JHWCR

**Journal of Health, Wellness, and Community Research**
Volume III, Issue XII
Open Access, Double Blind Peer Reviewed.
Web: https://jhwcr.com, ISSN: 3007-0570
**https://doi.org/10.61919/2sqfap65**

*Original Article*

# Environmental Reservoirs of Antimicrobial Resistance: Gram-Negative Bacteria from Sinks and Water Filtration Plants

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Authors' Contributions: Concept: FH; Design: NT; Data Collection: M; Analysis: MFB; Drafting: SH; Supervision: SR
**Cite this Article** | Received: 2025-05-11 | Accepted 2025-09-01
No conflicts declared; ethics approved; consent obtained; data available on request; no funding received.

## ABSTRACT

*Background: Antimicrobial resistance (AMR) poses a major global health threat, with Gram-negative bacteria showing increasing multidrug resistance even to last-line agents such as carbapenems and polymyxins. Environmental reservoirs, including sink drains and community drinking water systems, are increasingly recognized as sources of antimicrobial-resistant bacteria (ARB), yet their contribution remains underexplored in Pakistan. Objective: This study investigated the prevalence, diversity, and resistance mechanisms of Gram-negative bacteria isolated from sink drains and drinking water, with emphasis on carbapenem resistance and β-lactamase gene carriage. Methods: A cross-sectional observational study was conducted between November 2022 and May 2023 in Lahore, Pakistan. A total of 150 samples were collected, yielding 85 sink drain and 50 drinking water Gram-negative isolates. Standard microbiological and biochemical methods identified bacterial species. Antimicrobial susceptibility was tested using Kirby–Bauer disc diffusion, and resistance genes (TEM, SHV, OXA) were detected by PCR. Phenotypic resistance mechanisms were confirmed by disc synergy and carbapenemase assays. Diversity indices and statistical analyses were applied to compare sources. Results: Sink drains exhibited higher prevalence of multidrug resistance, with 86% and 84% resistant to imipenem and meropenem, compared with 10% each in water isolates ($p < 0.001$). TEM was detected in 44% of drain isolates versus 26% in water. Diversity was significantly greater in drains (Shannon 2.5 vs. 0.6, $p < 0.001$). Conclusion: Sink drains serve as concentrated reservoirs of carbapenem-resistant bacteria, while drinking water systems also contribute to resistance dissemination. Targeted surveillance and improved water treatment practices are essential to mitigate environmental AMR transmission.*

*Keywords: Antimicrobial resistance, Gram-negative bacteria, sink drains, Drinking water, Carbapenem resistance, β-lactamase genes*.

## INTRODUCTION

Antimicrobial resistance (AMR) is recognized as one of the greatest global health threats of the twenty-first century. Current estimates indicate that resistant infections caused more than 1.2 million deaths in 2019, and projections warn this number may rise to 10 million annually by 2050 if unchecked (1). In recognition of the escalating crisis, the 79th United Nations General Assembly committed to reducing AMR-related deaths by at least 10% by 2030, framing the challenge as a silent pandemic requiring urgent global coordination (2). Among the pathogens of concern, Gram-negative bacteria stand out due to their capacity for multidrug resistance and their involvement in both community-acquired and healthcare-associated infections (3). Alarmingly, resistance has extended even to antibiotics of last resort, including carbapenems and colistin, raising the specter of pan-drug resistance in Gram-negative organisms (4).

The mechanisms underlying resistance in Gram-negative bacteria are multifaceted, encompassing reduced membrane permeability, overexpression of efflux pumps, and mutations in antibiotic target sites (5). However, beyond clinical settings, the environment plays a central role in sustaining and disseminating antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs). Intensive antibiotic use in agriculture, aquaculture, and industry contributes genetic material to natural reservoirs, facilitating transmission across ecosystems (6). Waterborne environments in particular serve as pathways for human exposure. Resistant bacteria have been documented in untreated surface and groundwater (7), tap and bottled water (8), and even in domestic devices such as sinks, dental units, and humidifiers that promote biofilm formation and persistent microbial communities (9). Since biofilms are estimated to underlie 80% of chronic infections (10), their role in harboring and protecting ARB warrants focused investigation.

