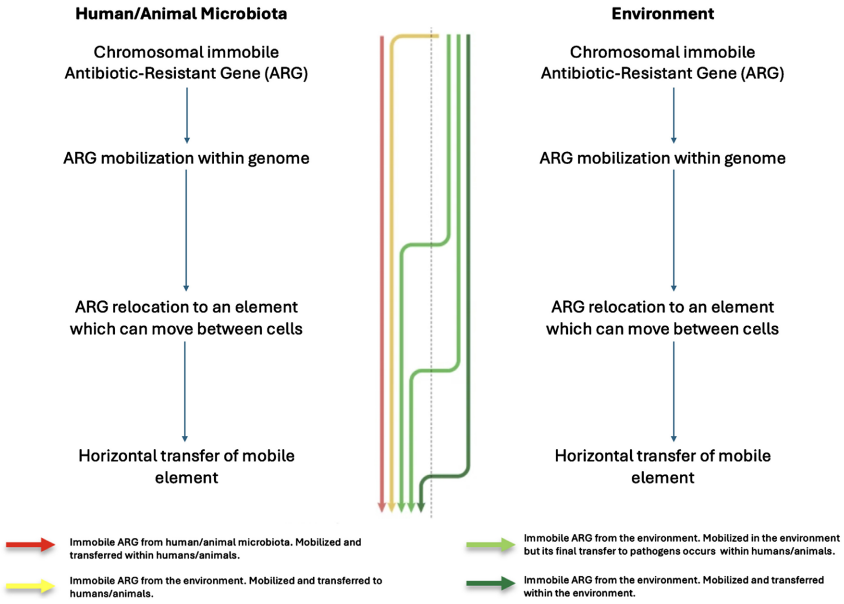

Figure 2. Role of the environment in the emergence and mobilization of resistance genes to pathogens through horizontal gene transfer. Adapted from: [47].

ARGs are spread among bacteria by conjugation between bacteria which is the transfer of DNA and is facilitated by plasmids or by integrative conjugative elements (ICEs) [46]. Some conjugative plasmids can even mobilize genes from plasmid recipients back to plasmid donors, a process called retromobilization, expanding their ecological role [46]. Plasmids vary in size, and are categorized as conjugative, mobilizable, or non-mobilizable based on their ability to encode or use conjugative machinery [46]. They contain genes essential for conjugation, alongside other genes responsible for stable maintenance, regulatory control, and replication of the plasmid [46]. Additionally, plasmids often possess "accessory" regions, housing genes that promote their own or their host's survival, such as

toxin-antitoxin genes, metal and antibiotic resistance genes, virulence genes, as well as metabolic and catabolic functions [46]. These accessory regions can vary between plasmids due to recombination events, resulting in mosaic plasmids. Mosaic plasmids contain genetic elements from different sources and are more prevalent among clinically relevant genera like *Escherichia*, *Klebsiella*, or *Salmonella* [46]. They also carry a higher proportion of transposase and antibiotic resistance genes [46]. In another paper by Li and Zhang (2023) [47], stressors that influence the transfer of plasmid mediated ARGs in the environment are discussed. These include environmental pollutants, including metals, pharmaceuticals, disinfectants, and microplastics [47].

3.1. Limitations and Knowledge Gaps

Despite the growing body of literature linking AMR to environmental sources, substantial limitations persist across studies. Many investigations vary in sampling methods, microbial targets, and analytical techniques, making it difficult to compare findings or generalize trends across regions [48]. This heterogeneity limits our ability to quantify the relative importance of each exposure route. Moreover, uncertainties remain around how frequently environmental ARGs are transferred to clinically relevant pathogens, particularly under real-world conditions. Without consistent risk characterization, it is also challenging to assess the true human health burden posed by these environmental reservoirs. A clearer understanding of exposure dynamics, coupled with standardized monitoring frameworks, is needed to inform effective mitigation strategies and guide policy efforts.

3.2. Consequences of Environmental Spread and State Regulation

A review paper by Kraemer *et al.* (2019) [35] discussed international policies aimed at mitigating antibiotic and AMR spread in the environment and gaps in existing policies. Each country is taking measures to address the issue of antibiotic pollution and antimicrobial resistance (AMR) in different ways and could serve as strategies to consider. Canada has implemented a domestic plan called " Tackling Antimicrobial Resistance and Antimicrobial Use: A Pan-Canadian Framework for Action". which focuses on surveillance, stewardship, and innovation [35]. It addresses the misuse of antibiotics in the health and agriculture sectors and aims to develop policies and regulations to combat AMR [35]. Additionally, inappropriate and widespread use of antibiotics is a major contributor to AMR in India [35]. The country has started monitoring and developing policies to address antibiotic pollution and AMR. India's medical societies adopted national recommendations to promote antibiotic stewardship, which were later incorporated into the 2017 National Action Plan on Antimicrobial Resistance. This plan is based on the WHO Global Plan and aims to tackle AMR through various strategies [35]. Europe has been at the forefront of tackling the antibiotic pollution and AMR crisis [35]. The European Union (EU) implemented the "EU One Health Action Plan against AMR" in 2017 [35]. The plan focuses on making the EU a best prac-

