REVIEW ARTICLE

Microbial resistance in Wastewater ecosystems: A review of Co-resistance to antibiotics and heavy metals

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A significant worldwide concern that jeopardises human health, animal health, and environmental balance is antimicrobial resistance (AMR). As reservoirs and pathways for antibiotic-resistant bacteria (ARB) and resistance genes, wastewater ecosystems are essential to the development, upkeep, and spread of resistance determinants. With an emphasis on the particular difficulties faced by quickly urbanising and water-scarce locations like Saudi Arabia, this review reviews the state of the art regarding the co-selection and co-resistance of antibiotics and heavy metals within wastewater environments. This review describes the main sources of antibiotics and heavy metals in wastewater, such as pharmaceutical production, medical institutions, residential and commercial discharges, and runoff from cities and farms. Also, examine the variety of molecular defences bacteria use against these stresses. In addition to the critical role that horizontal gene transfer and mobile genetic elements play in the spread of multidrug resistance, particular attention is paid to the interaction of co-regulation (coordinated genetic responses), co-resistance (physical linkage of resistance genes), and cross-resistance (shared resistance mechanisms). Even though wastewater treatment has advanced significantly, existing systems frequently fall short of fully eliminating ARB, resistance genes, and persistent contaminants, allowing them to spread throughout the environment. Because heavy metals can maintain or increase antibiotic resistance even when antibiotics are not present, their persistence is especially concerning. Mass gatherings, extensive water reuse, and the country's fast urbanisation all contribute to this issue by increasing the number and variety of resistance determinants in wastewater systems. Therefore, it is crucial to implement comprehensive mitigation methods that include cutting-edge treatment technologies, strong regulatory frameworks, proactive source management, and a One Health surveillance approach. Clarifying the ecodynamics of resistance, developing focused biotechnological treatments, and encouraging coordinated action across the environmental, medical, and agricultural domains must be the main goals of future study. The cycle of co-selection and environmental transmission of multidrug resistance may be broken by bridging both domains, protecting ecosystem sustainability and public health in the process.

Keywords: Antimicrobial resistance, Wastewater, Co-resistance, Heavy metals, Antibiotics, Horizontal gene transfer, Saudi Arabia, One Health.

INTRODUCTION

Antimicrobial resistance (AMR) has emerged as a pressing global concern in the 21st century, posing threats to human health, animal well-being, and the balance of ecosystems (La Rosa et al. 2025). The widespread and improper use of antibiotics in medicine, veterinary care, and agriculture is a key factor driving the rapid rise of AMR across the globe. Contributing elements include the unregulated availability of antibiotics without medical oversight, poor sanitation infrastructure, and the environmental release of unmetabolized antibiotics or their residues through human and animal waste, as well as industrial discharge. As a result, locations that handle human and animal waste—such as composting toilets, livestock operations, and aquaculture facilities—are considered major hotspots for the development

and

dissemination of antibiotic-resistant bacteria (ARB)(Ahmad et al. 2021; Agodi, 2024; La Rosa et al. 2025).

Municipal sewage systems and wastewater treatment plants (WWTPs) play a vital role as both sources and conduits for the spread of antibiotic resistance. Wastewater is frequently laden with antibiotic residues, nutrients, resistant bacteria, and antibiotic resistance genes (ARGs), creating an environment conducive to the development of multidrug resistance among environmental microbes. WWTPs, in particular, are rich in both organic and inorganic nutrients and maintain conditions—such as suitable temperature and pH—that encourage the growth of antibiotic-resistant bacteria (ARB). The dense microbial populations within these systems also facilitate close cell-to-cell contact, promoting interactions that lead to the spread of ARB and the evolution of ARGs (Berendonk et al. 2015, Krzeminski et al. 2019). Additionally, these conditions enhance horizontal gene transfer (HGT), further accelerating the dissemination of resistance (Savin et al. 2020a, Savin et al. 2020b). HGT allows bacteria to exchange genetic material via conjugation, transformation, and transduction—processes driven by mobile genetic elements (MGEs) such as plasmids, transposons, and integrons (Moralez et al. 2021).

When wastewater treatment is ineffective, ARB can accumulate and be released into the environment, spreading through direct water contact, agricultural irrigation, and contamination of water bodies and ecosystems (Vaz-Moreira et al. 2014, Berendonk et al. 2015). This intricate interaction between human activities and microbial adaptation significantly threatens both public health and environmental integrity, as WWTPs and similar environments foster the growth and transmission of resistant organisms. Addressing antimicrobial resistance, therefore, requires a holistic strategy that incorporates both environmental and health-related concerns, particularly focusing on antibiotic contamination in ecosystems. This broad perspective is supported by growing evidence linking environmental antibiotic exposure to resistance development. The World Health Organization's One Health approach emphasizes the interconnected nature of human, animal, and environmental health when tackling AMR(Organization, 2022, La Rosa et al. 2025). Understanding how ARGs spread from WWTPs and contribute to resistance is crucial for designing strategies to mitigate the environmental transmission of antibiotic resistance.

Antimicrobial agents including antibiotics, heavy metals, and biocides—are introduced into the environment through various pathways such as discharges from pharmaceutical manufacturing, hospital wastewater, human and livestock excretion, and agricultural runoff (Ebele et al. 2020). Common antibiotics detected in wastewater and surface waters include sulfamethoxazole (SMX), tetracycline (TET), erythromycin (ERY), ciprofloxacin (CIP), amoxicillin (AMX), and trimethoprim (TMP) (Chukwu et al. 2023).Heavy metals may naturally occur due to geological processes like rock weathering and soil deposition. However, high concentrations are typically linked to human activities such as industrial operations, mining, and domestic waste discharge. These contaminants often accumulate in surface waters (e.g., rivers and estuaries) and sediments, forming long-term environmental reservoirs (Fayiga et al. 2018).