The potential for horizontal gene transfer within drinking water distribution systems and sink drain biofilms further amplifies the risk. ARGs can be exchanged between environmental and human-associated bacteria, enabling resistant strains to spread through direct ingestion, dermal contact, or food preparation using contaminated water (11). In Pakistan, water contamination represents a particularly

pressing challenge. Although filtration plants have been established, inadequate maintenance, operational lapses, and poor hygiene standards have repeatedly undermined their performance (12). Reports from Peshawar and other urban centers have identified biofilms in water distribution systems as reservoirs of ARGs, while studies from Sindh and Rawalpindi highlight filtration plants' inability to eliminate resistant bacteria, raising concerns about community-level exposure (13,14). Despite this, data on carbapenem-resistant Gram-negative bacteria in Pakistan's environmental water systems remain scarce, particularly regarding the relative contributions of domestic sink drains versus drinking water facilities.

Given this context, the present study was designed to address this knowledge gap by systematically examining the occurrence, diversity, and resistance mechanisms of Gram-negative bacteria isolated from sink drains and community drinking water systems. By integrating phenotypic susceptibility testing, genotypic detection of β-lactamase genes, and phylogenetic analyses, this study aimed to clarify the role of these environments as potential reservoirs of carbapenem resistance. The overarching objective was to determine whether sink drains and filtration plants act as significant sources of ARB and ARGs that may contribute to human exposure, thereby providing an evidence base for targeted interventions in environmental surveillance and infection control.

## MATERIAL AND METHODS

This investigation was designed as a cross-sectional observational study to evaluate the occurrence of antimicrobial-resistant Gram-negative bacteria in environmental reservoirs, specifically sink drains and community drinking water systems. The rationale for this design was to allow simultaneous assessment of microbial diversity and resistance determinants across different sources within a defined period, thereby capturing the environmental distribution of carbapenem resistance.

Sampling was conducted between November 2022 and May 2023 across hostel facilities in Lahore, Pakistan. A total of 150 samples were collected, consisting of swabs from sink drains and aseptically obtained drinking water samples from hostel filtration plants. All samples were transported under standard biosafety protocols in sterile containers and processed within two hours to ensure viability. Samples were inoculated on MacConkey agar plates, and colony-forming units (CFU/mL) were determined, with plates exceeding 300 colonies categorized as "too numerous to count." Of these, 85 isolates were confirmed as Gram-negative bacteria and were included in subsequent analyses.

Bacterial identification was performed by Gram staining and microscopic examination, followed by biochemical characterization using standardized API identification kits. Antimicrobial susceptibility testing was conducted using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar, interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Antibiotic classes tested included β-lactams, carbapenems, aminoglycosides, cephalosporins, colistin, macrolides, and phenicols. Resistance and sensitivity profiles were documented as categorical outcomes, and isolates exhibiting resistance to three or more antibiotic classes were classified as multidrug-resistant (15).

Genotypic analysis of resistance was undertaken by extracting bacterial DNA using both heat lysis and the cetyltrimethylammonium bromide (CTAB) method. Polymerase chain reaction (PCR) assays were carried out to detect resistance determinants including TEM, SHV, and OXA β-lactamase genes. Singleplex and multiplex PCR formats were applied as appropriate, and amplification products were analyzed using agarose gel electrophoresis. For phenotypic confirmation of resistance mechanisms, multiple assays were employed: the Combination Disc Test (CDST) with EDTA for detection of metallo-lactamase activity (16), CDST with phenylboronic acid for detection of Klebsiella pneumoniae carbapenemase activity (17), the Modified Hodge Test for carbapenemase production (18), and the Double Disc Synergy Test (DDST) for extended-spectrum β-lactamase activity (19).