tice region, boosting research and innovation, and shaping the global agenda [35]. Europe has banned the use of antibiotics as growth promoters in livestock and food animals since 2006 [35], a policy that reportedly led to limited economic impact in high-income countries such as slight livestock productivity losses of 1 - 3% or modest short-term cost increases, closely offset by market price adjustments and also coincided with declines in AMR in livestock populations [49]. Policies and regulations have been implemented to address AMR in various sectors such as agriculture, aquaculture, human medicine, wastewater treatment, and the pharmaceutical industry [35]. The U.S. Food and Drug Administration (FDA) wants to improve the judicious use of antibiotics in humans and animals by having medically important antibiotics (MIAs)—as in those that are used in human medicine—to fall under the oversight of veterinarians [35]. Additionally, penicillins, tetracycline, and sulfas would now need to be prescribed instead of being readily available over the counter [35]. In addition to the FDA's initiatives, the Centers for Disease Control and Prevention (CDC) has implemented the National Action Plan for Combating Antibiotic-Resistant Bacteria (CARB), which outlines coordinated strategies to detect, respond to, and prevent AMR threats. This includes investments in public health infrastructure, expanding laboratory capacity, and enhancing data collection to monitor resistance trends across the country. The CDC also supports state and local health departments through programs like the Antibiotic Resistance (AR) Solutions Initiative, which funds regional efforts to contain and prevent outbreaks of resistant organisms [50]. These surveillance and stewardship programs have helped reduce deaths from antimicrobial resistane by approximately 18% overall and nearly 30% in hospitals during 2013-2019, though sustaining these gains requires ongoing investment in infrastructure and workforce [51]. At a broader level, the U.S. Department of Health and Human Services (HHS) is engaged in international collaborations to address AMR as a global health security threat. Through its Global Health Security Agenda (GHSA) and partnerships with the World Health Organization (WHO), the HHS Office of Global Affairs works to strengthen surveillance, research, and stewardship practices worldwide [52].

Although efforts are being made to strengthen surveillance systems, promote appropriate antibiotic use, and increase awareness about AMR, there are still gaps in existing policies, especially in regulating the release of antibiotics into the environment during pharmaceutical production [39]. Many action plans and policies lack specific measures to address antibiotic and antibiotic resistance gene pollution in natural environments [35]. This omission is concerning as localized antibiotic pollution from these facilities can contribute to the evolution of resistance, potentially leading to the global spread of antibiotic-resistant bacteria and genes [35]. Additionally, there are currently no policies in place in the studied regions that specifically target the pollution of wastewater treatment plants (WWTPs) by antibiotics and antibiotic resistance genes [35]. As existing WWTP designs do not effectively remove these substances, it is necessary to develop policies and tech-

nologies to regulate and reduce the presence of antibiotic resistance genes in WWTP effluents and biosolids [35]. Large-scale antibiotic pollution and resistance are significant public health challenges. While research on environmental dimensions of antibiotic resistance has increased, there are still many unknowns that require further investigation. Current studies focus on identifying resistance genes in the environment, but the associated risks of transmission and their impact on human health are not well understood. Antibiotic pollution can disrupt aquatic ecosystems and potentially affect human health. Effective policies should go beyond surveillance and consider the complex interconnections between human, animal, and environmental health. International cooperation and consistent policies are crucial to addressing this global problem.

In addition to federal and international efforts, tackling environmental antimicrobial resistance requires action at the state level. States have jurisdiction over hospital systems, agricultural practices, and waste management. These areas are directly tied to how antibiotics are used and released into the environment. Due to this, state-level policies and action are essential for addressing environmental AMR and filling in the gaps that federal strategies may not fully cover.

In the state of Indiana currently, there are steps being taken to combat environmental AMR. The Indiana Department of Health aims to educate and provide recommendations to healthcare facilities, providers, and patients in the state [53]. Also, there are Antimicrobial Stewardship programs which are used to ensure appropriate antimicrobial use [54]. The Indiana Department of health provides online resources geared for providers tackling outpatient care regarding antimicrobial stewardship guidance.