Rapid urbanization, population increase, and the country's arid environment, which require substantial reuse of treated wastewater for agriculture and urban landscaping, present Saudi Arabia with particular and urgent difficulties with relation to microbial resistance in wastewater ecosystems(Al-Otaibi et al. 2024). Heavy metals and antibiotic resistance genes (ARGs) have been found in surface waters and wastewater throughout the Kingdom, even in major cities like Riyadh and Jeddah, according to recent studies(Al-Otaibi et al. 2024, Wang et al. 2022).

For instance, studies conducted in Riyadh's Wadi Hanifah Valley have shown that surface waters are tainted with a range of heavy metals and multi-drug-resistant bacteria. ARGs associated with popular antibiotics such as tetracycline, sulphonamides, and β-lactams have also been often found. These ARGs' distribution is impacted by pharmaceuticals, industrial pollutants, and wastewater discharge inputs, all of which help environmental bacteria co-select and disseminate resistance characteristics(Al-Otaibi et al. 2024). The presence of heavy metals is enough to exert selective pressure, encouraging the persistence and horizontal transmission of resistance genes, even though their concentrations often stay below WHO standards.

These issues are made worse by the fact that Saudi Arabia is the location of some of the biggest mass gatherings in the world, most notably the yearly Hajj and Umrah pilgrimages, which have been demonstrated to bring antibiotic-resistant bacteria and novel ARGs into regional sewage systems(Wang et al. 2025). The introduction of clinically significant resistance genes, like *bla*_PER and *bla*_NDM, in viable opportunistic infections has been identified by wastewater surveillance during these episodes, highlighting the danger of AMR diffusion through untreated or insufficiently treated wastewater(Wang et al. 2025).

Wastewater treatment facilities (WWTPs) are essential for controlling these risks, however in Saudi Arabia, traditional treatment methods frequently fall short of fully eliminating heavy metals and ARGs, which raises questions about the safety of reusing water as well as the possibility of negative effects on the environment and public

health(Medina et al. 2025, Alotaibi, 2023, Alharbi et al. 2023). Anaerobic membrane bioreactors and other cutting-edge treatment technologies have demonstrated promise in lowering ARG abundance and restricting gene transfer; however, more study and funding are required to fully address the complexity and scope of the issue in the Kingdom.

All things considered, the co-resistance to heavy metals and antibiotics in Saudi Arabia's wastewater ecosystems is a developing concern that is influenced by regional environmental factors, cultural norms, and global health dynamics. Coordinated public health initiatives that are adapted to the particular circumstances of the Kingdom, sophisticated treatment options, and integrated surveillance will all be necessary to address this problem. This review aimed to review the sources, mechanisms, and environmental dynamics of co-resistance to antibiotics and heavy metals in wastewater ecosystems, with a focus on challenges and mitigation strategies in regions like Saudi Arabia under the One Health framework.

Sources of Antibiotics and Heavy Metals in Wastewater

Antibiotics

Pharmaceutical industries:
One of the main sources of antibiotics in wastewater is the pharmaceutical industry (Figure 1). If waste is not adequately handled, large amounts of antibiotics may be discharged into the environment during the manufacture of active pharmaceutical ingredients (APIs). Research has shown that pharmaceutical factory effluents contain incredibly high quantities of antibiotics, often surpassing the amount in patients' blood. The development and dissemination of antibiotic resistance genes (ARGs) in environmental bacteria are facilitated by these discharges(Kotwani et al. 2023, Tahrani et al. 2016, Rayan, 2023). Beta-blockers, hormones, antibiotics, lipid regulators, and nonsteroidal anti-inflammatory medications have been identified as the most prevalent pharmaceutical classes in the environment. A recent global assessment examined the prevalence of 61 medicines in 1052 places across 258 rivers in 104 nations with a combined population of 471.4 million people on all continents(Alharbi et al. 2023). Effective enforcement and openness are still lacking in many areas, despite industry pledges and global calls for more stringent regulation.

In the Kingdom of Saudi Arabia, in relation to the problem of microbial resistance in wastewater ecosystems, the first thorough evaluation of new trace organic compounds (TOrCs), such as medications and personal hygiene items, in the effluents of four wastewater treatment facilities in Western Saudi Arabia was conducted by Alidina et al. (2014). The results demonstrated that a variety of medications, including antibiotics, continue to exist in treated wastewater at levels similar to those observed in the

US. Higher TOrC concentrations were found in plants with less sophisticated treatment methods, and distinct chemical profiles were connected to population dynamics, including the number of foreign visitors(Alidina et al. 2014).

A 2017 study of Saudi Arabia's Red Sea coast found high levels of pharmaceuticals, including antibiotics, in seawater near sites with continuous sewage discharge. Researchers sampled surface seawater at seven locations, including sites directly influenced by continuous sewage release, and detected 13 PPCPs in 26 samples. The most abundant compounds were metformin, diclofenac, acetaminophen, and caffeine, with concentrations exceeding 3 µg/L for several substances. Substantial amounts of antibiotics were also detected in all samples. The study highlighted that coastal lagoons with low water exchange, such as Al-Arbaeen and Al-Shabab in Jeddah, exhibited the highest concentrations of PPCPs due to ongoing sewage releases. The presence and distribution of these compounds indicate high release rates and raise concerns about long-term environmental risks, especially to fragile marine ecosystems and nearby protected coral reefs(Ali et al. 2017).

A recent study carried out in Riyadh offers important new information about the presence and destiny of pharmaceuticals, including antibiotics, in the city's wastewater treatment facilities (WWTPs). Over the course of 16 months, researchers collected 144 samples from influents and effluents at three large WWTPs to evaluate 15 medicines and one metabolite. The study discovered that, on average, the pharmaceutical concentrations in the influents and effluents were higher than those documented in earlier Saudi or international investigations. Interestingly, ciprofloxacin, a common antibiotic, was one of the most prevalent substances found, with mass loads as high as 20.7 mg/day/1000 people and effluent concentrations as high as 33.2 µg/L(Alharbi et al. 2023).

