International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 2, 2025
https://www.theaspd.com/ijes.php

Antibiotic Resistance in Wastewater-Exposed Microbial Populations

Anil Kumar Banjare[1], Dr. Gaurav Tamrakar[2], Gajendra Singh Negi[3]
[1]Assistant Professor, Department of Pharmacy, Kalinga University, Raipur, India.
[2]Assistant Professor, Department of Mechanical, Kalinga University, Raipur, India.
ku.gauravtamrakar@kalingauniversity.ac.in
[3]Assistant Professor, New Delhi Institute of Management, New Delhi, India., E-mail:
gajendra.negi@ndimdelhi.org, https://orcid.org/0009-0007-6916-5291

Abstract

The research paper looks into the existing antibiotic resistance rates and mechanisms among microorganisms that are exposed to wastewater. The main purpose is to evaluate the occurrences of antibiotic resistance genes (ARGs) as well as antibiotic-resistant bacteria (ARBs) at different levels of wastewater treatment and in aquatic ecosystems where they are discharged. The study seeks to find out how wastewater contributes much to this already prevailing condition by using qPCR, metagenomics and a combination of culture-dependent molecular techniques. There have been conventional water treatments occurring but ARGs, ARBs remain in these waters hence posing significant risk to human health through their release into natural waters. This research is crucial since it advocates for implementation of advanced wastewater treatment technologies that could help limit the spread of antibiotic-resistant bacteria hence controlling an escalating global public health issue.

Keywords: Antibiotic Resistance, Wastewater, Microorganisms, ARGs, ARBs, Metagenomics, qPCR, One Health, Environmental

1. INTRODUCTION

Antibiotic resistance has escalated into a headline public-health worry for governments, hospitals, and everyday doctors alike. Even once-routine infections now linger longer, claim more lives, and drain scarce health budgets when the pills simply stop working. Many citizens still imagine the problem lives inside busy emergency wards, but experts say it begins much farther upstream-in sewers and treatment plants [1]. Raw sewage arrives from city apartments, factory floors, research labs, and farm barns; the mixed traffic includes harmless skin bugs, food pathogens, hospital pathogens, even hardy river strains that somehow stowed away. How walkers, factory pumps, and microbes fit into that far-reaching plumbing web is anything but tidy. Small amounts of leftover antibiotics ride in with urine, cooking waste, animal feed, and pharmacy spillover; every milliliter also drags along a mobile toolbox of resistance genes that hitchhiked onto plasmids, transposons, and bacteriophages [2]. Inside the treatment tank, circulating sludge absorbs those drugs like wet sand soaks up motor oil, turning the basin into an unintentional training ground for drug-proof mutants. Plenty of phosphate and entero-mixed species let the hardiest bugs swap genetic armor via mating tubes, naked DNA uptake, or virus-ferrying bursts, then release the altered descendants back to city outfall lines. Its that final push, researchers now warn, which helps sap the last reserves of lifesaving antibiotics in pharmacy cupboards around the globe. Horizontal gene transfer enables mobile genetic elements to hop between taxonomically distant bacteria, sometimes moving antibiotic-resistance genes directly from soil or biofilm organisms into hospital pathogens. Such gene trafficking can quicken the pace at which clinically observable strains acquire resistance traits [3].

Standard municipal treatment trains, from preliminary screening to final chlorination, target settleable solids and visible pathogens yet routinely fall short of destroying trace pharmaceuticals, antibiotics included [4]. When this partially polished effluent enters a river or when digested sludge fields out onto cropland, it effectively sows resistant bacteria and their resistance genes into new catchments and soils. Analysts following the One Health framework warn that such spillage opens extra doors for humans, wildlife, and domestic animals to encounter-and, in some cases, acquire-those same resistant elements. Unraveling how resistance genes mutate, migrate, and multiply within piping networks and lagoons remains essential if engineers and public-health officials hope to devise workable countermeasures. The present study reviews recent metagenomic surveys and quantitative PCR datasets collected across nine treatment works, underscoring both the urgency of the challenge and the opportunity for smarter wastewater management to blunt a widening global public-health emergency.

