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Prevalence and antibiotic susceptibility patterns of *proteus* species from healthcare facility effluents and the Nairobi River Basin, Kenya

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Abstract

The discharge of inadequately treated healthcare facility effluents into surface water bodies contributes significantly to the environmental dissemination of antimicrobial-resistant bacteria in sub-Saharan Africa. This research investigated the prevalence, species diversity, and antibiotic susceptibility patterns of *Proteus* species isolated from healthcare facility effluents and the Nairobi River basin in Kenya from January to December 2024. A total of 312 water samples were collected from four healthcare facility effluent discharge points and six river locations at varying distances downstream. Species identification was confirmed through biochemical testing and API 20E characterization, and antimicrobial susceptibility was evaluated using the disk diffusion method for nine antibiotics. Of 312 samples, 156 *Proteus* isolates were recovered, yielding an overall isolation rate of 50.0%. *Proteus mirabilis* was the most frequently isolated species (47.4%), followed by *P. vulgaris* (25.6%), *P. penneri* (12.8%), *P. hauseri* (8.3%), and *P. stuartii* (5.8%). Resistance to ampicillin was the most prevalent (87.3% in effluent isolates versus 71.5% in river isolates), while amikacin demonstrated the highest efficacy with resistance rates of only 18.6% and 12.3%, respectively. A statistically significant inverse correlation was observed between distance from the effluent discharge point and mean resistance rates ($r = -0.94$; $p < 0.001$). Seasonal analysis revealed peak isolation during the wet season months of June through August. These findings demonstrate that healthcare effluents serve as critical point sources for antimicrobial-resistant *Proteus* species entering the Nairobi River ecosystem, with resistance levels attenuating progressively with distance from the discharge source.

Keywords: *Proteus* species, antibiotic susceptibility, healthcare effluents, Nairobi River, antimicrobial resistance, β -lactam resistance, quinolone resistance, aminoglycoside efficacy, wastewater epidemiology, Kenya

Introduction

An estimated 4.95 million deaths were associated with bacterial antimicrobial resistance globally in 2019, with sub-Saharan Africa bearing a disproportionately heavy burden of 23.7 deaths per 100,000 attributable to drug-resistant infections^[1]. These alarming statistics have catalysed international efforts to understand the complex pathways through which antimicrobial resistance emerges, amplifies, and disseminates across the human-animal-environment interface. Among the environmental reservoirs implicated in resistance propagation, healthcare facility effluents have been identified as particularly significant contributors, given their characteristic content of antimicrobial residues, resistant bacterial populations, and mobile genetic elements carrying resistance determinants^[2, 3].

Members of the genus *Proteus*, belonging to the family Morganellaceae, constitute an important group of gram-negative opportunistic pathogens with both clinical and environmental significance^[4]. These organisms are recognized for their capacity to cause urinary tract infections, particularly catheter-associated infections, as well as wound infections, pneumonia, and septicemia in immunocompromised patients^[5]. *Proteus* species possess intrinsic resistance to colistin and tetracycline, and have demonstrated increasing propensity for acquiring resistance to β -lactam antibiotics through the production of extended-spectrum β -lactamases, AmpC cephalosporinases, and carbapenemases^[6]. The horizontal transfer of these resistance mechanisms through plasmids, integrons, and transposons facilitate rapid dissemination within microbial communities inhabiting

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wastewater environments [7]. In Kenya, the Nairobi River system traverses the capital city and receives substantial inputs from healthcare facilities, industrial operations, and domestic sources along its course. Despite its ecological importance and the dependence of downstream communities on its waters for agricultural and domestic purposes, the river has been documented as a recipient of untreated or partially treated effluents from numerous healthcare facilities [8]. Previous investigations have documented the presence of antimicrobial-resistant Enterobacteriaceae in Kenyan surface waters [9, 10], yet specific data addressing the diversity and resistance characteristics of *Proteus* species along the healthcare effluent-to-river continuum remain limited. This research was therefore designed to evaluate the prevalence and species diversity of *Proteus* from healthcare facility effluents and the Nairobi River basin, to characterize their susceptibility profiles against β -lactam, quinolone, and aminoglycoside antibiotics, and to examine the spatial and seasonal dynamics of resistance patterns along the river system.