Hospitals and healthcare facilities:
Hospitals and clinics release effluents that contain resistant bacteria and unmetabolized antibiotics, which contributes to antibiotic pollution (Figure 1). Hospital wastewater frequently has higher amounts of antibiotics and ARGs than household sources. Research has indicated that these effluents can favor multi-resistant bacteria, which can then infiltrate municipal sewage systems. Numerous studies have reported the detection of various antibiotics and antibiotic resistance genes (ARGs) in hospital wastewater across Saudi Arabia. In one recent study, untreated wastewater from Ministry of Health-designated hospitals was analyzed, revealing a consistently high relative abundance of ARGs, including those associated with resistance to β-lactam antibiotics and other critical drug classes. The findings suggested that the elevated presence of these genes was closely tied to continuous antibiotic usage and horizontal gene transfer occurring within the hospital wastewater microbial community(Wang et al. 2022). Another investigation

identified multidrug-resistant bacterial strains—such as *Staphylococcus haemolyticus*, *Enterococcus faecalis,* and *Escherichia coli*—in effluents from hospital wastewater treatment plants. These results underscore the significant role of hospitals as key contributors to the environmental release of antibiotic-resistant bacteria and resistance genes(Mehanni et al. 2023).

Studies have demonstrated that both hospital and municipal wastewater in Saudi Arabia harbor significant concentrations of antibiotic-resistant bacteria, including species of *Klebsiella*, *Acinetobacter*, *Enterobacter*, and *Pseudomonas*. Many of these strains are known to produce extended-spectrum β-lactamases (ESBLs) and carbapenemases. Resistance genes such as *bla_TEM, bla_SHV, bla_CTX-M, bla_OXA-48,* and *bla_NDM-1* have been detected in hospital wastewater, highlighting the potential for environmental dissemination of multidrug resistance(Irfan et al. 2023). A wide range of antibiotics, including amoxicillin, ofloxacin, and roxithromycin, have been found in hospital effluents using targeted analysis. The concentrations of these antibiotics in influents and effluents range from 158 to 1025 ng/L and 99.9 to 411 ng/L, respectively. These results demonstrate that one of the main point sources of pharmaceutical pollution in urban sewage systems is hospital discharges(Alharbi et al. 2023, Al-Maadheed et al. 2019).

Domestic wastewater:

A significant percentage of the antibiotics that people eat are eliminated unmetabolized and end up in the sewage system as household wastewater (Figure 1). Therefore, a significant source of antibiotics and resistance genes in the environment, particularly in densely populated urban areas, is municipal wastewater. Because wastewater treatment plants (WWTPs) are not entirely successful in eliminating these substances, they get up in downstream surface waters and treated effluents(Mishra et al. 2025, Samrot et al. 2023). Domestic wastewater primarily receives antibiotics through human excretion of unmetabolized drugs and the disposal of unused medications. Recent studies have shown that municipal wastewater in Saudi Arabia contains a wide range of antibiotic-resistant bacteria and resistance genes. For example, analysis of municipal and treated wastewater samples revealed the presence of clinically significant Gram-negative bacteria including *Klebsiella*, *Acinetobacter*, *Enterobacter*, and *Pseudomonas* species many of which exhibited resistance to key antibiotics such as cefotaxime, amoxicillin-clavulanate, imipenem, meropenem, and trimethoprim-sulfamethoxazole. Extended-spectrum β-lactamase (ESBL) and carbapenemase-producing bacteria were also identified, with genes such as *bla_TEM*, *bla_SHV*, *bla_CTX-M*, *bla_OXA-48*, and *bla_NDM-1* detected in isolates from municipal wastewater(Irfan et al. 2023). These findings underscore the role of domestic sewage as a reservoir and conduit for

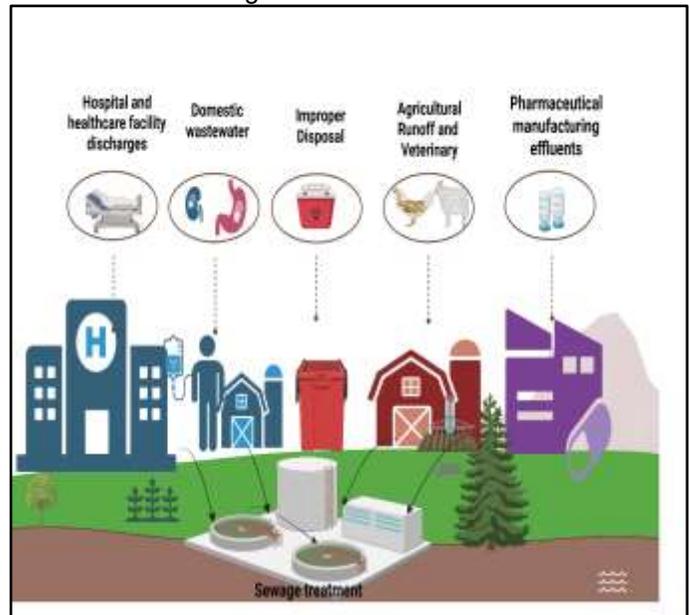antibiotic resistance genes in the environment.

Figure 1: Sources of heavy metals (Created by Biorender)

Heavy Metals

2.2.1 Industrial effluents:

Industrial operations are the main source of heavy metal pollution in wastewater. Major contributing sectors include electroplating, mining, chemical manufacturing, petroleum refining, battery production, textiles, tanneries, and pulp and paper industries (Figure 2).. These activities release substantial quantities of metals—such as lead (Pb), cadmium (Cd), copper (Cu), mercury (Hg), chromium (Cr), nickel (Ni), zinc (Zn), arsenic (As), and iron (Fe)—through direct effluent discharge, improper waste handling, leaks, and accidental spills (Oladimeji et al. 2024, Qasem et al. 2021). For instance, textile manufacturing frequently employs metal-laden dyes, while electroplating and mining are significant sources of chromium, cadmium, and lead pollution. Similarly, chemical processing and petroleum refining contribute diverse metals to wastewater streams (Oladimeji et al. 2024, Qasem et al. 2021).

