

Environmental Antibiotic Resistance in Resource-Limited Settings: Sources, Co-Selection Mechanisms, and Sustainable Mitigation Strategies; A Review

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A B S T R A C T

Antibiotic resistance has become an important public health issue globally, with environmental factors considered crucial for the transmission and distribution of ARGs. Specifically, resistant bacteria, mobile genetic elements, and co-selected pollutants tend to concentrate in wastewater, thus contributing to the maintenance and spread of antibiotic resistance. This review aims to compile the most recent findings on the occurrence and dissemination of ARGs, their potential co-selection, and their detection in wastewater globally and specifically in Pakistan. This review highlights the effect of non-antibiotic pollutants on the maintenance of ARGs, the horizontal transmission of genes, and the difference between DNA- and culture-based assessments of the resistome. Innovative mitigation approaches, in addition to conventional ones, will be considered. The present study shows that conventional methods of decontamination have the capability of lowering ARG levels to a certain degree, but there is no uniformity in their performance, which is sometimes ineffective in practice. In contrast, nature-based or combined methods of treatment appear to be quite successful in destroying these types of genes in reality, particularly in developing countries like Pakistan. Quantitative modeling of ARG elimination, reliable monitoring systems, and long-term field-scale validation of mitigating devices are still severely constrained. This review highlights the need for comprehensive, One-Health-oriented surveillance and the creation of predictive and economical treatment options to manage antibiotic resistance in wastewater systems successfully.

Keywords: Antibiotic resistance genes, wastewater, horizontal gene transfer, resistome, co-selection, nature-based treatment.

Introduction

World Health Organization (WHO) forecasts indicate that if current trend continues, improperly used antimicrobial drugs may be responsible for up to 10 million deaths per year globally by 2050, thus making antimicrobial resistance (AMR) one of the major global health concerns.¹ Other than clinical settings, the environment serves as a hidden reservoir of pre-existing Antimicrobial resistant genes (ARGs). These are abundant in the freshwater, marine, and soil environments, where there is a continuous production and transfer of resistance determinants among the resident microorganisms. In addition to acting as mixing sites for resistance genes from various sources, wastewater treatment systems provide an ideal medium for horizontal gene transfer. Conjugation, transformation, and transduction promote antibiotic resistant gene (ARG) persistence and spread.^{2,3} In Pakistan, extensive use of

antibiotics combined with poor sanitation results in highly diverse regional statistics on resistance due to a limited focus on community and environmental reservoirs of bacteria. The current research on the prevalence of antibiotic-resistant bacteria (ARB) in Pakistan mainly employs susceptibility phenotypic tests in approximately 70% of cases and rarely uses molecular techniques for ARG detection.⁴ There is also a lack of comprehensive One Health research addressing all environmental, animal, and human reservoirs.⁵ As a result, existing data provide limited insights into possible mechanisms of resistance dissemination and co-selection of genes by other chemicals apart from antibiotics or effective prevention measures.

Thus, the objective of the proposed research is to gather and analyze data on the origins and distribution patterns, transmission routes, and co-selection processes of ARGs in wastewater treatment systems in Pakistan and other countries⁶

Identification of research gaps and assessment of innovative treatments will help to direct further studies on the problem towards surveillance, quantitative evaluation of treatment effectiveness, and development of natural and inexpensive mitigation approaches. This review proposes an integrated environmental-AMR framework linking pollutant-driven selective pressure with horizontal gene transfer (HGT) and ARG persistence, with a regional focus on Pakistan.

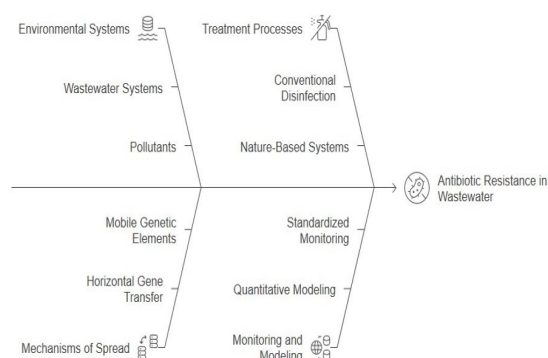


Figure 1. Conceptual framework of antibiotic resistance in wastewater systems.