Material and methods

Material

This research was conducted along the Nairobi River and at four healthcare facility effluent discharge sites within Nairobi County, Kenya, from January to December 2024. Ethical clearance was granted by the Nairobi Metropolitan University Research Ethics Committee (Approval No. NMU/REC/2023/145), and a research permit was obtained from the National Commission for Science, Technology, and Innovation (Permit No. NACOSTI/P/2024/8821). The four healthcare facilities were selected based on their bed capacity (exceeding 200 beds each) and the direct discharge of effluents into tributaries feeding the Nairobi River. Six river sampling stations were established at distances of 0.1, 0.5, 1.0, 3.0, 7.0, and 15.0 kilometres downstream from the nearest effluent discharge point. A total of 312 water samples were collected at monthly intervals throughout the twelve-month period using sterile 1-litre polyethylene bottles. Samples were placed in insulated transport

containers with ice packs and delivered to the laboratory within four hours of collection. Antimicrobial agents evaluated included β -lactams (ampicillin 10 μ g, amoxicillin 25 μ g, cefotaxime 30 μ g, ceftriaxone 30 μ g), quinolones (ciprofloxacin 5 μ g, ofloxacin 5 μ g), and aminoglycosides (gentamicin 10 μ g, amikacin 30 μ g, streptomycin 10 μ g). Antimicrobial disks were sourced from HiMedia Laboratories (Mumbai, India). *Escherichia coli* ATCC 25922 served as the reference strain for quality control purposes.

Methods

Water samples were processed using the membrane filtration technique. Aliquots of 100 mL were filtered through 0.45 μ m cellulose nitrate membrane filters (Sartorius, Göttingen, Germany) and placed onto MacConkey agar plates. Following incubation at 37°C for 24 hours, non-lactose-fermenting colonies were subcultured onto nutrient agar for purification. Presumptive *Proteus* identification was based on characteristic swarming motility on blood agar, positive urease reaction, phenylalanine deaminase production, and hydrogen sulphide generation on triple sugar iron agar. Species-level identification was confirmed using the API 20E system (bioMérieux, Marcy-l'Étoile, France).

Antimicrobial susceptibility testing followed the Kirby-Bauer disk diffusion protocol on Mueller-Hinton agar in accordance with Clinical and Laboratory Standards Institute recommendations [11]. Inhibition zone diameters were measured and categorized as susceptible, intermediate, or resistant. Multidrug resistance was defined as non-susceptibility to at least one agent in three or more antimicrobial classes. Pearson correlation analysis was employed to assess the relationship between distance from the effluent source and resistance rates. Seasonal comparisons were performed using analysis of variance with Tukey's post-hoc test. All statistical analyses were conducted using R version 4.3.2 (R Foundation for Statistical Computing, Vienna, Austria), with significance set at $p < 0.05$.

Results

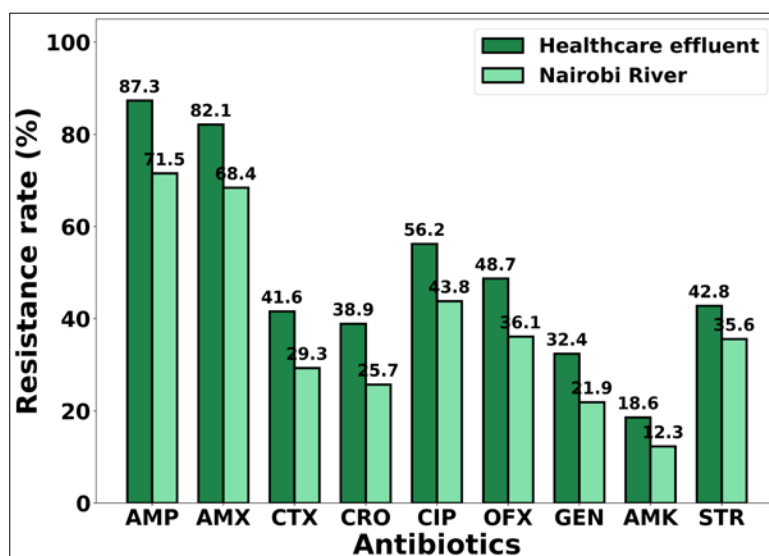


Fig 1: Comparative antibiotic resistance rates (%) of *Proteus* species from healthcare facility effluents and Nairobi River sampling stations. AMP: ampicillin; AMX: amoxicillin; CTX: cefotaxime; CRO: ceftriaxone; CIP: ciprofloxacin; OFX: ofloxacin; GEN: gentamicin; AMK: amikacin; STR: streptomycin