Industrial zones in Saudi Arabia, particularly in major cities such as Riyadh, Jeddah, Jubail, and Yanbu, are recognized sources of heavy metals in wastewater. Industries like electroplating, mining, textiles, and petrochemicals discharge metals such as lead, cadmium, copper, and mercury into municipal and industrial wastewater streams. Government and industry reports highlight ongoing efforts to improve industrial waste management and treatment, especially as the country expands its wastewater recycling and industrial waste processing capacity(Bajaal, 2023).

In a multi-year study, the physicochemical characteristics of wastewater from treatment plants in Riyadh were assessed, along with the levels of important

heavy metals like lead, cadmium, copper, and zinc. The study confirmed that industrial discharges, urban runoff, and maybe agricultural activities contribute to the metal load in municipal wastewater by detecting detectable quantities of these metals in both influent and effluent samples. Additionally, the study observed operational and seasonal fluctuations in metal concentrations, highlighting the necessity of ongoing monitoring and customized treatment plans(Elfeky et al. 2025). According to a thorough analysis, the main sources of heavy metals in wastewater in Saudi Arabia are the industrial sectors, including petrochemicals, mining, textiles, and electroplating. Both urban and rural areas were found to be contaminated with metals due to urban runoff and the use of treated wastewater for irrigation. The evaluation emphasized that many treatment facilities lack the necessary equipment to eliminate heavy metals, which can linger in effluents and endanger public health and the environment(Alahdal et al. 2021).

Urban runoff:

Stormwater runoff from urban areas adds to the concentrations of heavy metals in wastewater(Figure 2). Metals from construction materials, roads, and air deposition are washed into sewage systems by rainwater. Common causes include runoff from industrial areas, corrosion of building materials, and car emissions that release lead, zinc, and copper. This diffuse input can have a big impact, particularly in places that are heavily inhabited or industrialized(Oladimeji et al. 2024, Qasem et al. 2021).

Agricultural runoff:

Fertilizers and pesticides, which may contain trace metals including arsenic, cadmium, and lead, are the main way that agricultural activities contribute to the heavy metal burden in wastewater. Metal buildup in soils and subsequent discharge into water bodies can be exacerbated by irrigation with tainted water and fertilization with manure or sewage sludge(Oladimeji et al. 2024, Qasem et al. 2021) (Figure 2).

Many regions use wastewater for agricultural purposes, which has an impact on ecosystem and human health. Through absorption channels including the consumption of plant material (in the food chain), heavy metals in contaminated wastewater present a number of dangers to humans. In addition to increasing drainage and soil aeration, heavy metals also kill the organisms in the soil that fix nitrogen. Long-term wastewater irrigation might eventually cause heavy metal deposition in the soil, even though metal concentrations in household wastewater effluents are often quite low (Wuana and Okieimen, 2011).
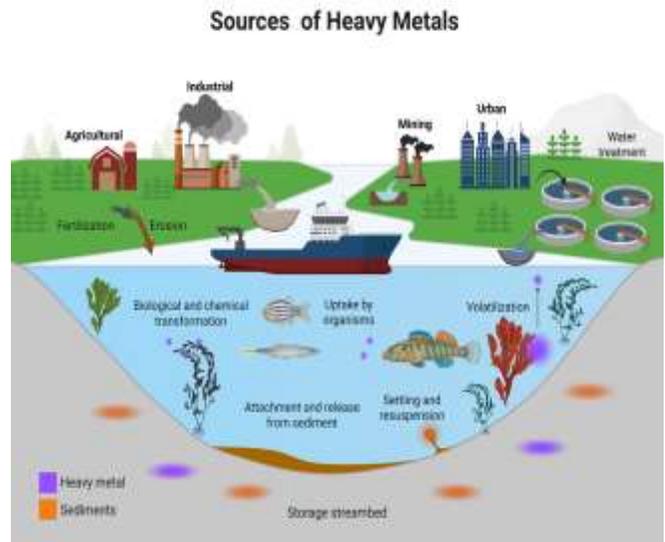

Figure 2: Sources of heavy metals (Created by Biorender)

Mechanisms of Resistance

Antimicrobial resistance is becoming an increasingly serious issue in medicine as a result of the various ways that bacteria have evolved to withstand medications(Belay et al. 2024). The following are important resistance mechanisms (Figure 3): (1) enzymatic inactivation, in which bacteria produce enzymes like β-lactamases that break down antibiotics like cephalosporins and penicillins; (2) drug target modification, as demonstrated by modified penicillin-binding proteins in MRSA, which prevent antibiotics from binding effectively; (3) decreased permeability, which is frequently accomplished by altering the structure of membrane porins, thereby limiting antibiotic entry—this is particularly common in Gram-negative bacteria; (4) active efflux, which uses specialized pumps to remove antibiotics from the cell; and (5) biofilm formation, in which bacterial communities create a matrix that protects them from antibiotics and the immune response(Belay et al. 2024). Treatment plans may be made more difficult by these mechanisms, which can operate singly or in concert.

Bacteria inhabiting metal-contaminated environments have developed a diverse array of resistance strategies to mitigate the toxic effects of heavy metals. These mechanisms function either by actively excluding, transforming, sequestering, or precipitating metal ions, thereby enabling microbial survival and ecological persistence. Bacteria use mechanisms like efflux pumps to eliminate toxic ions, enzymatic detoxification to change toxic ions into less toxic forms, metal sequestration through protein binding (e.g., metallothioneins), reduced uptake through transport protein modification, and metal transformation into insoluble forms to resist heavy metals. Mobile genetic elements frequently contain genes that give resistance to both heavy metals and antibiotics(Belay et al. 2024). This allows for co-selection, in which exposure to

heavy metals can also favor bacteria that are resistant to antibiotics.