Sources of Antibiotic Resistance Genes

ARGs come from a variety of environmental reservoirs that cross ecological, animal, and human niches. ARGs are prevalent in natural environments, including soil and marine waters; soil serves as a long-term sink for metals and antibiotics that co-select resistance, and marine *E. coli* often carry heavy-metal resistance genes coupled to ARGs on plasmids.^{7,8} Runoff, sewage, and animals provide ARGs to freshwater systems, such as rivers, lakes, and sediment. Research on lake sediments has shown that ARGs are highly prevalent and have unique resistome profiles.⁹ Donor species and mobile genetic elements (MGEs) are known to co-occur in wastewater treatment facilities (WWTPs), which facilitates the initial mobilization and amplification of ARGs that are then released into downstream rivers and biofilms.^{10,11}

Applying biosolids to agricultural fields creates a feedback loop between human waste and soil ecosystems by spreading conjugative plasmids carrying ARGs and integrons that are clinically relevant.¹² Manure from livestock is capable of acting as a sink for heavy metals as well as antibiotics, leading to co-selection of ARGs and MGEs. In this regard, heavy metals including zinc, copper, and cadmium have been repeatedly shown to play a role in this phenomenon.¹³ Clinically important ARGs such as *bla*-NDM and different *mcr* mutants are delivered to urban water matrices through hospital and municipal effluent streams, where they can be transferred to environmental

microorganisms by conjugation, transformation, or transduction.¹⁴ The interactions among soil, freshwater bodies, and marine ecosystems, wastewater treatment plants (WWTPs), biosolids, agricultural runoff, and clinical effluents make it possible to maintain a globally interactive resistome. However, existing models are inadequate in their ability to determine source abundance, HGT rate, and the microbial community structure. Carbapenem-resistant UPEC (ST405/ST167) harboring *bla*CTX-M-15 and *bla*NDM-5 were found in a hospital-based study conducted in Faisalabad and Lahore, Pakistan.¹⁵

ARG dynamics

A conceptual model of pollutant-mediated ARG dynamics involves the interaction among three components: pollutant load, rate of HGT and ARGs maintenance through stress-mediated physiological/Genetic mechanisms.

1. Pollutant load (Heavy metals, polycyclic aromatic hydrocarbons, Disinfectants, Micro and Nano-plastics) exerts sub-inhibitory stress on bacteria causing Reactive oxygen species generation, cell membrane damage, and the activation of SOS/ Oxidative stress regulons. This leads to induction of conjugation pili assembly, Type IV secretion system, and transfer genes increasing HGT (Conjugation, Transformation, transduction).¹⁶
2. The rise in the rate of HGT helps in the transfer of ARGs from donor cells to recipient cells; especially where both genes reside on a mobile genetic element. The association of the two leads to a loop in which the pollutant selects the MGE which in turn maintains the ARG even in the absence of antibiotics.¹⁷
3. The persistence of the ARG is achieved by the dual role of (i) continued influx of ARG copies due to increased HGT; and (ii) the selective maintenance of ARG carrying MGEs due to exposure to pollutants.¹⁸

According to the model, a dose-response curve should be generated such that a low to medium level of pollutants results in maximum HGT (hormesis), while high levels lead to the loss of viability in cells. While experimental data are consistent with these relationships, quantitative descriptions incorporating the concentration of pollutants, kinetics of HGT, and retention/degradation of ARGs are rare.

Antibiotic Resistance Gene Spread

Horizontal gene transfer (HGT), a technique that permits the transmission of genetic material across bacteria irrespective of reproduction, is the main way that antibiotic resistance genes (ARGs) spread.¹⁹ In both environmental and clinical contexts, numerous investigations consistently identify three main HGT

pathways: conjugation by direct cell-to-cell plasmid transfer, transformation through the uptake of extracellular DNA, and transduction mediated by bacteriophages.²⁰