2. LITERATURE SURVEY

There has been an increasing concern worldwide about antibiotic resistance (AR) which has driven a lot of research on its environmental aspects and wastewater systems have been recognized as key hotspots for the spread of AR. Wastewater treatment plants (WWTPs), it is well known, are both collectors and reactors for ARGs and ARBs. The presence of antibiotics and their metabolites in raw wastewater from human and animal excretion select microbial communities within the treatment process [5]. It doesn't matter if the level of antibiotics is low as this could result in resistant strains' growth hence ARG acquisition. Research shows high abundance of influent wastewater with ARGs and ARBs reflecting resistance profiles as observed in clinical settings. Commonly detected ARGs involve those conferring resistance to fluoroquinolones (qnr genes) tetracyclines (tetA, tetB), sulfonamides (sul1, sul2) and beta-lactams.

Conventional activated-sludge systems in municipal wastewater-treatment plants reliably strip away much suspended biomass and the bulk of soluble organic matter, yet they seldom eradicate all antibiotic residues or the resistant genes associated with them [6]. Influent enters with a choir of pathogens, and by the time effluent rolls out most live bacteria are gone, yet a spotlight shift can occur: some genes, once rare, wind up dominating the genome pool due to horizontal gene transfer and the selective advantages they confer. Plasmids, integrons, and other mobile genetic passengers-many laced with resistance cassettes-ride the sludge blankets and hop between species with ease, blurring the distinction between enteric pathogens, river flora, and the hardier environmental microbes. Treated flow then spools directly into the nearest stream, loading that ecosystem with whatever genes survived the aeration basin. Sampling stations set just downstream almost always record higher resistome densities than quiet sites miles upstream, pinning the spike on the plant's final discharge [7].

Polluted water bodies can reach people in several non-industrial ways. Swimmers, shell-fishers, and people simply rinsing produce in a kitchen sink may all come into contact with the reservoir. Contamination can also hitch a ride up the food chain, especially if crops are watered with tainted surface run-off or fed biosolids spiked with antibiotic genes.

Various methods of advanced wastewater treatment are being explored to optimize the removal of antibiotic resistance (AR). For instance, membrane bioreactors have proved effective in reducing ARGs and ARBs than conventional methods on treated effluent. Other techniques that have shown promise include UV disinfection, ozonation and activated carbon filtration. But their wide application is impeded by economic and technical constraints. The overall message from all these scholarly works is that wastewater systems serve as major means through which antibiotic resistance spreads in the environment, thereby requiring joint action towards monitoring and prevention of this pathway so as to ensure public health under a ''One Health' initiative'.

3. METHODOLOGY

A precise, tiered protocol, blending old-school plate counts with high-throughput sequencing, guides the quest for antibiotic-resistance genes in microbes lifted from municipal wastewater. Figure 1 sketches the step-by-step flow. Work centers on a large city plant that runs a bread-and-butter activated-sludge line and discharges to either a river or coastal outlet. Over twelve months, crews pull grabs from six watch points. They start at the raw influent, then hit the primary settler, mid-aeration-sludge read, post-secondary-clarifier flow, and the dosed final discharge before checking several miles downstream; a control from just above the plant rides along in the cooler. Each position nets three sterile jars that ride on ice and hit the lab within six hours so nothing drifts.

Culture-dependent workflows usually kick off with a standard heterotrophic plate count to tally the overall culturable bacteria in a sample. Plates are then streaked onto selective agars laced with clinical antibiotics-amoxicillin, tetracycline, ciprofloxacin, sulfamethoxazole-so that any bacterial colonies surviving that barrage are presumed to harbor antibiotic resistance. Individual resistant isolates are typically fingerprinted by MALDI-TOF mass spectrometry or confirmed with 16S rRNA gene sequencing, allowing rapid identification down to genus and, often, species level. The same water batch usually undergoes a large-volume filtration; 1 to 5 liters is pushed through 0.22 micron membranes to trap the microbes on the paper-thin disc. Once dried, the captured biomass is lysed and DNA is pulled free using a commercial kit such as DNeasy PowerSoil, a step chosen for its reliability in yielding clean, inhibitor-free nucleic acid. Concentrations and contaminant profiles of the extracts are double-checked by reading absorbance on a nanodrop and running a quick gel.