Metal sequestration and precipitation, bacteria can bind toxic metal ions intracellularly using metal-chelating molecules such as metallothioneins, polyphosphates, and phytochelatins, reducing their bioavailability. In parallel, extracellular metabolites can mediate the precipitation of heavy metals into insoluble forms, rendering them non-toxic (Maret, 2024). Efflux pumps, including P-type ATPases and RND-type transporters, actively export heavy metal ions like Zn^{2+}, Cd^{2+}, and Cu^{2+} out of the cytoplasm, utilizing ATP hydrolysis. These pumps are often metal-specific and are regulated under stress conditions to maintain metal homeostasis (Ngendahimana, 2012). Enzymatic transformation, certain bacteria detoxify heavy metals by enzymatically converting them into less toxic or volatile forms. For example, mercury reductase (MerA) reduces toxic Hg^{2+} into volatile elemental mercury (Hg^0), which then diffuses out of the cell (Golysheva et al. 2025, Singh and Kumar, 2020). Modification of Membrane Permeability, bacteria may reduce the uptake of metal ions by modifying the outer membrane composition, downregulating porins, or altering the lipid bilayer structure. This mechanism limits the initial entry of metals into the cell (Niño-Martínez et al. 2019).

Biofilms provide a protective matrix (EPS) in which metal ions are trapped and immobilized, thereby shielding individual bacterial cells from direct exposure to toxic concentrations. This collective defense mechanism is commonly observed in metal-polluted environments (Barik et al. 2021, Syed et al. 2023). Some bacteria facilitate the formation of insoluble mineral particles via biological processes, thereby neutralizing toxic metal ions. An example is the microbial reduction of hexavalent chromium (Cr^{6+}) to the less toxic trivalent form (Cr^{3+}), often coupled with nanoparticle formation (Chaturvedi et al. 2020, Dong et al. 2021). Over all, Heavy metal resistance genes are frequently organized into operons (e.g., mer, czc), often located on plasmids or mobile genetic elements. This facilitates horizontal gene transfer and rapid adaptation among microbial communities in metal-contaminated habitats (Halema et al. 2024, Sánchez-Corona et al. 2025, Hassen and Abbassi, 2025)

Overall, bacterial resistance pathways to heavy metals and antibiotics are contrasted in Table 1. Both rely on genetic mobility to spread resistance and use efflux pumps to eliminate harmful compounds. Heavy metal resistance usually uses sequestration and enzymatic detoxification, whereas antibiotic resistance usually involves target change and biofilm formation. For both, decreased uptake is typical. Antibiotics are the primary cause of target route bypass. This synopsis focuses on common and unique bacterial defense mechanisms against heavy metal and antibiotic stress.
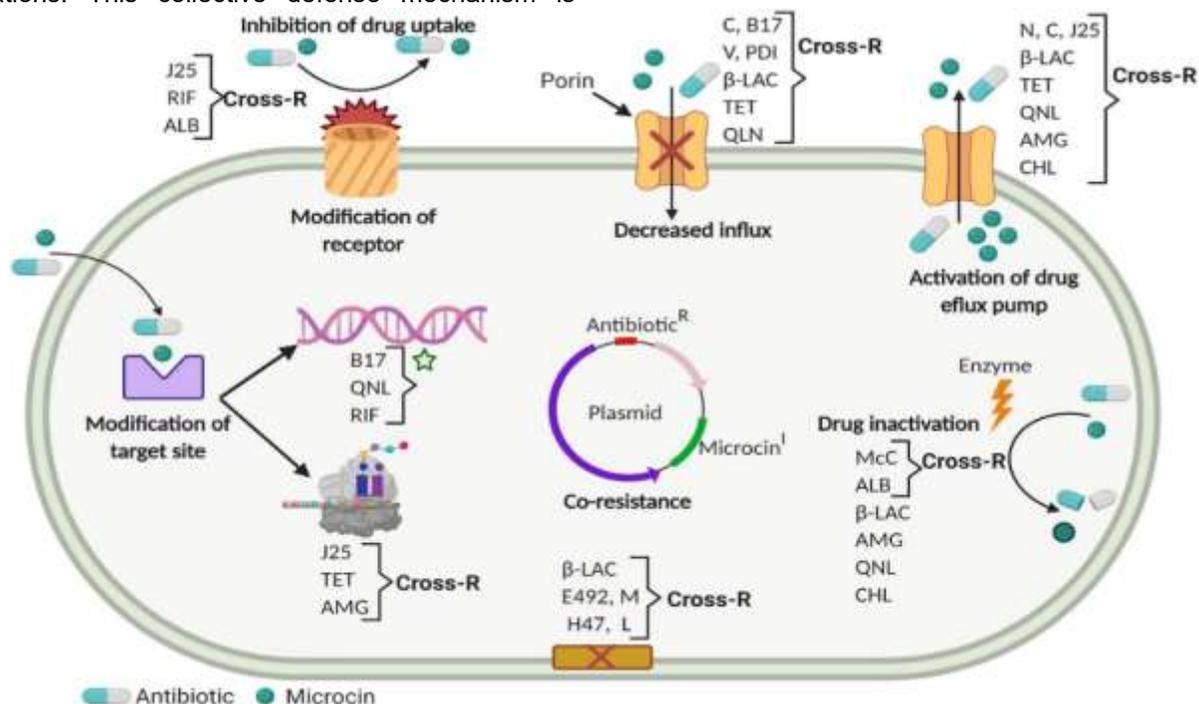

Figure 3: Schematic Overview of Bacterial Resistance Mechanisms Against Antibiotics and Microcins https://fity.club/lists/suggestions/mechanisms-of-antibiotic-resistance/

Table 1: Comparison of Mechanisms of Resistance to Antibiotics and Heavy Metals in Bacteria

Mechanism	Heavy Metals	Antibiotics
Efflux Pumps	Remove toxic metal ions	Remove antibiotics from cell
Enzymatic Inactivation/Detox	Reduce or transform metals	Destroy/modify antibiotics
Target Modification/Alteration	Often less common	Reduce drug binding
Decreased Uptake	Limit metal ion entry	Limit antibiotic entry
Bypass of Target	Not typically reported	Use alternate pathways
Sequestration & Precipitation	Common (protein/peptide binding)	Rare
Biofilm Formation	Not primary but can confer some	Shields from antibiotics
Genetic Mobility (Plasmids, etc.)	Common	Common