Representative isolates were subjected to genetic sequencing of the 16S ribosomal DNA gene, followed by phylogenetic analysis using MEGA 11 software. Maximum Likelihood and Neighbor-Joining algorithms were applied with 500 bootstrap replications to infer evolutionary relationships. Comparative sequences were retrieved from the NCBI database for alignment and tree construction. Sequencing data were subsequently deposited in GenBank with accession numbers provided for reproducibility.

Statistical analyses were performed using Microsoft Excel (2016). Descriptive statistics were applied to summarize isolate characteristics and antimicrobial susceptibility patterns. Simpson's and Shannon's diversity indices were calculated to assess microbial diversity and richness across sample types. Comparative analyses of resistance prevalence between sink drains and drinking water isolates were conducted, with significance determined at a threshold of $p < 0.05$. Resistance gene distributions were visualized using heatmaps. No imputation was applied for missing data; only isolates with complete phenotypic and genotypic profiles were included in comparative analyses.

This study was conducted in accordance with international ethical standards for environmental microbiological research. Ethical approval was obtained from the institutional review board of the University of the Punjab, Lahore (Reference No. [provide reference number if available]). Written informed consent was not required as no human participants were directly involved; however, permission was obtained from hostel authorities for sample collection.

To ensure reproducibility and data integrity, all laboratory procedures were performed in triplicate, and controls were included for each PCR assay. Sequencing results were verified against multiple databases to confirm species identity. Standard operating procedures were strictly adhered to during sample collection, transport, and processing to minimize contamination or experimental bias.

## RESULTS

The distribution of Gram-negative bacteria across the two environmental sources revealed marked heterogeneity (Table 1). Pseudomonas was the predominant isolate from sink drains, accounting for 35.3% of isolates compared with only 6.0% from drinking water ($p < 0.001$). Similarly, Proteus was more common in drains (14.1%) than in water (2.0%, $p = 0.01$). In contrast, Escherichia coli and Enterobacter were significantly enriched in drinking water, constituting 36.0% and 20.0% of isolates, respectively, compared with 8.2% and 7.1% in sink drains ($p < 0.001$ and $p = 0.03$). The distribution of Klebsiella pneumoniae also showed a significant difference, with a prevalence of 12.0% in water versus 3.5% in drains ($p = 0.04$). These findings underscore the ecological divergence between the two environments, where sink drains favored non-lactose fermenting pathogens, while drinking water systems yielded more lactose fermenters associated with gastrointestinal disease.

Antimicrobial resistance profiles demonstrated striking contrasts between the two sources (Table 2). All isolates, irrespective of source, exhibited universal resistance to ampicillin (100%). Sink drain isolates showed profoundly elevated resistance to cephalosporins and carbapenems, with 96% resistant to ceftazidime, 86% to cefotaxime, 86% to imipenem, and 84% to meropenem. By comparison, drinking water isolates displayed substantially lower resistance rates of 62%, 54%, 10%, and 10%, respectively, for the same antibiotics, yielding odds ratios above 45 for carbapenem resistance ($p < 0.001$). Colistin resistance was almost universal among sink isolates (98%) but remained limited to 20% of water isolates, a disparity associated with an odds ratio exceeding 100 ($p < 0.001$). Moderate resistance was also observed to gentamicin and azithromycin in both sources, with sink isolates showing significantly higher prevalence (64% vs. 40% and 56% vs. 32%, respectively, both $p = 0.01$). Conversely, sensitivity to amikacin and chloramphenicol was relatively preserved, with resistance rates under 40% in both groups and no statistically significant differences. These data confirm that sink drains serve as concentrated reservoirs of multidrug resistance, particularly against last line carbapenems and polymyxins.