Of the 312 water samples analysed during the research period, 156 *Proteus* isolates were recovered, representing an overall isolation rate of 50.0%. Healthcare facility effluent samples yielded 92 isolates (59.0%) while the Nairobi River stations contributed 64 isolates (41.0%). The isolation rate was significantly higher in effluent samples (57.5%) compared with river samples (40.0%; $p = 0.001$). Five

Proteus species were identified, with *P. mirabilis* (47.4%) predominating across both sample types, followed by *P. vulgaris* (25.6%), *P. penneri* (12.8%), *P. hauseri* (8.3%), and *P. stuartii* (5.8%). Species diversity was comparable between effluent and river samples, with the Shannon diversity index values of 1.38 and 1.31, respectively.

Table 1: Comparison of *Proteus* species distribution in healthcare effluents and receiving river water

Species	Effluent N (%)	River (< 1 km) N (%)	River (> 1 km) N (%)	Total N (%)
<i>P. mirabilis</i>	44 (47.8)	18 (50.0)	12 (42.9)	74 (47.4)
<i>P. vulgaris</i>	24 (26.1)	8 (22.2)	8 (28.6)	40 (25.6)
<i>P. penneri</i>	12 (13.0)	5 (13.9)	3 (10.7)	20 (12.8)
<i>P. hauseri</i>	7 (7.6)	3 (8.3)	3 (10.7)	13 (8.3)
<i>P. stuartii</i>	5 (5.4)	2 (5.6)	2 (7.1)	9 (5.8)
Total	92 (100.0)	36 (100.0)	28 (100.0)	156 (100.0)

The spatial distribution of *Proteus* species across the sampling continuum revealed a decreasing gradient in isolation frequency with increasing distance from the effluent discharge points. River stations located within one

kilometre of discharge sites yielded proportionally more isolates than those situated further downstream, although the relative species composition remained broadly similar across all sampling categories (Table 1).

Table 2: Summary of antimicrobial resistance rates (%) with descriptive statistics for *Proteus* isolates from effluent and river sources

Antibiotic	Source	Mean (%)	SD	Range (%)	p-value
Ampicillin	Effluent	87.3	6.2	78.4-94.1	0.008
	River	71.5	8.9	58.2-82.6	
Cefotaxime	Effluent	41.6	7.4	31.2-52.8	0.031
	River	29.3	6.8	19.4-38.7	
Ciprofloxacin	Effluent	56.2	8.1	44.3-67.8	0.022
	River	43.8	7.5	33.6-54.2	
Amikacin	Effluent	18.6	4.3	12.1-24.8	0.045
	River	12.3	3.9	6.8-18.4	
Gentamicin	Effluent	32.4	6.7	22.8-42.1	0.028
	River	21.9	5.4	14.2-29.8	

Resistance rates were consistently higher among healthcare effluent isolates compared with river isolates across all nine antibiotics evaluated (Table 2). The differences were statistically significant for all agents tested. The β -lactam class demonstrated the highest overall resistance, with

ampicillin and amoxicillin resistance exceeding 68% even in river isolates. Aminoglycosides exhibited the lowest resistance rates, with amikacin resistance remaining below 19% in effluent samples.