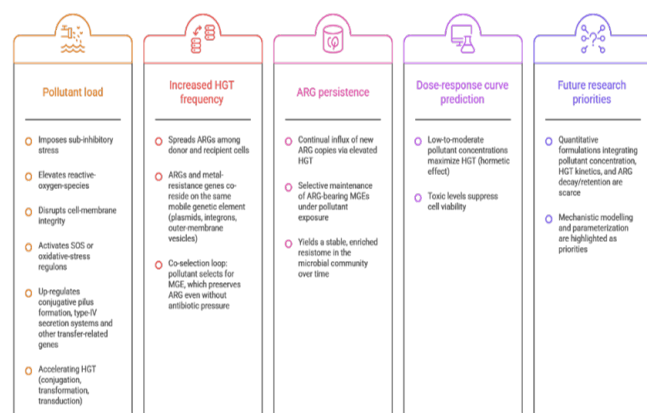


Figure 2. Conceptual model of pollutant mediated antibiotic resistant genes dynamics.

Conjugation is generally thought to be the primary pathway for ARG transmission among these mechanisms, but transformation and transduction are becoming more acknowledged as important in particular ecological contexts.²¹ Additionally, a variety of mobile genetic elements (MGEs), such as integrons and insertion sequences, as well as alternate transfer pathways like outer-membrane vesicles, increase the genetic exchange repertoire and hasten the spread of ARGs across taxonomic boundaries.²² Particularly, wastewater habitats have been identified as hotspots where mobilizing materials and origin species coexist, promoting the initial mobilization of ARGs, while downstream WWTP effluents increase the abundance of ARGs in rivers, sediments, and biofilms.^{11, 23}

A crucial gap for predictive risk models is the absence of quantitative estimates of the relative contributions of each mechanism across many habitats, despite the fact that these works collectively describe the pathways and contexts of ARG dissemination. Horizontal gene transfer in anthropogenically contaminated environments is the main cause of the spread of antibiotic resistance genes (ARGs) in Pakistan. Mobile genetic elements facilitate the exchange of ARGs, especially sulfonamide resistance genes, in hotspots like the River Ravi, wastewater, and hospital settings.²⁴

Co-selection of ARGs by Environmental Pollutants

Antibiotic-resistance genes (ARGs) are frequently co-selected by non-antibiotic contaminants, a process that has been observed in a variety of environments.^{25,26} Metal-resistance genes (MRGs) and ARGs co-occur on the same mobile genetic

elements in approximately 17% of bacterial genomes, according to large-scale genomic surveys. Plasmids carrying both traits are significantly more likely to be conjugative and to encode toxin-antitoxin systems, especially in isolates associated with humans and animals (5–7% of plasmids) compared to environmental plasmids (<0.7%).²⁷

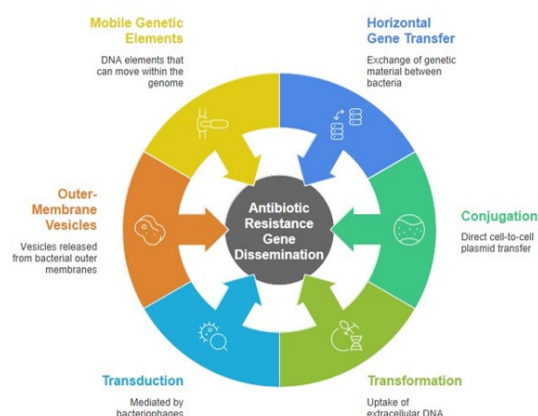


Figure 3. Antibiotic resistance genes dissemination in the environment.

Exposure to nickel or copper increased total ARG and MRG abundances by approximately 2.5 times, according to environmental studies of wastewater and activated-sludge reactors. Low metal concentrations produced a stronger co-occurrence effect than high concentrations, showing dose-dependent selection pressure.^{27,28} Metal residues like Cd, Zn, Cu, and as explained more of the ARG variance in agricultural contexts (5.7%) than measured antibiotic residues (0.7%), while integrons like *intI-1* often connected metal and antibiotic resistance circuits.²⁹ Resistance to heavy metals was widespread among *Escherichia coli* marine isolates, and 40% of antibiotic-resistant genomes harbored HMRGs and ARGs together on plasmids, thus showing that heavy metal pollution, no matter how low it is, contributes to genetic association in natural waters. Nonetheless, the degree of co-selection depends on the nature of the pollutant and environment: for example, copper strongly hindered plasmid transfer from an *IncF* plasmid, decreasing its efficiency twice. At the same time, biocides containing chlorine favored the presence of *chtR* genes that promoted the persistence of ARGs on plasmids in sewer biofilm ecosystems.^{30,31} Despite these convergent findings, there are yet no quantitative models that combine pollutant concentrations, HGT rates, and community composition to forecast the proportionate contribution of each co-selection pathway. This is a crucial gap for risk assessment and mitigation techniques.^{25,26} Intensive uses of glyphosate and organophosphate pesticides in wheat-growing districts