Co-selection models of resistance

There are three recognized co-selection models: co-resistance, cross-resistance, and co-regulation(Gillieatt and Coleman, 2024). Co-resistance occurs when resistance genes are physically connected, usually on the same MGE, and are inherited as a single package at the same time (Fig. 3A). An efflux pump that eliminates both metals and antibiotics is an example of cross-resistance, which occurs when a single mechanism confers resistance to both agents (Fig. 3B). Last but not least, co-regulation creates a single transcriptional response by combining resistance genes that share a promoter or regulator (Fig. 3C)(Gillieatt and Coleman, 2024). There is no mutual exclusion between these mechanisms. Regardless of antibiotic exposure, any of these co-selection techniques may enable ARGs and, hence, resistance phenotypes to endure under heavy metal selection.

The minimum selective concentration (MSC), and minimum co-selected concentration (MCC) are some key ideas for comprehending co-selection between metals and antibiotics. According to (Seiler and Berendonk, 2012) , the minimum inhibitory concentration (MIC) is the lowest concentration of a drug that prevents a particular bacterium from growing. According to (Yu et al. 2017), the MSC is the lowest concentration of an agent at which the fitness benefit of resistance above the fitness cost. The minimum concentration of one drug that will favor resistance to another is known as the MCC (Arya et al. 2021). Though there aren't many examples, MSC and MCC values should, in theory, be on par with or less than MIC values. This is important since metals and antibiotics probably surpass the MSC (Gullberg et al. 2014, Zhang et al. 2018) or MCC

(Gillieatt and Coleman, 2024), even if they hardly ever reach the MIC in the environment (Wales and Davies, 2015, Zhang et al. 2018). The combination of agents, the kind of microbial community, and the environmental circumstances will all have a significant impact on the MSC and MCC values (Gillieatt and Coleman, 2024).
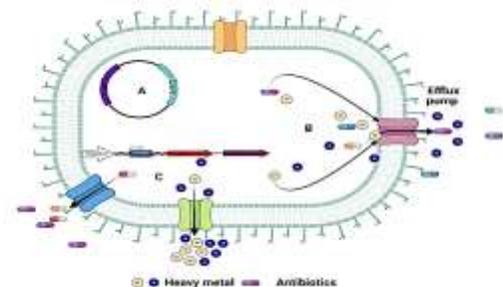

Figure 4: Mechanisms of co-selection are compared.
(A) Co-resistance, which is exhibited by the ARG and MRG genes living on the same plasmid and being inherited at the same time. (B) An efflux pump ejecting metals and antibiotics demonstrates cross-resistance. (C) Co-regulation as evidenced by the expression of both an MRG and an ARG upon metal induction. (Created by Biorender)

Co-resistance

Co-resistance occurs when resistance genes for antibiotics and heavy metals are physically linked together on the same mobile genetic elements such as plasmids, integrons, or transposons(Edet et al. 2023, Simjee et al. 2024). When antibiotics or heavy metals are present in the

environment, this genetic linkage enables bacteria to be selected for numerous resistance features at the same time. For instance, bacterial populations in wastewater systems can acquire co-resistance by horizontal gene transfer (HGT) when a plasmid containing both metal resistance genes (MRGs) and antibiotic resistance genes (ARGs) is present. According to studies, bacterial isolates from heavy metal-contaminated settings frequently demonstrate co-resistance, or multi-drug resistance (MDR) to antibiotics. The survival and spread of resistant strains can be aided by the selection pressure that heavy metals like cadmium, lead, copper, and zinc can exert, maintaining antibiotic resistance even in the absence of antibiotic exposure(Edet et al. 2023, Simjee et al. 2024).

Co-resistance refers to the physical linkage of antibiotic resistance genes (ARGs) and metal resistance genes (MRGs) on the same mobile genetic elements (MGEs), such as plasmids, transposons, or integrons, enabling simultaneous inheritance and selection. Multiple studies have documented consistent correlations between specific ARGs (e.g., *sul1*, *tet(M)*, *blaTEM*, *ermB*) and MRGs (e.g., *czcA*, *copA*, *merA*), often co-localized within conjugative plasmids isolated from wastewater and industrially impacted sites (Gillieatt and Coleman, 2024). Plasmid transfer experiments demonstrate that exposure to metals like copper and zinc can increase conjugation frequencies and transfer of ARG-MRG-bearing plasmids, facilitating rapid dissemination of multidrug resistance across diverse bacterial communities.

Co-resistance usually occurs when metal and antibiotic resistance genes are physically connected on mobile genetic elements like integrons, transposons, or plasmids, allowing for horizontal transmission and simultaneous selection. Several genes have been linked to heavy metal resistance, such as the arsenic and antimony resistance gene ars, which is present in a variety of taxa, including *Pseudomonadota* and the CFB Group; cue (copper resistance), which is common in *Pseudomonadota*; and mer (mercury resistance), which is present in *Actinomycetota*, *Bacillota*, and *Pseudomonadota*. Efflux pump genes like cop (copper resistance) and czc (cadmium, cobalt, zinc resistance) are commonly found in *Bacillota* and *Pseudomonadota*, demonstrating the ubiquitous bacterial ability to use heavy metal efflux mechanisms(Gillieatt and Coleman, 2024).

Antibiotic resistance genes also mediate survival through chemical modification (aac, ant, aph for aminoglycosides), enzymatic degradation (bla genes for β-lactams), efflux pumps (acr, mdt, mef), target modification or replacement (erm genes for macrolides and mec/PBP2a for β-lactams). These ARGs are widespread across the same key bacterial taxa implicated in metal resistance, particularly Actinomycetota, Bacillota, Pseudomonadota, and the CFB Group. Because metals can favor organisms with both metal resistance genes (MRGs) and antibiotic resistance genes (ARGs), co-resistance is strongly supported by this overlapping host range and frequent co-localization on MGEs.

