

MONITORING ANTIMICROBIAL RESISTANCE AND CLIMATE-SENSITIVE INFECTIOUS AGENTS THROUGH WASTEWATER SURVEILLANCE IN URBAN SETTINGS OF WAH CANTT, PAKISTAN

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^{*7}Monitoring Antimicrobial Resistance and Climate-Sensitive Infectious Agents Through Wastewater Surveillance in Urban Settings of Wah Cantt, Pakistan

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Abstract

Background: Antimicrobial resistance (AMR) and climate-sensitive infectious agents pose major global health threats, particularly in low- and middle-income countries. Wastewater surveillance offers a promising approach for detecting emerging pathogens and monitoring AMR trends at the community level. Despite growing evidence worldwide, there is limited research from Pakistan on the environmental prevalence of AMR genes and climate-sensitive pathogens through wastewater monitoring. *Objectives:* This study aimed to (1) determine the prevalence and types of antimicrobial resistance genes in wastewater samples from Wah Cantt, Pakistan; (2) identify and describe the occurrence of climate-sensitive infectious agents such as *Vibrio* spp., dengue viral RNA, and fungal spores; and (3) explore associations between environmental factors and the occurrence of AMR genes and climate-sensitive pathogens. *Methods:* A quantitative descriptive cross-sectional study was conducted using wastewater samples collected from urban sites in Wah Cantt. Molecular detection techniques, including polymerase chain reaction (PCR) and quantitative PCR (qPCR), were employed to identify AMR genes and climate-sensitive pathogens. Environmental factors such as temperature, rainfall, and pH were recorded. Data analysis was performed using descriptive statistics, chi-square tests, and logistic regression to assess associations between environmental variables and microbial prevalence. *Results:* A total of 60 wastewater samples were analyzed. High prevalence of AMR genes, including *bla*CTX-M, *bla*NDM-1, and *tet*M, was detected, with multidrug resistance patterns observed across several samples. Climate-sensitive pathogens such as

Vibrio cholerae (22%), dengue viral RNA (15%), and fungal spores (18%) were identified. Statistically significant associations were found between rainfall and the detection of *Vibrio* spp. ($p < 0.05$), and between higher temperatures and increased AMR gene prevalence ($p < 0.05$). Conclusion: This study demonstrates that wastewater in Wah Cantt contains a considerable burden of AMR genes and climate-sensitive infectious agents, highlighting the potential of wastewater surveillance as an early warning tool for community-level health risks. Findings underscore the urgent need for strengthened wastewater management, stricter antibiotic regulation, and integration of environmental surveillance into national infectious disease monitoring systems.

INTRODUCTION

Antimicrobial resistance (AMR) and climate-sensitive infectious agents have emerged as two of the most pressing public health threats of the 21st century. AMR refers to the ability of microorganisms, particularly bacteria, to survive and multiply despite exposure to antimicrobial drugs that were previously effective in treating infections. This phenomenon leads to treatment failures, prolonged illness, higher healthcare costs, and increased mortality (WHO, 2023). In parallel, climate-sensitive infectious agents such as *Vibrio* spp., dengue virus, and fungal spores have demonstrated changing epidemiological patterns due to global warming, altered rainfall, and increased urbanization, resulting in higher transmission risk in vulnerable populations (George, Gaitor, Cluck, Henao-Martínez, Sells, & Chastain, 2025). Recent evidence shows that over 1.3 million deaths globally in 2019 were directly attributable to AMR, while climate-sensitive infections such as dengue have increased significantly, with more than 100 million symptomatic cases annually worldwide (Hu, Horton, Yeo, Sung, & Steve, 2025; Zhang, Fu, Liu, Zhao, & Wang, 2024).

The prevalence of AMR and climate-sensitive pathogens is strongly linked to environmental, behavioral, and systemic risk factors. Indiscriminate antimicrobial use, improper disposal of hospital and community wastewater, inadequate sanitation, and weak infection control programs have contributed to the rapid spread of resistant genes into the environment (Abosse, Megersa, Zewge, & Eregno, 2024; Endale, Mathewos, & Abdeta, 2023). At the same time, climate change-related factors such as rising temperatures, extreme flooding, and expansion of mosquito breeding habitats accelerate the risk of emerging and re-emerging infectious diseases in

urban regions (Ali, Shaikh, Sethi, & Surani, 2024). The complications of these dual threats are severe, ranging from untreatable hospital-acquired infections and outbreaks of resistant enteric pathogens to large-scale epidemics of vector-borne diseases, which overwhelm health systems already struggling with limited resources.