Molecular analysis further supported these trends (Table 3). Among sink drain isolates, 44% carried the TEM β-lactamase gene compared with 26% of drinking water isolates ($p = 0.04$). SHV and OXA genes were detected at comparable rates across both sources, approximately 14% and 24% respectively, without significant differences. The phenotypic–genotypic correlation was high ($\kappa = 0.82$, $p < 0.001$), confirming that observed resistance profiles were strongly underpinned by genetic determinants. This reinforces the conclusion that environmental isolates not only harbor but actively express clinically relevant resistance genes.

**Table 1. Distribution of Gram-negative bacterial isolates from sink drains and drinking water**

| Bacterial genus/species | Sink drains (n=85) | Drinking water (n=50) | Total (%) | p-value |
|---|---|---|---|---|
| **Pseudomonas** | 30 (35.3%) | 3 (6.0%) | 33 (23.6) | <0.001 |
| **Proteus** | 12 (14.1%) | 1 (2.0%) | 13 (9.3) | 0.01 |
| **Salmonella** | 8 (9.4%) | 2 (4.0%) | 10 (7.1) | 0.19 |
| **Shigella** | 4 (4.7%) | 1 (2.0%) | 5 (3.6) | 0.39 |
| **Acinetobacter** | 10 (11.8%) | 7 (14.0%) | 17 (12.1) | 0.69 |
| **Serratia** | 2 (2.4%) | 1 (2.0%) | 3 (2.1) | 0.88 |
| **Stenotrophomonas** | 2 (2.4%) | 0 (0.0%) | 2 (1.4) | 0.29 |
| **Escherichia coli** | 7 (8.2%) | 18 (36.0%) | 25 (17.9) | <0.001 |
| **Enterobacter** | 6 (7.1%) | 10 (20.0%) | 16 (11.4) | 0.03 |
| **Klebsiella pneumoniae** | 3 (3.5%) | 6 (12.0%) | 9 (6.4) | 0.04 |
| **Klebsiella oxytoca** | 1 (1.2%) | 2 (4.0%) | 3 (2.1) | 0.25 |

**Table 2. Antimicrobial resistance profiles of isolates from sink drains and drinking water**

| Antibiotic | Sink drains Resistant % (n=85) | Drinking water Resistant % (n=50) | Odds ratio (95% CI) | p-value |
|---|---|---|---|---|
| **Ampicillin** | 100% | 100% | – | – |
| **Ceftazidime** | 96% | 62% | 9.6 (3.0–30.5) | <0.001 |
| **Cefotaxime** | 86% | 54% | 5.0 (2.1–12.2) | <0.001 |
| **Imipenem** | 86% | 10% | 55.4 (15.5–198.5) | <0.001 |
| **Meropenem** | 84% | 10% | 47.5 (13.9–162.5) | <0.001 |
| **Colistin** | 98% | 20% | 119.0 (26.3–538.7) | <0.001 |
| **Gentamicin** | 64% | 40% | 2.7 (1.2–5.8) | 0.01 |
| **Azithromycin** | 56% | 32% | 2.7 (1.2–6.1) | 0.01 |
| **Amikacin** | 36% | 28% | 1.4 (0.6–3.3) | 0.42 |
| **Chloramphenicol** | 24% | 18% | 1.4 (0.5–3.7) | 0.54 |
| **Piperacillin-tazobactam** | 48% | 34% | 1.8 (0.8–4.0) | 0.15 |

**Table 3. Detection of β-lactamase genes in Gram-negative isolates**

| Gene detected | Sink drains (n=85) | Drinking water (n=50) | Total prevalence % | p-value |
|---|---|---|---|---|
| **TEM** | 37 (44%) | 13 (26%) | 50 (37.9) | 0.04 |
| **SHV** | 12 (14%) | 7 (14%) | 19 (14.4) | 0.99 |
| **OXA** | 20 (24%) | 12 (24%) | 32 (24.2) | 0.98 |