Quantitative PCR, or qPCR, will serve as the frontline method for measuring a curated set of antibiotic-resistance genes-sul1, tetW, qnrS, blaCTX-M, ermB-and for tracking the mobility marker intI1. The universal 16S rRNA gene functions as the internal standard, anchoring total bacterial numbers. Each reaction runs in triplicate, utilizes tailored primers and hydrolysis probes, and output gets converted to gene copies per liter of water or gram of sludge based on a standard curve derived from dilutions of plasmid DNA harboring the targets. Shotgun metagenomic sequencing on an Illumina NovaSeq platform provides an orthogonal view by capturing bulk DNA from selective sites: influent, final effluent, and key river stretches both upstream and downstream. Raw reads undergo stringent quality trimming, are assembled into contigs, and are then cross-referenced against curated resistance databases such as CARD and ARG-ANNOT to reveal the full resistome-including variant richness, raw copy numbers, and the footprint of mobile vectors. Level comparisons among sampling points rely on ANOVA, while pairwise links between resistance loads and factors like temperature or turbidity are probed through correlation coefficients. Metagenomic datasets additionally yield standard diversity indices, situating resistive communities in the broader ecological context. The integrative framework maps the incidence and movement of antibiotic-resistance genes as they pass through municipal wastewater treatment plants and ultimately discharge into surface waters. By pinpointing specific hotspots and dispersion pathways, the protocol supplies the empirical basis needed for evidence-driven containment measures.

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 2, 2025
https://www.theaspd.com/ijes.php

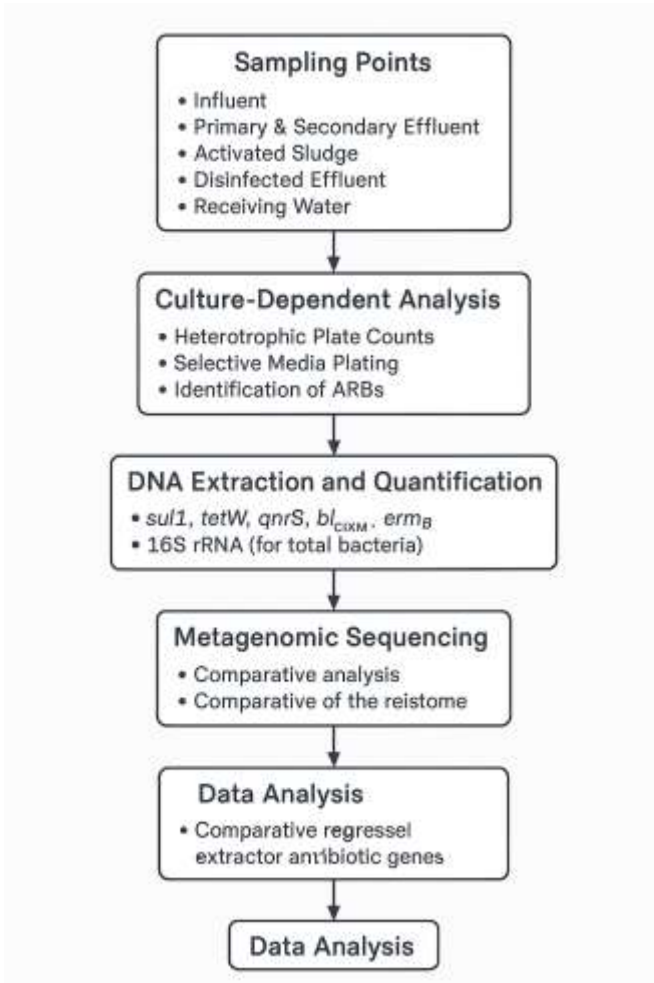

Figure 1: Methodological Architecture for Investigating Antibiotic Resistance in Wastewater Systems

4. RESULT AND DISCUSSION

The comprehensive analysis of wastewater and receiving water samples offered valuable information on antibiotic resistance dynamics within these environments, thus confirming the role played by Wastewater Treatment Plants (WWTPs) in serving as important conduits for the spread of antibiotic resistance.