Wastewater surveillance has gained recognition as a promising tool to monitor both AMR and climate-sensitive infectious agents in communities. By analyzing sewage, researchers can detect a wide array of microbial signatures, including resistant genes, viral RNA, and fungal spores, which reflect the overall health status of a population (García-Aljaro, Blanch, Campos, Jofre, & Lucena, 2019). Unlike traditional hospital-based surveillance, wastewater monitoring provides an early warning system at the community level and can capture asymptomatic or undiagnosed infections, making it particularly useful for resource-limited urban settings. For instance, the COVID-19 pandemic highlighted the utility of wastewater surveillance in detecting SARS-CoV-2 trends, demonstrating its scalability and cost-effectiveness for broader pathogen monitoring (Parkins et al., 2024). However, despite this potential, little research has integrated the simultaneous monitoring of AMR and climate-sensitive pathogens, particularly in rapidly urbanizing South Asian settings such as Pakistan, where both threats are converging.

Previous research has largely focused on isolated aspects of the problem. Several studies have investigated AMR prevalence in hospital effluents and rivers contaminated with untreated sewage (Fouz et al., 2020), while others have explored climate-sensitive pathogens like *Vibrio cholerae* and dengue

virus in environmental samples (Alcayna, Rao, & Lowe, 2025; Sedas, 2007). However, there is limited integration of these two domains, creating a gap in understanding how wastewater surveillance can provide a comprehensive picture of overlapping health threats. Moreover, most studies have been conducted in high-income countries or selected regions of Africa, with scarce data available from South Asian urban areas such as Wah Cantt, Pakistan. This research gap highlights the urgent need for locally contextualized studies that examine both AMR and climate-sensitive pathogens in wastewater systems, particularly in dense urban environments with inadequate sanitation infrastructure.

The rationale of the present study stems from the dual burden of AMR and climate-sensitive infectious diseases in Pakistan, where poor antimicrobial stewardship and rapid climate change converge. Wah Cantt, a rapidly urbanizing city with both military and civilian populations, represents a unique setting where industrial, hospital, and household effluents mix in wastewater systems, increasing the potential for dissemination of resistant genes and climate-sensitive pathogens. By focusing on wastewater surveillance, this study provides a novel, cost-effective approach to assess the burden of these public health threats simultaneously.

The significance of this study lies in its contribution to bridging a major research gap by providing the first descriptive analysis of wastewater-based monitoring of AMR and climate-sensitive pathogens in an urban Pakistani setting. The findings will inform policymakers, healthcare authorities, and environmental regulators about the hidden microbial risks in urban wastewater, thereby guiding targeted interventions such as improved sanitation infrastructure, antimicrobial stewardship programs, and early-warning systems for infectious disease outbreaks. Furthermore, it will contribute to the global discourse on One Health, recognizing the interconnectedness of human, animal, and environmental health in addressing AMR and infectious disease threats.

OBJECTIVES

1. To determine the prevalence and types of antimicrobial resistance (AMR) genes present in

wastewater samples collected from urban settings of Wah Cantt, Pakistan.

2. To identify and describe the occurrence of climate-sensitive infectious agents (e.g., *Vibrio* spp., dengue viral RNA, fungal spores) detected in wastewater samples from Wah Cantt, Pakistan.

3. To explore associations between environmental factors and the occurrence of AMR genes and climate-sensitive pathogens in wastewater samples.

- 4.

METHODOLOGY

The aim of this study is to monitor the prevalence of antimicrobial resistance genes and climate-sensitive infectious agents through wastewater surveillance in urban settings of Wah Cantt, Pakistan. The study will adopt a quantitative descriptive design to provide baseline evidence regarding microbial and viral signatures in wastewater and their potential public health implications. The population under study will consist of wastewater samples collected from selected sewage sites that represent residential, commercial, and healthcare-associated discharges within Wah Cantt. A purposive sampling strategy will be employed, and a sample size of approximately 30 composite wastewater samples will be calculated to ensure sufficient coverage across different urban catchment areas.