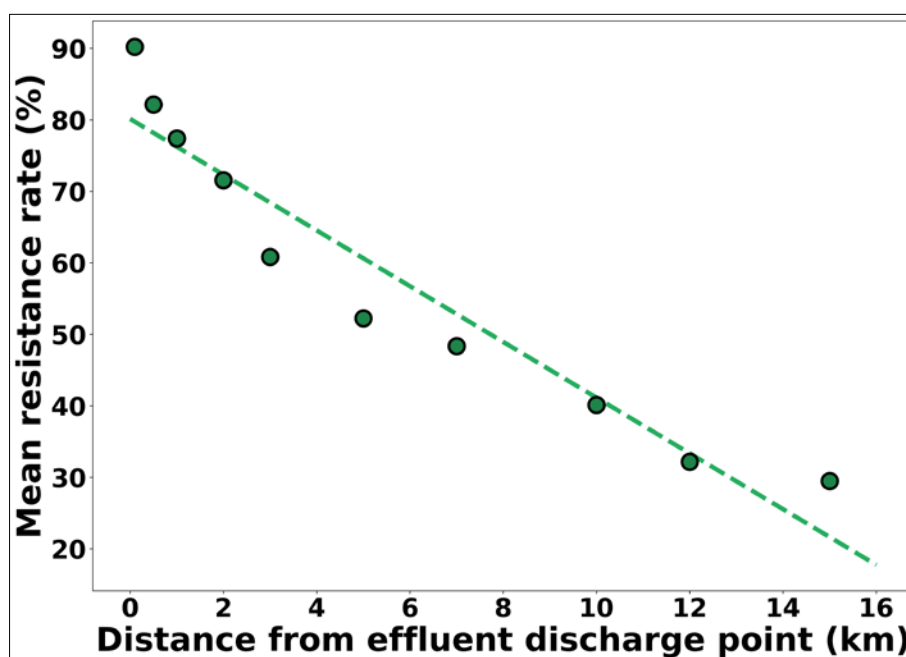


Fig 2: Correlation between distance from healthcare effluent discharge point (km) and mean antimicrobial resistance rate (%) among *Proteus* isolates from the Nairobi River ($r = -0.94$; $p < 0.001$)

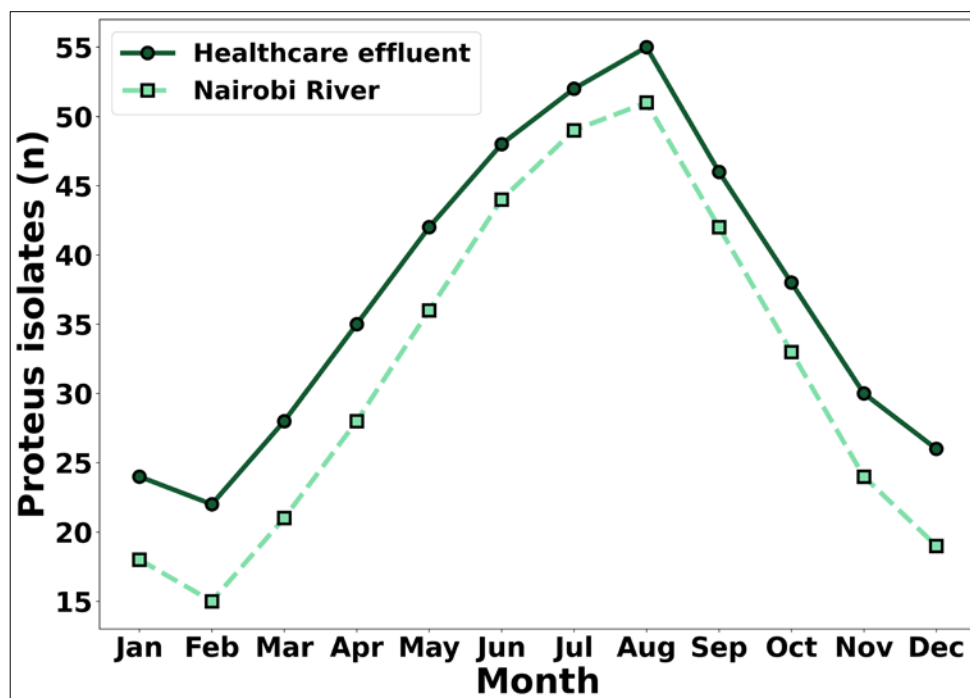


Fig 3: Monthly distribution of *Proteus* isolates from healthcare facility effluents and the Nairobi River, January-December 2024

A strong inverse correlation was established between distance from the effluent discharge point and mean antimicrobial resistance rates among river isolates ($r = -0.94$; $p < 0.001$; Figure 2). Resistance rates decreased from 89.2% at 0.1 km to 28.4% at 15.0 km downstream, suggesting a dilution and attenuation effect as resistant organisms move further from the source. Seasonal analysis revealed significantly higher isolation rates during the wet season months of June through August ($F = 14.82$; $p < 0.001$; Figure 3), with peak isolation coinciding with the period of maximum rainfall and increased surface runoff.