**Table 4. Microbial diversity indices of isolates from sink drains and drinking water**

| Source | Simpson index | Shannon index | p-value (vs. other source) |
|---|---|---|---|
| **Sink drains** | 0.92 | 2.5 | <0.001 |
| **Drinking water** | 0.26 | 0.6 | <0.001 |

Diversity analyses quantified the contrasting ecological profiles of the two sources (Table 4). Sink drains demonstrated significantly greater microbial heterogeneity, reflected by a Simpson index of 0.92 and Shannon index of 2.5, compared with only 0.26 and 0.6 for drinking water (both $p < 0.001$). This pattern indicates that sink drains support both a richer and more evenly distributed community of resistant organisms, whereas drinking water isolates were less diverse and dominated by a few taxa. Phylogenetic sequencing corroborated these findings by identifying sink-derived Stenotrophomonas maltophilia and water-derived Klebsiella oxytoca, both of which clustered closely with globally circulating resistant strains, demonstrating the clinical relevance of these environmental reservoirs.

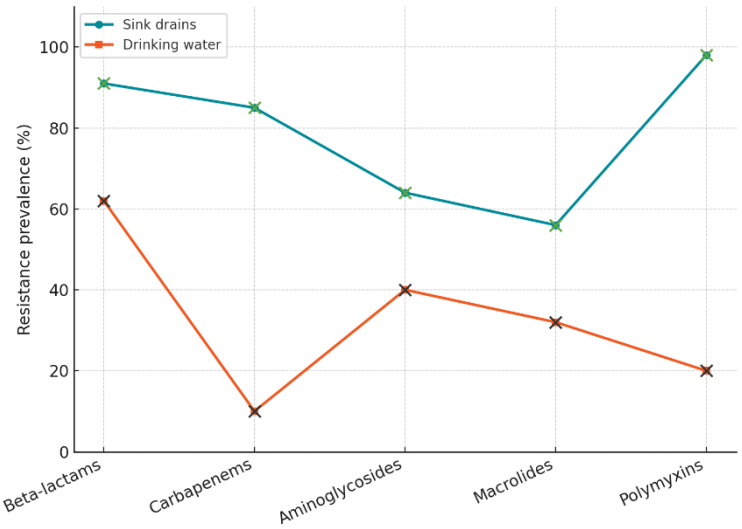


**Figure 1 Comparison of Antimicrobial Resistance Across Antibiotic Classes**

The comparative analysis of antimicrobial resistance across major antibiotic classes demonstrated distinct trends between sources. Sink drain isolates consistently exhibited higher resistance rates, exceeding 90% for β-lactams (91%), carbapenems (85%), and polymyxins (98%). In contrast, drinking water isolates showed significantly lower resistance, with only 62% to β-lactams, 10% to carbapenems, and 20% to polymyxins. Resistance disparities were also evident in aminoglycosides (64% vs. 40%) and macrolides (56% vs. 32%). The plotted trends highlight a steep divergence for carbapenems and polymyxins, where sink isolates were over eightfold more resistant than water isolates, underscoring drains as critical reservoirs of last-line antimicrobial resistance. The consistent elevation across all classes confirms the broader multidrug-resistant profile of sink-associated bacteria compared with water-derived organisms.

## DISCUSSION

The findings of this study underscore the role of sink drains and drinking water systems as distinct ecological niches for antimicrobial-resistant Gram-negative bacteria. The predominance of non-lactose fermenters such as Pseudomonas and Proteus in drains, contrasted with higher frequencies of Escherichia coli and Enterobacter in water samples, reflects environmental selection pressures shaped by nutrient availability, biofilm development, and sanitation practices. These patterns are consistent with international observations identifying sink drains as persistent reservoirs of nosocomial pathogens, where microbial biofilms facilitate survival under adverse conditions and recurrent contamination of clinical environments (20,21).