Cross-resistance

A single resistance mechanism that confers resistance to both heavy metals and antibiotics is known as cross-resistance. Multidrug efflux pumps, which are membrane proteins that remove a variety of harmful substances from bacterial cells, such as metals and antibiotics, are a typical example. One gene or system can offer dual protection because of its overlapping functioning. In co-contaminated environments, such as wastewater, cross-resistance mechanisms give bacteria a fitness advantage that increases their survival and spread(Simjee et al. 2024, Gillieatt and Coleman, 2024).

Additionally, studies show that wastewater ecosystems face a considerable co-selection pressure. According to microcosm studies, the abundance of both ARGs and MRGs is increased more by the combined exposure to low quantities of metals (such as copper, zinc, and cadmium) and antibiotics (such as ampicillin and tetracycline) than by each agent alone. Rapid dissemination within microbial communities is made possible by these exposures, which encourage the growth of integrons and transposons that co-carry the resistance genes. Additionally, heavy metals can occasionally increase the rate of plasmid transfer, which increases horizontal gene transfer events that spread co-resistance(Gillieatt and Coleman, 2024).

Cross-resistance arises when a single mechanism, commonly broad-spectrum efflux pumps such as the RND family (e.g., *MdtABC*, *MacAB*, *CusCFBA*), mediates resistance to structurally diverse antibiotics and metals simultaneously. Functional genetic studies confirm that deletion or overexpression of these efflux systems alters bacterial susceptibility to both classes of compounds in organisms like *Escherichia coli*, *Salmonella enterica*, and *Agrobacterium tumefaciens* (Gillieatt and Coleman, 2024). Such efflux pumps provide bacteria with a physiological advantage in co-contaminated environments by extruding multiple toxicants efficiently, which is supported by metagenomic data showing high prevalence of genes encoding these pumps in wastewater resistomes.

Co-regulation:

Co-regulation involves transcriptional regulatory networks whereby exposure to one stressor (e.g., heavy metals) triggers expression changes in genes conferring resistance to another (e.g., antibiotics). For example, in *Pseudomonas aeruginosa*, the metal-responsive two-component system CzcR-CzcS induces expression of the CzcCBA efflux pump and represses the porin OprD, reducing carbapenem uptake and conferring antibiotic resistance (Gillieatt and Coleman, 2024). Furthermore, metal exposure can activate global stress responses (e.g., SOS and oxidative stress pathways) in *E. coli*, increasing mobility of MGEs and integrons that carry ARGs, thus indirectly enhancing resistance gene acquisition and expression. These nuanced regulatory effects suggest that

co-regulation contributes to resistance acquisition dynamics in environmental bacterial communities. Significantly, provided the complex selective pressures found in contaminated soils, industrial effluents, and wastewater treatment plants (WWTPs), these co-selection mechanisms frequently work in concert and are not mutually exclusive. Subinhibitory concentrations of metals and antibiotics can both promote the horizontal transmission and abundance of ARGs and MRGs, according to experimental microcosm investigations. Metals often exhibit stronger or more lasting selection than antibiotics alone (Gillieatt & Coleman, 2024). The relative contributions of each model, however, differ depending on the environmental setting, antibiotic and metal types, bacterial taxa, and the genetic makeup of resistance determinants.

Overall, the main ways that bacteria resist heavy metals and drugs are illustrated in Table 2. Co-resistance allows metal and antibiotic resistance genes to propagate together on mobile elements. Single mechanisms that guard against both agents, such as broad-spectrum efflux pumps, are the source of cross-resistance. Co-regulation is the process by which exposure to metals activates genes that confer resistance to antibiotics. All of these processes work together to help multi-resistant bacteria persist and spread.

Horizontal Gene Transfer in Wastewater Systems

In wastewater systems, horizontal gene transfer (HGT) is an important variable in the spread of genes that confer resistance to heavy metals and antibiotics. The exposure of varied microbial communities to antibiotics, heavy metals, and other pollutants at wastewater treatment facilities (WWTPs) creates an environment that is conducive to the exchange of genetic material across bacteria. HGT happens by a number of processes, such as transduction (transfer mediated by bacteriophage), transformation (uptake of free DNA), and conjugation (transfer of plasmids and other mobile genetic components)(Brown et al. 2024). Because plasmids frequently carry both metal resistance genes (MRGs) and antibiotic resistance genes (ARGs), enabling co-selection and the simultaneous transfer of multiple resistances, they are especially significant in WWTPs. Metagenomic investigations have revealed that influent, activated sludge, and digested sludge samples from wastewater treatment plants contain high concentrations of plasmid-encoded ARGs and MRGs, indicating that these environments serve as reservoirs and hotspots for the propagation of resistance genes. Due to high microbial densities and tight cell-to-cell contact, which promote genetic exchange, the biofilm-rich environments found in WWTPs further increase HGT rates(Li et al. 2015).

Multidrug-resistant bacteria have the potential to spread into receiving water bodies and, eventually, the wider environment due to the fact that both native environmental bacteria and possible pathogens can develop resistance through horizontal gene transfer (HGT) in wastewater environments. Notably, high-throughput research shows that HGT frequently has a greater impact on the distribution of ARGs among WWTP microbial communities than vertical transmission, or cell division(Gómez Calderón et al. 2013). The extensive exchange of resistance genes in wastewater systems emphasizes how crucial it is to optimize treatment procedures in order to reduce genetic exchange opportunities and selective pressures, ultimately reducing the risks to public health posed by environmental reservoirs of resistance(Fang et al. 2024).