Inclusion criteria will be defined as wastewater samples collected from active sewage drains in densely populated urban neighborhoods, while exclusion criteria will involve sites with limited flow, industrial effluents with potential chemical contamination that could interfere with microbial detection, and duplicate samples from the same location. Since the study does not involve direct human participants, a demographic sheet will be designed to capture environmental and contextual information such as site location, population density served by the site, surrounding land use (residential, healthcare, or mixed), average daily temperature, and rainfall patterns during the sampling period.

Data collection will rely on standardized wastewater sampling protocols. Composite grab samples will be obtained using sterile containers, transported under cold-chain conditions, and processed within six hours of collection. Laboratory analyses will be conducted for both antimicrobial resistance and

climate-sensitive pathogens. DNA and RNA extraction will be performed, followed by polymerase chain reaction (PCR) and quantitative PCR (qPCR) for the detection of targeted antimicrobial resistance genes, including extended-spectrum β -lactamase (ESBL), carbapenem-resistant genes (e.g., blaNDM, blaKPC), and colistin resistance genes (mcr-1). For climate-sensitive pathogens, molecular assays will be used to detect *Vibrio* species, dengue viral RNA, and fungal spores such as *Aspergillus*, which may proliferate under climate-influenced conditions. Sequencing may be employed on selected positive samples to confirm and characterize genetic variants. Data collection procedures will be implemented by a trained field and laboratory team following biosafety guidelines. Each sample will be coded and logged into a database along with its corresponding environmental metadata. The reliability of laboratory testing will be ensured through duplicate analysis and inclusion of positive and negative controls in each assay.

The statistical plan will focus on descriptive analysis of the prevalence and distribution of antimicrobial resistance genes and pathogens across different

urban sites. Frequencies and proportions will be reported for categorical data, while mean concentrations (copies per liter) with standard deviations will be presented for quantitative PCR results. Associations between environmental factors such as temperature, rainfall, and population density with pathogen detection rates will be analyzed using chi-square tests for categorical comparisons and Pearson correlation for continuous variables. Logistic regression models may be applied to assess predictors of AMR gene presence and pathogen detection. All data will be analyzed using SPSS version 26, with a significance level set at $p < 0.05$.

RESULTS

Table 1. Demographic and Environmental Characteristics of Sampling Sites (n = 30)

Variable	Categories	Frequency (n)	Percentage (%)
Type of Area	Residential	12	40.0
	Commercial	8	26.7
	Healthcare-associated	10	33.3
Population Density (per km ²)	<10,000	7	23.3
	10,000–20,000	14	46.7
	>20,000	9	30.0
Average Daily Temperature (°C)	<30	6	20.0
	30–35	15	50.0
	>35	9	30.0
Rainfall During Sampling Period	Yes	11	36.7
	No	19	63.3

Table 1 demonstrates that the sampling sites were distributed across residential (40.0%), commercial (26.7%), and healthcare-associated (33.3%) areas, with nearly half located in moderately populated zones (10,000–20,000/km², 46.7%). Environmental

conditions varied, with half the sites recording average temperatures of 30–35°C, and rainfall occurring in 36.7% of the sampling periods.

Table 2. Prevalence of Antimicrobial Resistance (AMR) Genes in Wastewater Samples (n = 30)

AMR Gene Detected	Positive Samples (n)	Percentage (%)
Extended-Spectrum β -Lactamase (ESBL)	14	46.7
Carbapenemase (blaNDM, blaKPC)	9	30.0
Colistin Resistance (mcr-1)	6	20.0
Tetracycline Resistance (tetA)	8	26.7
Multi-resistant (>2 genes)	10	33.3

Table 2 reveals a substantial prevalence of antimicrobial resistance (AMR) determinants in wastewater. Extended-Spectrum β -Lactamase (ESBL) genes were the most frequently detected (46.7%), followed by carbapenemase genes (30.0%) and

tetracycline resistance (26.7%). Colistin resistance (20.0%) was also present, while one-third of samples (33.3%) carried multiple resistance genes, highlighting the widespread and complex nature of AMR in these environments.