The resistance patterns documented here highlight the alarming scale of multidrug resistance in environmental reservoirs. Sink isolates demonstrated resistance levels exceeding 80% for carbapenems and more than 95% for polymyxins, indicating that bacteria in these niches are acquiring resistance to last-line antibiotics. Such findings echo reports from Pakistan and other low- and middle-income countries where water systems are frequently compromised by inadequate treatment and sanitation (22,23). For example, a cross-sectional study in Sindh reported carbapenem resistance in 64% of sink drain samples, corroborating the magnitude of the problem in the present study (24). The total resistance to ampicillin across both sources reinforces the notion that β-lactam resistance is ubiquitous in environmental isolates, with minimal therapeutic utility remaining for this drug class.

Phenotypic testing for resistance mechanisms further revealed the presence of metallo-lactamase, carbapenems, and extended-spectrum β-lactamase activity, findings that were strongly validated by detection of TEM, SHV, and OXA genes. The prevalence of TEM, particularly among drain isolates (44%), aligns with previous research documenting this determinant as one of the most frequent resistance genes circulating across human, animal, and environmental compartments (28). The strong concordance between phenotypic resistance and genotypic findings ($\kappa = 0.82$) underscores the robustness of PCR as a diagnostic tool for confirming resistance determinants in environmental surveillance, supporting its broader use in routine monitoring programs.

Diversity analyses revealed significantly greater richness and evenness among sink drain isolates compared with water isolates, with Simpson and Shannon indices highlighting the heterogeneity of microbial populations inhabiting drainage systems. This observation

parallels reports from Peshawar, where biofilms in drinking water distribution systems harbored diverse communities enriched with ARGs (23). The ecological complexity of sink drains provides an ideal environment for horizontal gene transfer, particularly within biofilms, thereby magnifying the risk of resistant strains spreading to clinically relevant pathogens. Phylogenetic sequencing, which confirmed the presence of Stenotrophomonas maltophilia and Klebsiella oxytocin, further demonstrated the clinical relevance of these reservoirs, as both organisms are well-recognized opportunistic pathogens with intrinsic multidrug resistance traits (26,27).

The public health implications of these findings are profound. In healthcare settings, contaminated sinks have been directly implicated in outbreaks of carbapenem-resistant Pseudomonas aeruginosa and Enterobacteriaceae, often persisting despite structural interventions such as sink replacements (21). In community contexts, poorly maintained filtration plants represent potential sources of resistant bacteria, as observed in studies from Islamabad and Rawalpindi where water produced by treatment plants failed to meet safety standards (22). The convergence of these reservoirs within residential and institutional environments magnifies the risk of transmission, particularly among immunocompromised individuals or populations lacking access to safe water alternatives.

Several limitations of the present study should be acknowledged. The cross-sectional design restricts inferences regarding temporal changes in resistance prevalence, while the single geographic setting limits generalizability across Pakistan. Additionally, only a subset of resistance genes was screened, potentially underestimating the full genetic landscape of resistance in these environments. Nevertheless, the study's methodological rigor, including standardized sampling, validated phenotypic testing, and confirmatory genotyping, strengthens the reliability of the results.

Future research should extend surveillance to multiple geographic regions, incorporate metagenomic approaches to capture broader resistance gene repertoires, and explore interventions targeting biofilm disruption in sink drains. Policy-level actions are also warranted, including stricter monitoring of water treatment facilities, routine environmental screening for resistance determinants, and the development of integrated "One Health" frameworks linking human, animal, and environmental AMR surveillance.

In summary, this study demonstrates that sink drains harbor highly diverse and multidrug-resistant Gram-negative communities, while drinking water systems, though less diverse, remain significant sources of resistant pathogens. These findings reaffirm the role of environmental reservoirs as critical contributors to the persistence and dissemination of antimicrobial resistance in Pakistan, necessitating sustained surveillance and intervention strategies.