Table 2: Mechanisms of Co-Resistance, Cross-Resistance, and Co-Regulation in Bacterial Resistance

| Model	Genetic Linkage Needed?	Example Mechanisms	Basis	Implication in Resistance Spread
Co-resistance	Yes	ARGs and MRGs together on plasmids	Physical linkage of ARGs and MRGs on MGEs	Simultaneous selection and HGT of multiple resistances
Cross-resistance	No	Broad-specificity efflux pumps	Single mechanism protects against both classes	One gene/system causes resistance to diverse agents
Co-regulation	No	Metal-induced transcription activating ARGs	Shared or connected regulatory responses	Exposure to one agent induces resistance to another
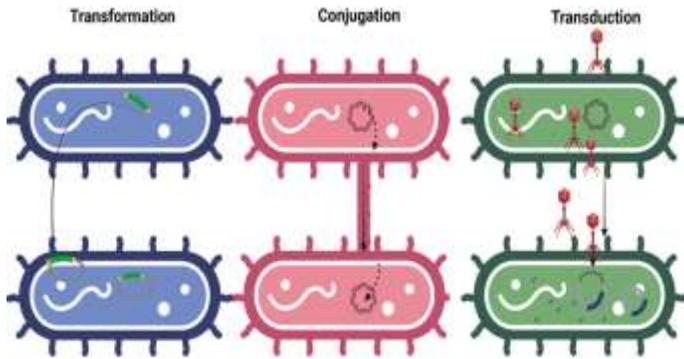
Figure 5: Mechanisms of horizontal gene transfer. Mechanisms of conjugation and horizontal gene transfer DNA transfer from a donor cell to a recipient via a plasmid. (Created by Biorender)

Environmental and Health Implications

In wastewater treatment facilities (WWTPs), resistant bacteria frequently survive because antibiotic-resistant bacteria (ARB) and antibiotic-resistant genes (ARGs) are not entirely eliminated by traditional treatment methods. UV, ozonation, chlorination, activated sludge, biofilm processes, and other physical and chemical treatments reduce but do not totally eliminate resistant bacteria or ARGs, leaving some resistant organisms to persist in the effluent (Uluseker et al. 2021).

Antibiotic resistance can be transmitted into rivers, lakes, and groundwater when resistant bacteria and resistance genes are discharged into natural waters through WWTP effluents. In the aquatic environment, this discharge is a major point source of antimicrobial resistance (AMR). According to studies, multi-drug resistant bacteria, such as resistant E. coli and other clinically significant pathogens, are still present in treated wastewater and contribute to environmental reservoirs of resistance(Nappier et al. 2020). There are several ways for humans and animals to be exposed to these resistant organisms: drinking tainted water, irrigating crops with treated wastewater, and using impacted surface waters for recreational purposes(La Rosa et al. 2025). Even in the absence of direct antibiotic exposure, co-contaminants like antibiotics and other chemicals in wastewater produce selective pressure that sustains and increases resistance. In areas downstream of WWTPs, this selection pressure enables resistant microorganisms to endure and proliferate(Mutuku et al. 2022). Overall, WWTPs lessen but do not eradicate genes and bacteria that are resistant. When treated wastewater is discharged into natural waters, resistant organisms spread and can expose people and animals through food and water chains. Additionally, co-contaminants maintain the selective pressure that favors resistant populations(Fouz et al. 2020).

Mitigation Strategies

Regulatory frameworks, bioremediation, improved treatment technologies, and source control are all integrated into a comprehensive strategy to mitigate antibiotic resistance in wastewater. The goal of source control is to minimize the selective pressure for the development of resistance by reducing the release of heavy metals and antibiotics. Advanced oxidation processes (AOPs) like ozonation and photocatalysis break down antibiotics and resistance genes, but they may also yield unidentified byproducts that need more research. Membrane filtration is one example of an advanced treatment technology that physically eliminates bacteria and resistance genes. While bioremediation uses microorganisms that may break down antibiotics and immobilize metals, hence reducing resistance genes under controlled conditions, constructed wetlands function as sustainable natural systems that use plant-microbe interactions to remove contaminants. To stop resistance from spreading throughout the ecosystem, policies and regulations regulate discharge restrictions and encourage sustainable practices. Although there are still few large-scale and long-term studies, recent reviews point out that combinations of these strategies, including innovative applications like nanomaterials, biochar amendment, and coagulation processes, show promising removal efficiencies of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs). Overall, to address the complexity of resistant pollution in wastewater and safeguard public health, effective mitigation necessitates combining physical removal, biodegradation, and oxidation treatments backed by strict regulatory oversight.

Future Directions

Integrated surveillance that combines environmental, clinical, and agricultural data, thorough risk assessment frameworks to comprehend the effects of co-resistance, and innovative biotechnological treatments like gene-editing tools and engineered microbes are key components of future efforts to combat antibiotic resistance. By improving the identification, assessment, and selective elimination of resistance genes, these strategies hope to safeguard public health and lessen the spread of antibiotic resistance in the environment.

CONCLUSION

Antibiotics and heavy metals from various sources exacerbate antimicrobial resistance (AMR) in wastewater, which poses a major risk to the environment and public health worldwide. Wastewater treatment facilities are hotspots for the development and spread of resistance through co-resistance, cross-resistance, and co-regulation, particularly in areas such as Saudi Arabia. Although resistant genes and microorganisms are frequently impossible to eradicate with conventional treatments, they continue to spread throughout the ecosystem, which is made worse by behaviors like water reuse and big public

gatherings. Advanced therapies, improved oversight and regulation, prudent chemical use, and a One Health strategy that integrates the environmental, public health, and agriculture sectors are all necessary to meet this problem. To stop the spread of resistance and protect ecosystems and public health, combined action and ongoing research are crucial.

CONFLICT OF INTEREST

We declare that there is no conflict of interest.

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AUTHOR CONTRIBUTIONS

A.M.Q contributed to the conceptualization, literature review, and drafting of the manuscript. S.M.A supervised the project, the conceptualization, the literature review, the manuscript structure, drafting of the manuscript and performed critical revisions. Z.F.S.A assisted in data collection, synthesis of literature, and writing. Bassam Al Johny contributed to the manuscript editing. R.A participated in literature screening and manuscript editing. All authors read and approved the final version of the manuscript.

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