were Pakistan (Faisalabad and Sargodha) was linked to increased *sul1*, *sul2*, and *tetA* genes in irrigation canal biofilms. Metagenomics evidence revealed co-occurrence of ARGs and pesticide-degrading genes (*phn*, *opd*) on the same plasmids, indicating co-resistance caused by herbicide exposure.³²

Co-selective agent	Source	Mechanism	Impact on ARGs	References
Heavy metals (Cu, Zn)	Industry, sludge	Co-resistance	ARG enrichment	(¹⁷)
Biocides	Hospitals, households	Cross-resistance	Increased MDR	(³³)
Micro plastics	Domestic waste	Biofilm formation	ARG persistence	(³⁴)
Sub-lethal antibiotics	WWTPs	Selective pressure	HGT stimulation	(¹⁷)

Culture-Based versus Resistome Approaches

Both DNA-based (qPCR, metagenomics) and culturable-isolate (culture-based) methods predominate in the investigation of antibiotic-resistant genes, but they have different advantages and disadvantages. Culturomics supplies isolates phenotypic testing and broadens the list of cultivable microorganisms, but it is constrained by the challenges of cultivating numerous environmental bacteria and by prior selection of media, which results in a bias toward fast-growing, well-known species.^{33,34} DNA-based techniques completely avoid cultivation: shotgun metagenomics provides a thorough, culture-independent overview of ARGs, mobile genetic elements, and taxonomic affiliations throughout the entire population, while quantitative PCR provides excellent sensitivity for specific ARGs with low DNA input.^{35,36} Comparative evidence suggests that while metagenomics can identify novel ARGs and their ecological context, it might overlook functional phenotype information and require large amounts of DNA. In contrast, culture-dependent studies frequently underestimate resistome diversity because uncultivable organisms remain hidden.^{33,35}

Thus, the best approach for a comprehensive resistome profile has been suggested to be a hybrid workflow that combines metagenomics to capture the larger, uncultured resistome with culturomics to obtain functional isolates.^{34,35} However, there is still a fundamental void for future risk-assessment frameworks in the form of defined protocols and quantitative models that directly evaluate detection capabilities, bias rates, and cost-effectiveness of these approaches. A ten-year retrospective investigation of *E. coli* from a tertiary hospital in Lahore

(Pakistan) revealed nearly complete resistance to ampicillin (98%), high cephalosporin resistance ($\geq 82\%$), and an increasing trend of imipenem resistance, all of which were caused by routine disc diffusion AST.³⁷ With reference to Pakistan a resistome containing *bla*NDM-5, *bla*OXA-181, *bla*CTX-M-15, and colistin-resistant mutations was found in the whole-genome sequencing of 32 wastewater isolates, including *Escherichia coli* and *Klebsiella pneumoniae*. These isolates showed high resistance to cefotaxime (91%), ciprofloxacin (75%), and imipenem (62.5%).³⁸

Aspect	Culture-based methods	DNA-based methods
Detectable organisms	Only culturable	Culturable + unculturable
Data type	Phenotypic resistance	Genotypic resistance
Sensitivity	Low-moderate	High
Cost	Low	Moderate high
Skill requirement	Basic microbiology	Molecular + bioinformatics
Use in Pakistan	Widely used	Rare
Major limitation	Misses resistome	DNA $\neq$ viability
References	41	42

Treatment and Mitigation of Antibiotic Resistance

Conventional treatment technologies:

While resistant bacteria can be eliminated using traditional disinfection techniques such as ozonation, ultraviolet light exposure, and chlorination, the effectiveness of the procedures in removing ARGs differs. Although suboptimal levels may favor resistant organisms and encourage horizontal gene transfer, free-chlorine therapy ($\approx 30 \text{ mg L}^{-1}$) produces up to 1.5-log ARG elimination.^{39,40} Modest ARG reductions (0.3–0.6 log) are produced by UV alone ($\approx 250 \text{ mJ cm}^{-2}$), and the percentage of resistant cells may even rise.^{39,41} On the other hand, ozonation routinely outperforms both UV and chlorination, with 4-5 log ARG loss at $1 \text{ mg L}^{-1} \text{ O}_3$ for 5 min.^{42,43} However, all the techniques demand that precise amounts of the substances be applied to ensure their effectiveness, but they often result in the presence of leftover ARGs.

Nature-based and low-cost treatment approaches:

These voids are filled by inexpensive, nature-based solutions. By utilizing extended hydraulic retention, plant-microbe interactions, and sorption on biochar surfaces, constructed

wetlands (CWs) with biochar filter medium reduce ARB by >99% and reduce ARG loads by 0.8–2.3 log.^{44,45} In Pakistan, hybrid subsurface-flow CWs achieved >98% ARB reduction and 60–90% eradication of physicochemical pollutants with optional chlorination (30 mg L⁻¹) for ultimate polishing.⁴⁶ Microalgae-based systems improve water quality and lower ARGs while offering a sustainable alternative to urban streams.⁴⁷ CWs are especially attractive in Pakistan because amended biochar may be produced locally from agricultural residues, reducing capital and operating expenses.⁴⁴

Pakistan-Specific Gaps in Antibiotic Resistance Research

The current state of research on antibiotic resistance in Pakistan indicates several deficiencies existing simultaneously and are interconnected. For example, provinces such as Balochistan do not get enough attention from researchers since the majority of studies available in literature focus on major urban areas of the country.⁴⁸ AMR surveillance in Pakistan is hindered by challenges such as lack of coordination at the national level, unclear laboratory networks, and weak AST capability. Mentorship enhanced laboratory network capabilities for AST but revealed ongoing challenges with data collection and LIS utilization.⁴⁹ Environmental and animal aspects of One-Health are often neglected despite being recognized as sources of pathogens.⁵

Treatment approach	ARG reduction	Cost	Suitability for Pakistan	References
Chlorination	Low–moderate	Low	Widely used	(⁵⁷)(⁵⁸)
UV treatment	Low	Moderate	Limited	(⁵⁹)
Constructed wetlands	Moderate–high	Low	Highly suitable	(⁶⁰) (⁶¹)
Biochar amendment	High (adsorption)	Low	Locally feasible	(⁶²)
Algal polishing	Moderate	Low	Climate-friendly	(⁶³)

In the current research, there is a tendency to prioritize therapeutic results over primary prevention interventions, while the availability of funds and infrastructure is one more obstacle preventing the implementation of multicenter and longitudinal projects.⁵⁰ Healthcare providers' lack of knowledge, weak infection prevention and control measures, and insufficient assessment of antimicrobial stewardship programs affect the implementation of the National Action Plan (NAP).⁵¹

For these reasons, policy-oriented research approaches continue to be limited because of such limitations as a lack of

breakpoints in disk diffusion tests and a lack of multisectoral research.⁵²

Conclusion

Resistance to antibiotics in the environment is one of the greatest public health challenges facing us today, and wastewater and associated reservoirs have emerged as key sites for the survival and dissemination of antibiotic resistance genes (ARGs). This review examines the topic from a One-Health point of view, covering the origin, transport, co-selection, and control of ARGs, with a specific focus on Pakistan. One of its major contributions is the synthesis of scattered information from various disciplines, together with an evaluation of culture-based and molecular approaches to ARG detection.

While there is an increase in knowledge, major challenges still exist in regard to prediction modeling, surveillance standardization, and scaling up of treatment methods. It is recommended that future endeavors focus on the development of a comprehensive and cost-effective monitoring system, as well as a natural solution intervention strategy. In general, this review is geared towards achieving an interconnected analysis of ARG ecology, which can lead to the successful management of AMR.

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