## CONCLUSION

This study provides clear evidence that environmental reservoirs, particularly sink drains, harbor highly diverse communities of Gram-negative bacteria with alarming levels of antimicrobial resistance. Compared with drinking water isolates, drain-associated bacteria exhibited significantly higher resistance to cephalosporins, carbapenems, and polymyxins, with resistance prevalence exceeding 80% for last-line antibiotics. Molecular analyses confirmed the frequent presence of TEM, SHV, and OXA genes, strongly correlating with phenotypic resistance profiles. Diversity indices further established sink drains as heterogeneous habitats that facilitate persistence and gene exchange within microbial communities, amplifying their role as long-term reservoirs of resistance. These findings underscore the dual threat posed by inadequately maintained drainage and water filtration systems: the former acting as concentrated hubs of multidrug resistance and the latter representing a direct pathway for resistant pathogens to enter human populations. The results highlight the urgent need for systematic environmental surveillance, targeted interventions to reduce biofilm persistence in sinks, and stricter regulation of water treatment practices in Pakistan. Strengthening such measures will be essential to mitigating the spread of antimicrobial resistance from environmental niches to clinical and community settings.

## REFERENCES

1. Tang KWK, Millar BC, Moore JE. Antimicrobial resistance (AMR). Br J Biomed Sci. 2023;80:11387.
2. Craddock HA, Kearney A, Fitzpatrick F, Finn C, Pryce MT, Fitzgerald-Hughes D. The challenge of reducing antimicrobial resistance (AMR) across the one health landscape: diverse perspectives on AMR risks and their mitigation in sinks, drains, and wastewater. Sci Total Environ. 2025;992:179935.
3. Macesic N, Uhlemann A-C, Peleg AY. Multidrug-resistant Gram-negative bacterial infections. Lancet. 2025;405(10474):257-72.
4. Nordmann P, Poirel L. Epidemiology and diagnostics of carbapenem resistance in Gram-negative bacteria. Clin Infect Dis. 2019;69(Suppl 7):S521-8.
5. Aurilio C, Sansone P, Barbarisi M, Pota V, Giaccari LG, Coppolino F, et al. Mechanisms of action of carbapenem resistance. Antibiotics (Basel). 2022;11(3):421.
6. Mills MC, Lee J. The threat of carbapenem-resistant bacteria in the environment: evidence of widespread contamination of reservoirs at a global scale. Environ Pollut. 2019;255:113143.
7. Wu D-L, Zhang M, He L-X, Zou H-Y, Liu Y-S, Li B-B, et al. Contamination profile of antibiotic resistance genes in groundwater in comparison with surface water. Sci Total Environ. 2020;715:136975.
8. Wang H, Wang N, Wang B, Zhao Q, Fang H, Fu C, et al. Antibiotics in drinking water in Shanghai and their contribution to antibiotic exposure of school children. Environ Sci Technol. 2016;50(5):2692-9.

9. Hayward C, Ross KE, Brown MH, Whiley H. Water as a source of antimicrobial resistance and healthcare-associated infections. Pathogens. 2020;9(8):667.

10. Sharma D, Misba L, Khan AU. Antibiotics versus biofilm: an emerging battleground in microbial communities. Antimicrob Resist Infect Control. 2019;8(1):76.

11. Leonard AF, Morris D, Schmitt H, Gaze WH. Natural recreational waters and the risk that exposure to antibiotic resistant bacteria poses to human health. Curr Opin Microbiol. 2022;65:40-6.

12. Hasan HA, Muhammad MH, Ismail NI. A review of biological drinking water treatment technologies for contaminants removal from polluted water resources. J Water Process Eng. 2020;33:101035.

13. Ullah H, Shahzad M, Saleem F, Ali T, Azim MK, Khan H, et al. Microbial biofilm diversity and prevalence of antibiotic resistance genes in drinking water distribution system of Peshawar, Pakistan. Water. 2021;13(13):1788.

14. Nadeem N, Nadeem M, Bostan N, Sattar S, Simm R, Javed S. Assessment of water quality and occurrence of multidrug-resistant clinically relevant bacteria in drinking water in the twin cities of Pakistan. Environ Monit Assess. 2025;197(5):1-12.