While these efforts are a step in the right direction, Indiana's current approach to environmental AMR still has major limitations. According to the Indiana Department of Health, most of the state's initiatives focus on increasing awareness and providing educational resources for healthcare providers and the public [55]. While awareness is important, there is a lack of concrete policies or regulations that directly target the environmental release of antibiotics or resistance genes. Stronger enforcement strategies which address antibiotic use in agriculture, pharmaceutical waste, and wastewater treatment are largely missing.

3.3. Emerging Policy Approaches in Low- and Middle-Income Countries (LMICs)

While most regulatory responses to AMR in the environment have emerged from high-income countries, initiatives in low- and middle-income nations are also growing. For example, India released a National Action Plan on Antimicrobial Resistance in 2017 that included a "One Health" framework explicitly targeting environmental contamination from pharmaceutical manufacturing and agricultural runoff. The state of Telangana, a major hub for antibiotic production, implemented effluent treatment plant requirements for pharmaceutical industries, aiming to reduce environmental discharges of active pharmaceutical ingredients [56] [57]. Although enforcement remains a challenge, such policies demonstrate

that LMICs can adopt targeted interventions with global implications for AMR mitigation. Future efforts should focus on enhancing monitoring capacity, transparency, and international support to scale similar initiatives across resource-limited settings.

4. Stopping Pathogenic Bacteria from Passing Mdr Genes to Non-Pathogenic Bacteria

There are several recent studies which highlight treatments and processes to combat AMR.

A paper by Azuma *et al.* (2022) [58], discusses the inactivation of antibiotic-resistant bacteria in wastewater by ozone-based advanced water treatment processes [58]. Many recent studies examined methods such as Fenton treatment, ultrasonication, electrolysis, TiO_2 photocatalysis, persulfate oxidation, graphitic carbon nitride (g-C_3N_4) treatment, UV/chlorine treatment, and ozone (O_3) treatment for water disinfection [58]. However, among these techniques, ozone (O_3) is notable for its strong oxidation capability and effectiveness in decolorization, deodorization, and sterilization [58]. Ozone treatment has demonstrated efficacy not only in inactivating pathogenic microorganisms but also in removing micropollutants such as pharmaceutical residues and endocrine disruptors from wastewater [58]. This study's results were that this treatment inactivates antimicrobial resistant and sensitive bacteria after ten minutes of treatment [58].

Another paper explored the potential of bacteriophages as a strategy for controlling antibiotic-resistant pathogens and understanding microbial interactions in wastewater treatment systems. Bacteriophages are viruses that infect and kill bacteria and are a potential alternative to antibiotics [59]. This paper cites their advantages which include their ability to target specific bacteria, their low toxicity, their effectiveness against ARBs and biofilms, and their ability to reproduce in response to pathogen density [59]. They also discuss various studies in which bacteriophages were able to successfully treat antibiotic-resistant bacteria in wastewater. For example, they have been effective against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*, reducing bacterial populations and antibiotic resistance [59]. Another application of bacteriophages that is discussed in this paper is against membrane fouling, or formation of biofilms on membrane bioreactor systems which are used in the treatment of wastewater [59]. Several studies discussed in this paper demonstrate the ability of bacteriophages to target bacteria in biofouling. The authors of this paper concluded that lytic phages are preferred to avoid horizontal gene transfer [59].

Additionally, a study by Iakovides *et al.* (2019) [60] discussed continuous ozonation of wastewater as a promising treatment for the elimination of the selected antibiotics, ARBs, and ARGs [60]. The schematic of this treatment process is seen in **Figure 3**.