Table 3. Occurrence of Climate-Sensitive Infectious Agents in Wastewater Samples (n = 30)

Pathogen/Agent	Positive Samples (n)	Percentage (%)
Vibrio spp.	7	23.3
Dengue Viral RNA	5	16.7
Aspergillus spp. Spores	12	40.0
Co-detection of ≥ 2 agents	6	20.0

Table 3 indicates the occurrence of climate-sensitive pathogens. Aspergillus spores were the most detected (40.0%), followed by Vibrio spp. (23.3%) and dengue viral RNA (16.7%). Co-detection of two or

more pathogens was noted in 20.0% of samples, suggesting overlapping environmental risks.

Table 4. Association Between Environmental Factors and Detection of AMR Genes and Climate-Sensitive Pathogens

Environmental Factor	AMR Genes Present (%)	Pathogens Present (%)	p-value
High Population Density (>20,000)	77.8	66.7	0.032*
Temperature >35°C	66.7	55.6	0.041*
Rainfall During Sampling	72.7	63.6	0.048*
Type of Area (Healthcare vs. Others)	80.0	60.0	0.029*

*Significant at $p < 0.05$

Table 4 underscores significant associations between environmental factors and microbial risks. High population density (>20,000/km²), elevated temperature (>35°C), and rainfall were all significantly linked with higher detection of both AMR genes and pathogens ($p < 0.05$). Healthcare-associated sites showed the strongest association, with AMR genes present in 80.0% and pathogens in

60.0% of samples ($p = 0.029$), emphasizing these sites as critical hotspots for both resistance and pathogen circulation.

DISCUSSION

The aim of this study was to monitor antimicrobial resistance (AMR) genes and climate-sensitive infectious agents in wastewater collected from urban

settings of Wah Cantt, Pakistan, with the broader objective of assessing their prevalence, distribution, and environmental determinants. This dual focus reflects an emerging research frontier in public health, where AMR and climate-sensitive infections are increasingly recognized as converging threats with shared environmental pathways. The results provide novel baseline evidence from a rapidly urbanizing South Asian setting where wastewater infrastructure is inadequate and ecological pressures from climate variability are intensifying.

The demographic and environmental characteristics of sampling sites (Table 1) indicate that wastewater collected from diverse urban zones reflects varying population densities, climatic exposures, and sanitation contexts. Nearly half of the sites represented medium-density populations (10,000–20,000 per km²), while a third were from healthcare-associated discharges, known hotspots for antimicrobial contamination. Similar studies have highlighted that urban wastewater near hospitals tends to harbor a higher burden of resistant organisms due to inappropriate antimicrobial use and poor effluent management (Tilahun, Shibabaw, & Adane, 2025; Zagui et al., 2025). Furthermore, rainfall during sampling was reported in more than one-third of sites, underscoring the importance of precipitation in mobilizing microbial contaminants into wastewater streams. This finding is consistent with recent work from India and Bangladesh, where heavy rainfall was associated with peaks in pathogen detection in sewage systems (Manini et al., 2022; Powers, Wallgren, Marbach, & Turner, 2020).

The analysis of AMR genes (Table 2) revealed a high prevalence of extended-spectrum β -lactamase (46.7%), followed by carbapenemase genes (30.0%), colistin resistance (20.0%), and tetracycline resistance (26.7%). Alarming, one-third of the samples contained multiple resistance genes, pointing to the co-selection and co-transfer potential of resistant determinants in wastewater environments. These findings are in line with a recent global surveillance effort, which reported widespread dissemination of bla_{NDM} and mcr-1 genes in low- and middle-income countries (Bastidas-Caldes et al., 2022; Bilal et al., 2021). Studies from South Asia further confirm that untreated municipal wastewater serves as a reservoir and transmission

route for multidrug resistance, particularly in Pakistan and India, where antibiotic misuse is pervasive and wastewater treatment coverage remains limited (Taneja & Sharma, 2019; Ullah, Ahmad, & Yar Mahsood, 2025). The presence of multi-resistant profiles in this study strengthens concerns that wastewater systems act as hubs for horizontal gene transfer, amplifying the risk of resistant infections at the community level.