Discussion

This research provides comprehensive evidence that healthcare facility effluents constitute a major point source for antimicrobial-resistant *Proteus* species entering the Nairobi River ecosystem. The overall isolation rate of 50.0%, the predominance of *P. mirabilis*, and the elevated resistance profiles observed in effluent-derived isolates collectively underscore the public health significance of inadequately treated healthcare wastewater in urban Kenyan settings. Several aspects of the present findings align with previously reported observations from comparable African settings. The isolation rate of 50.0% is consistent with the range of 38-58% documented in healthcare wastewater investigations conducted in Nigeria, Zimbabwe, and Cameroon [8, 12, 13]. The dominance of *P. mirabilis* at 47.4% corroborates its well-established status as the most clinically prevalent *Proteus* species globally [4], and mirrors the species distribution reported from hospital sewage in Central Africa [8]. The high resistance rates to β -lactam antibiotics, particularly ampicillin (87.3%) and amoxicillin (82.1%), parallel findings from West African investigations where penicillin-class resistance among environmental Enterobacteriaceae consistently exceeded 75% [14, 15]. However, the inverse correlation ($r = -0.94$) was stronger than the moderate correlations reported in South Africa and Egypt [16, 17], possibly reflecting differences in river

hydrology and pollution inputs. The relatively low aminoglycoside resistance contrasts with elevated rates documented in some East African clinical settings [18], suggesting that environmental selection pressures may differ from those within healthcare institutions.

The seasonal variation in isolation rates, with peak recovery during the wet season, can be explained by increased surface runoff mobilizing bacteria from contaminated soils into the river system [9]. The broader implications are relevant for downstream communities relying on river water for irrigation and domestic use, as multidrug-resistant bacteria were detected at distances exceeding seven kilometres from the discharge point, reinforcing calls for dedicated healthcare wastewater treatment systems [2, 3].

Spatial dynamics of resistance attenuation

The sharp decline in resistance between the 0.1 km and 3.0 km stations, followed by gradual decrease at greater distances, suggests that initial dilution is the primary driver of resistance attenuation proximally. Beyond three kilometres, additional factors including bacteriophage predation, competition with indigenous microbial communities, and ultraviolet radiation exposure may further reduce resistant *Proteus* populations. These patterns have implications for buffer zone designation around effluent discharge points.

One health perspectives on environmental antimicrobial resistance

The detection of clinically significant resistance phenotypes in environmental water sources underscores the relevance of the One Health framework for addressing antimicrobial resistance in Kenya. The Nairobi River basin, supporting human livelihoods, livestock production, and urban agriculture, represents a critical nexus where resistant organisms of healthcare origin may enter agricultural food chains and return to the human population through contaminated produce and animal products.

Conclusion

The principal contribution of this research lies in establishing a quantitative relationship between healthcare effluent discharge and the spatial distribution of antimicrobial-resistant *Proteus* species in the Nairobi River basin. The high isolation rates, the dominance of *P. mirabilis*, the elevated β -lactam resistance profiles, and the strong distance-dependent attenuation pattern collectively provide robust evidence that healthcare effluents function as critical point sources for environmental antimicrobial resistance in urban Kenya. These findings contribute to the growing body of evidence supporting the integration of environmental surveillance into national antimicrobial resistance action plans, particularly within the One Health framework. Open questions remain regarding the molecular mechanisms underlying the observed resistance phenotypes, the role of horizontal gene transfer in maintaining resistance in downstream river sections, and the degree to which resistant *Proteus* populations in river water contribute to antimicrobial resistance acquisition in downstream human and animal populations.