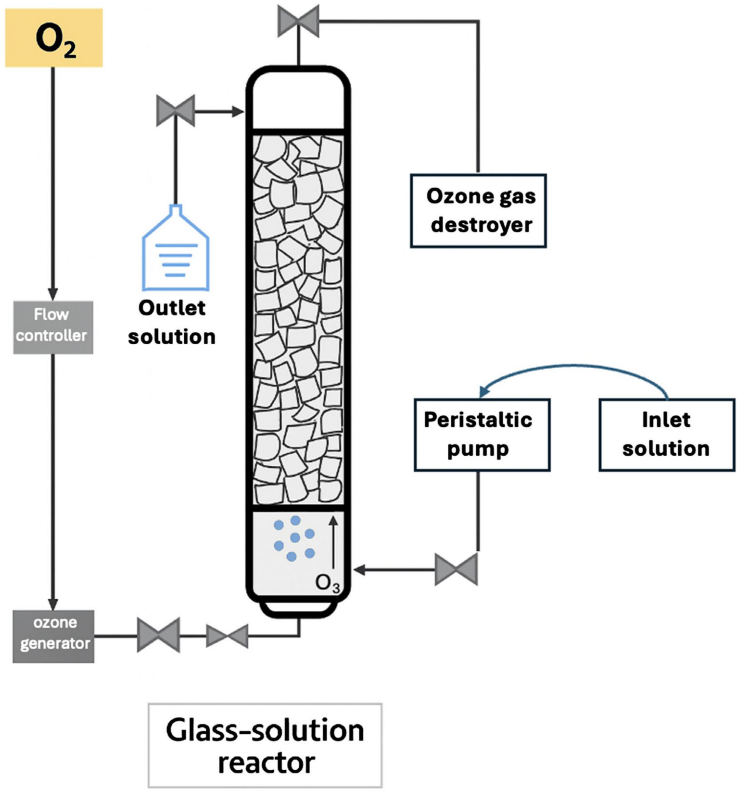

Figure 3. Schematic of an ozonation reactor used for continuous treatment of wastewater to eliminate antibiotics, antibiotic-resistant bacteria (ARBs), and antibiotic resistance genes (ARGs). Adapted from: [60].

A paper by Le *et al.* (2018) [61], discusses the removal of antibiotic residues, ARBs, and ARGs by membrane bioreactor systems. This research paper presents extensive data on the presence of 19 antibiotics, 10 types of antibiotic-resistant bacteria, and 15 antibiotic resistance genes (ARGs) in the untreated wastewater influent as well as during various treatment stages of conventional activated sludge (CAS) and membrane bioreactor (MBR) systems. The findings indicate that MBR systems demonstrated superior performance compared to CAS systems in terms of removing antibiotic-resistant bacteria, antibiotic resistance genes, and the majority of the target antibiotics [61].

Also, the paper titled "Increasing antimicrobial susceptibility of MDR *Salmonella* with the efflux pump inhibitor 1-(1-Naphthylmethyl)-piperazine" discusses the importance of developing effective measures against *Salmonella* due to its multidrug resistance. Bacterial antibiotic resistance is frequently mediated by efflux pumps [62]. In this research, the efficacy of two compounds, 1-(1-Naphthylmethyl)-Piperazine (NMP) and Phenylalanine-arginine β-naphthylamide (PaβN), in inhibiting efflux pump activity was investigated [62]. Specifically, their potential to synergistically enhance the effectiveness of the antibiotic tetracycline against tetracycline-resistant *Salmonella* was examined [62]. The findings revealed that these Efflux Pump Inhibitors (EPIs) were able to significantly reduce *Salmonella*'s

resistance to tetracycline, with a 16 to 32-fold decrease observed in multiple tetracycline-resistant isolates [62].

Lastly, a review by Alaa Qumsani (2023) examines the potential of nanoparticles (NPs) as a highly efficient method to combat bacteria. NPs act both as carriers for natural antibiotics and antimicrobials as well as active agents against bacteria [38]. Moreover, the surface engineering of nanocarriers allows for targeted delivery and modification of various resistance mechanisms [38]. However, a limitation of this drug-delivery method is clearance through the reticuloendothelial system [38]. Overall, however, the use of NPs is a promising technique for overcoming bacterial resistance.

5. Conclusions

While antimicrobial resistance (AMR) is monitored in clinical settings, there is a lack thereof in environmental settings and AMR in environmental settings poses a significant threat to public health. Understanding the environmental aspect of AMR is crucial to understanding the routes of dissemination, selection of resistant microorganism, and risks posed to human health [63].

This review highlights the issue of antimicrobial resistance in the environment, with an emphasis on water sources. A significant area of concern is wastewater treatment plants, especially in hospital plants, which serve as hotspots for the selection of ARGs and their diffusion in the environment [41]. Of particular concern is the role of horizontal gene transfer in facilitating resistance gene dissemination among pathogenic and non-pathogenic bacteria. This literature review also discussed multidrug resistant *Salmonella* strains. NTS is spread through many ways including the fecal-oral route, contaminated food, animal contact, and rarely person-to-person [64]. Future steps involve reviewing recent studies which highlight treatments and processes to combat AMR. Ideas discussed in this literature review are ozone-based advanced water treatment processes, bacteriophages, ozonation, membrane bioreactor systems, and efflux pump inhibitors. These strategies show promise in combating AMR, however, improvement in local government policies in regulating antibiotic pollution and enhancements to wastewater treatment capabilities, are necessary in solidifying the battle against AMR.

Publishing this review is essential to raise awareness of the increasing difficulty in treating antibiotic-resistant infections. Without intervention, the continued spread of AMR could render current treatments ineffective, leading to severe public health consequences.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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