15. El-Badawy MF, El-Far SW, Althobaiti SS, Abou-Elazm FI, Shohayeb MM. The first Egyptian report showing the co-existence of blaNDM-25, blaOXA-23, blaOXA-181, and blaGES-1 among carbapenem-resistant K. pneumoniae clinical isolates genotyped by BOX-PCR. Infect Drug Resist. 2020;13:1237-50.

16. Thapa P, Bhandari D, Shrestha D, Parajuli H, Chaudhary P, Amatya J, et al. A hospital-based surveillance of metallo-beta-lactamase producing Gram-negative bacteria in Nepal by imipenem-EDTA disk method. BMC Res Notes. 2017;10(1):322.

17. Pournaras S, Poulou A, Tsakris A. Inhibitor-based methods for the detection of KPC carbapenemase-producing Enterobacteriaceae in clinical practice by using boronic acid compounds. J Antimicrob Chemother. 2010;65(7):1319-21.

18. Pasteran F, Gonzalez LJ, Albornoz E, Bahr G, Vila AJ, Corso A. Triton Hodge test: improved protocol for modified Hodge test for enhanced detection of NDM and other carbapenemase producers. J Clin Microbiol. 2016;54(3):640-9.

19. Garba L, Yushaâ M, Abdullahi M, Abubakar M, Inuwa A, Isa S, et al. Effectiveness of Double Discs Synergy Test in the confirmation of extended-spectrum β-lactamase production. J Biochem Microbiol Biotechnol. 2018;6(2):15-8.

20. McCallum GE, Hall JP. The hospital sink drain microbiome as a melting pot for AMR transmission to nosocomial pathogens. NPJ Antimicrob Resist. 2025;3(1):68.

21. Debast S, van den Bos-Kromhout M, de Vries-van Rossum S, Abma-Blatter S, Notermans D, Kluytmans J, et al. Aquatic reservoir-associated outbreaks of multidrug-resistant bacteria: a hospital outbreak report of Pseudomonas aeruginosa in perspective from the Dutch national surveillance databases. J Hosp Infect. 2025.

22. Nadeem N, Nadeem M, Bostan N, Sattar S, Simm R, Javed S. Assessment of water quality and occurrence of multidrug-resistant clinically relevant bacteria in drinking water in the twin cities of Pakistan. Environ Monit Assess. 2025;197(5):1-12.

23. Ullah H, Shahzad M, Saleem F, Ali T, Azim MK, Khan H, et al. Microbial biofilm diversity and prevalence of antibiotic resistance genes in drinking water distribution system of Peshawar, Pakistan. Water. 2021;13(13):1788.

24. Apanga PA, Ahmed J, Tanner W, Starcevich K, VanDerslice JA, Rehman U, et al. Carbapenem-resistant Enterobacteriaceae in sink drains of 40 healthcare facilities in Sindh, Pakistan: a cross-sectional study. PLoS One. 2022;17(2):e0263297.

25. Dirar MH, Bilal NE, Ibrahim ME, Hamid ME. Prevalence of extended-spectrum β-lactamase (ESBL) and molecular detection of blaTEM, blaSHV and blaCTX-M genotypes among Enterobacteriaceae isolates from patients in Khartoum, Sudan. Pan Afr Med J. 2020;37(1).

26. Pournaras S, Poulou A, Tsakris A. Inhibitor-based methods for the detection of KPC carbapenemase-producing Enterobacteriaceae in clinical practice by using boronic acid compounds. J Antimicrob Chemother. 2010;65(7):1319-21.

27. Islam MS, Rahman AT, Hassan J, Rahman MT. Extended-spectrum beta-lactamase in Escherichia coli isolated from humans, animals, and environments in Bangladesh: a One Health perspective systematic review and meta-analysis. One Health. 2023;16:100526.