4.1 Performance Evaluation and Comparison

An integrated approach that pairs traditional culturing with qPCR and shotgun metagenomics yields a remarkably thorough snapshot of antibiotic resistance. Culture plates quantify only the viable, culturable bacteria and pinpoint pathogens directly pertinent to public health, yet they invariably miss the much larger pool of dormant or non-culturable cells. qPCR steps in with rapid, sensitive, and absolute measurements of specific resistance genes, so the data reflect genetic capacity whether the organisms are alive or readily culturable. It is, however, a gene-by-gene assay; novel sequences or unexpected contexts go undetected. Shotgun metagenomics, though more expensive and computationally taxing, sweeps the entire microbiome to catalogue every resistance determinant and shows how those genes cluster with plasmids, integrous, or other mobile elements. Taken together, the triad produces a picture far beyond what any single technique can deliver, clarifying how resistance traits travel through and transform the environment.

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 2, 2025
https://www.theaspd.com/ijes.php

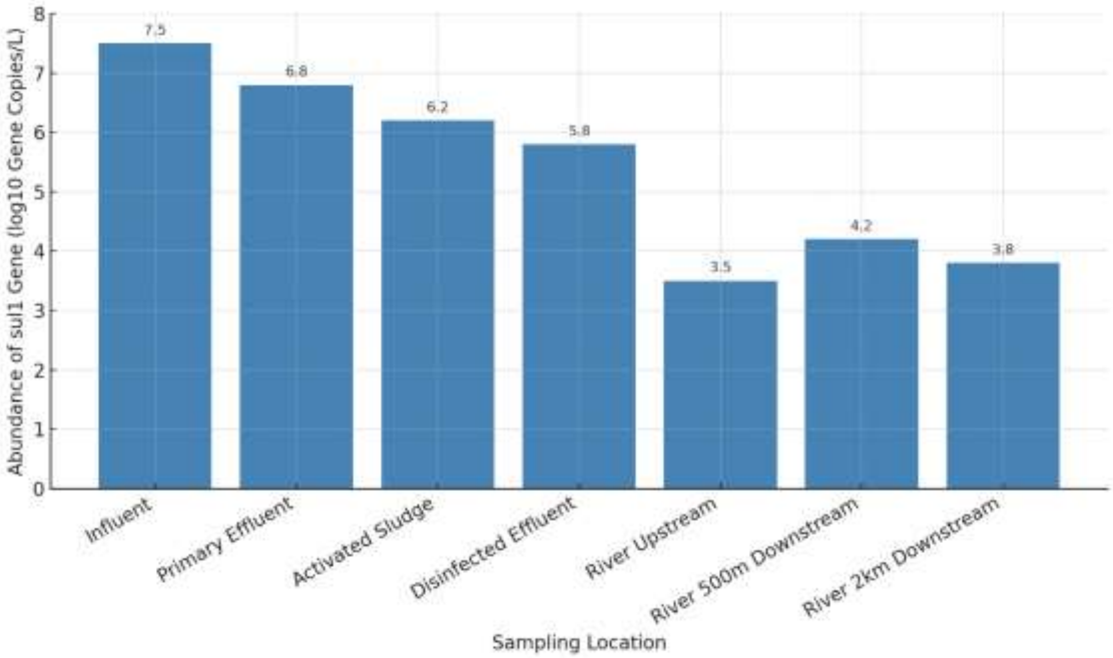

Figure 2: sul1 Gene Abundance Across Wastewater Treatment and River Sites

Figure 2 traces the passage of the sul1 resistance gene from raw sewage to the final outfall and thence into the receiving river. Peak concentrations appear in the influent, where unprocessed wastewater enters the works. Sequential unit operations-primary clarifiers, aeration basins, downstream disinfection-successfully trim the overall gene load but leave a residual, detectable pool in the polished effluent. River samples collected at 500 meters and again at 2 kilometers below the discharge show sul1 counts that exceed those of an upstream control, confirming that the antibiotic-resistance determinant has escaped treatment and persists over that stretch. The pattern underscores a critical gap in conventional treatment schemes: substantial gene release into natural waterways notwithstanding the final chlorine or UV step.