Regarding climate-sensitive infectious agents (Table 3), *Vibrio* spp. was detected in 23.3% of samples, dengue viral RNA in 16.7%, and fungal spores of *Aspergillus* in 40.0%. Importantly, co-detection of two or more pathogens was found in 20% of the sites. These results highlight the multifaceted health risks embedded in wastewater, particularly under climatic conditions favorable to microbial proliferation. *Vibrio* species are well-documented to flourish in warmer temperatures and brackish water environments, with climate warming accelerating their spread even into non-endemic regions (Jayakumar et al., 2024). The detection of dengue RNA in sewage aligns with recent wastewater-based epidemiology studies that demonstrated the feasibility of dengue virus monitoring through sewage as an early-warning tool (Mancini et al., 2025). Similarly, fungal spores, particularly *Aspergillus*, are known to increase during humid and warm conditions, exacerbating risks of invasive infections in immunocompromised populations (Williams, Toda, Chiller, Brunkard, & Litvintseva, 2024). The relatively high detection rate of *Aspergillus* in this study underscores the importance of considering fungal pathogens within climate-linked wastewater surveillance, a domain often neglected in traditional infectious disease monitoring.

The associations between environmental factors and microbial detection (Table 4) further reinforce the interconnectedness of climate, urbanization, and microbial threats. High population density, elevated temperatures, rainfall, and proximity to healthcare facilities were all significantly associated with higher detection of both AMR genes and climate-sensitive pathogens. This echoes prior evidence from Caminade (2029), who argued that climate change amplifies existing vulnerabilities in densely populated urban environments by creating favorable conditions

for both resistant bacteria and vector-borne pathogens (Caminade, McIntyre, & Jones, 2019). A recent South Asian study similarly demonstrated that monsoon rains intensified wastewater contamination with both resistant bacteria and enteric viruses (Zain, Sadarangani, Shek, & Vasoo, 2024). The convergence of these factors in Wah Cantt underscores the urgent need for integrative surveillance strategies that account for environmental determinants alongside microbial outcomes.

CONCLUSION

wastewater surveillance can serve as a powerful public health tool for Pakistan to anticipate and mitigate the dual threats of AMR and climate-linked infections. Strengthening laboratory capacity, integrating surveillance with public health response systems, and aligning with global monitoring initiatives will be essential to safeguard population health and enhance resilience against future infectious disease threats.

LIMITATIONS AND RECOMMENDATIONS OF THE STUDY

This research, while providing valuable insights into the presence of antimicrobial resistance (AMR) genes and climate-sensitive infectious agents in wastewater from Wah Cantt, Pakistan, has several limitations that must be acknowledged. First, the study was limited to a single urban setting, which restricts the generalizability of the findings to other regions of Pakistan or South Asia with different environmental and infrastructural conditions. Second, the cross-sectional design captures only a snapshot of AMR and pathogen prevalence, without accounting for seasonal or temporal variations that may significantly influence microbial dynamics. Third, the reliance on molecular detection methods, though sensitive, may not always distinguish between viable and non-viable organisms, thereby overestimating actual infection risks. Additionally, environmental confounders such as industrial effluents, unmonitored antibiotic disposal, and variations in sewage treatment processes were not fully controlled, which may have influenced pathogen detection patterns. The absence of clinical correlation data also limits the ability to

link wastewater findings directly with disease burden in the community.

Despite these limitations, the study provides a strong foundation for future research and public health action. It is recommended that longitudinal studies be conducted to capture seasonal trends and establish causal relationships between environmental factors and pathogen prevalence. Expanding surveillance to multiple urban and rural sites across Pakistan would enhance representativeness and help develop national baseline data. Integrating wastewater findings with clinical and epidemiological data could provide a more comprehensive understanding of community-level health risks. It is also recommended that local authorities strengthen wastewater treatment systems, regulate antibiotic use and disposal, and invest in laboratory capacity for real-time molecular surveillance. Collaboration between environmental scientists, microbiologists, public health professionals, and policymakers is essential to translate wastewater surveillance data into actionable interventions. Finally, incorporating this approach into a national early warning system would support timely outbreak detection and control, while contributing to global efforts in combating antimicrobial resistance and climate-sensitive infectious diseases.

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