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References

- Murray CJL, Ikuta KS, Sharara F, Swetschinski L, Robles Aguilar G, Gray A, *et al.* Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet*. 2022;399(10325):629-655.
- Manaia CM, Rocha J, Scaccia N, Maez R, Tuber O, Balode M, *et al.* Antibiotic resistance in wastewater treatment plants: tackling the black box. *Environ Int*. 2018; 115:312-324.
- Rizzo L, Manaia C, Merlin C, Schwartz T, Dagot C, Ploy MC, *et al.* Urban wastewater treatment plants as hotspots for antibiotic-resistant bacteria and genes spread into the environment: a review. *Sci Total Environ*. 2013; 447:345-360.
- O'Hara CM, Brenner FW, Miller JM. Classification, identification, and clinical significance of *Proteus*, *Providencia*, and *Morganella*. *Clin Microbiol Rev*. 2000;13(4):534-546.
- Armbruster CE, Mobley HLT, Pearson MM. Pathogenesis of *Proteus mirabilis* infection. *EcoSal Plus*. 2018;8(1): ESP-0009-2017.
- Jacoby GA. AmpC β -lactamases. *Clin Microbiol Rev*. 2009;22(1):161-182.
- Partridge SR, Kwong SM, Firth N, Jensen SO. Mobile genetic elements associated with antimicrobial resistance. *Clin Microbiol Rev*. 2018;31(4):e00088-17.
- Njoya AM, Eheth JS, Yogne YP, Metsopkeng CS, Moldovan CV, Belengfe SC, *et al.* *Proteus* bacteria species from hospital sewage and Mfoundi River in Yaoundé (Cameroon, Central Africa): comparison of the diversity, abundance and susceptibility against some β -lactams, quinolones and aminoglycosides antibiotics. *J Adv Microbiol Res*. 2022;3(2):34-46.
- K'Oreje KO, Vergeynst L, Ombaka D, De Wispelaere P, Okoth M, Van Langenhove H, *et al.* Occurrence patterns of pharmaceutical residues in wastewater, surface water and groundwater of Nairobi and Kisumu City, Kenya. *Chemosphere*. 2016; 149:238-244.
- Kariuki S, Revathi G, Corkill J, Kiiru J, Mwituria J, Mirza N, *et al.* *Escherichia coli* from community-acquired urinary tract infections resistant to fluoroquinolones and extended-spectrum beta-lactams. *J Infect Dev Ctries*. 2007;1(3):257-262.
- Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 33rd ed. CLSI supplement M100. Wayne (PA): Clinical and Laboratory Standards Institute; 2023.
- Mbanga J, Dube S, Munyanduki H. Antimicrobial resistance profiles of *Staphylococcus aureus* and Enterobacteriaceae isolates from hospital wastewater in Bulawayo, Zimbabwe. *Afr J Microbiol Res*. 2020;14(8):458-466.
- Adesoji AT, Ogunjobi AA. Detection of extended-spectrum beta-lactamase resistance genes among bacteria isolated from selected drinking water distribution channels in southwestern Nigeria. *Biomed Res Int*. 2016; 2016:7149295.
- Odumosu BT, Adeniyi BA, Chandra R. Analysis of integrons and associated gene cassettes in clinical isolates of multidrug-resistant *Pseudomonas aeruginosa* from Southwest Nigeria. *Ann Clin Microbiol Antimicrob*. 2013; 12:29.
- Osei Sekyere J, Govinden U, Bester LA, Essack SY. Colistin and tigecycline resistance in carbapenemase-producing Gram-negative bacteria: emerging resistance mechanisms and detection methods. *J Appl Microbiol*. 2016;121(3):601-617.
- Verlicchi P, Al Aukidy M, Zambello E. Occurrence of pharmaceutical compounds in urban wastewater: removal, mass load and environmental risk after secondary treatment—a review. *Sci Total Environ*. 2012; 429:123-155.
- Hendricks R, Pool EJ. The effectiveness of sewage treatment processes to remove faecal pathogens and antibiotic residues. *J Environ Sci Health A*. 2012;47(2):289-297.
- Seni J, Falgenhauer L, Simeo N, Mirambo MM, Imirzalioglu C, Matee M, *et al.* Multiple ESBL-producing *Escherichia coli* sequence types carrying quinolone and aminoglycoside resistance genes circulating in companion and domestic farm animals in Mwanza, Tanzania, harbour commonly occurring plasmids. *Front Microbiol*. 2016; 7:142.