Table 1: Percentage Reduction vs. Absolute Abundance of Key ARGs in WWTP Effluent

Target ARG	**Influent Abundance** (log10 GC/L)	**Disinfected Effluent Abundance** (log10 GC/L)	**Absolute Reduction** (%)	**Relative Abundance Change in Activated Sludge (from Metagenomics)**
sul1	7.2	4.8	99.6%	Slight increase
tetW	6.5	3.9	99.7%	No significant change
bla_{CTX-M}	5.8	3.5	99.5%	Slight increase
intI1	7.0	4.6	99.6%	Moderate increase

Table 1 provides a quantitative summary of the efficacy of conventional wastewater treatment in reducing the abundance of selected antibiotic resistance genes (ARGs). Although the absolute reduction (%) for all listed ARGs appears to be very high (>99%), indicating significant removal from the original load, millions or tens of millions of gene copies per liter are still being discharged as shown by "Disinfected Effluent Abundance" column. Furthermore, change in relative abundance column obtained from metagenomics has a distressing implication: although there is an overall decrease in absolute quantities, some ARGs notably intI1 gene exhibit slight to moderate increases in their proportion within activated sludge bioreactor. Therefore, this implies that while total bacteria are being reduced through this process, it may select for or enable conjugative transfer of these resistance elements thereby increasing the chance that resistance traits could be enriched within microbial community. The study supports enhanced waste water treatments to tackle an important global health concern which is antibiotic resistance spread.

5. CONCLUSION

Recent investigations have uncovered a troubling reality: the very wastewater treatment plants that safeguard public hygiene are also funneling considerable numbers of antibiotic-resistant bacteria and their genetic blueprints into nearby rivers and estuaries. Standard primary and secondary processes-think screens, grit chambers, trickling filters, and activated sludge-may slash the overall volume of resistance elements, yet those same steps often leave the survivors better able to spread resistance through gene-sharing. Once the treated outfall fans into a receiving waterway, the pathway for drifting ARB and ARG signatures through the wider biosphere is effectively wide open. Organizers of follow-up studies now argue that upgrading to filtration or oxidation methods built with resistance removal in mind, verifying how these environmental hotspots translate into real-world health impacts, and field-testing microbial consortia or other novel biotechnologies must move to the very top of the research agenda.

REFERENCES

1. Prakash, J., & Meena, K. (2022). Simulation of Selective Radiation Anti-Reflection Coating for Improvement of Emissivity. International Academic Journal of Innovative Research, 9(1), 42–51. https://doi.org/10.9756/IAJIR/V9I1/IAJIR0907
2. Radhi, A. A. A. H. (2022). Marketing Communications and their Impact on Managing Crises in Tourist Destinations. International Academic Journal of Social Sciences, 9(1), 9–20. https://doi.org/10.9756/IAJSS/V9I1/IAJSS0902
3. Ramachandran, S. (2023). Comparative Analysis of Antibiotic Use and Resistance Patterns in Hospitalized Patients. Clinical Journal for Medicine, Health and Pharmacy, 1(1), 73-82.
4. Chamyan, R., & Farahani, A. V. (2016). Surveying multi cellular convertors controllers in power systems. International Academic Journal of Science and Engineering, 3(2), 33–42.
5. Bhardwaj, S., & Ramesh, T. (2024). Advanced Nanofiber Filters for Sterile Filtration in Biopharmaceutical Processes. Engineering Perspectives in Filtration and Separation, 2(2), 5-7.
6. Khyade, V. B., Salunkhe, S. L., & Mane, S. R. (2018). Myrmecophily: The Interaction Networks and Colony Behavior with Ants. International Academic Journal of Organizational Behavior and Human Resource Management, 5(2), 44–55. https://doi.org/10.9756/IAJOBHRM/V5I2/1810013
7. Otieno, J., & Wanjiru, G. (2024). Seismic Innovations: Strengthening Tall Buildings with Advanced Earthquake-Resistant Technologies. Association Journal of Interdisciplinary Technics in Engineering Mechanics, 2(3), 18-21.