*Original Research Article*

**Genetic Diversity of Selected Antibiotic Resistant Bacterial Strains from Industrial Effluents in Nairobi County, Kenya.**

ABSTRACT

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| --- |
| Antibiotic resistance represents a critical global health challenge driven by the dissemination of resistant bacterial genes across households, livestock and environmental reservoirs. Horizontal gene transfer and mutations play substantial roles in the existence and persistence of antimicrobial resistance significantly diminishing the effectiveness of current antibiotic therapies. This study aimed to investigate the genotypic and phenotypic resistance profiles of bacterial strains isolated from industrial effluent samples. Conducted in Nairobi County, Kenya, between January and December 2024, the study involved analysis of four bacterial strains isolated from industrial effluent samples collected via systematic sampling across multiple industrial sites. Bacterial identification was performed using API® 20E biochemical identification kit (BioMérieux, France). Antibiotic susceptibility testing encompassed several antibiotic classes including cephalosporins, penicillins, aminoglycosides, sulfonamides, tetracyclines, fluoroquinolones and carbapenems. DNA extraction from antibiotic-resistant isolates was conducted using ZyppyTM Plasmid Miniprep Kit (Zymo Research, USA) following the manufacturer’s protocol. Conventional PCR assays targeted resistance genes *bla-TEM, bla-OXA, bla-KPC-1, bla-NDM* and *ParC.* Sequence alignment was performed using MUSCLE software while phylogenetic analyses were conducted with MEGA 11 employing the Maximum Likelihood method to infer evolutionary relationships. All tested bacterial isolates including *Pseudomonas aeruginosa, Escherichia coli,* *Aeromonas spp., Klebsiella pneumoniae,* and *Bacillus spp.,* exhibited100% resistance to penicillin. Resistance to tetracyclines, cephalosporins and sulfonamides was notably prevalent in *Aeromonas spp.* In contrast*,* carbapenems and aminoglycosides maintained substantial efficacy particularly against *Aeromonas spp.* and *Pseudomonas aeruginosa* strains*.* Multiplex PCR analysis revealed widespread distribution of resistance genes with *bla-TEM* being the most prevalent followed by *bla-KPC, bla-OXA, bla-NDM* and *ParC* underscoring the molecular basis for the observed resistance phenotypes. Phylogenetic analysis demonstrated high sequence homology with globally distributed pathogenic strains highlighting the clinical relevance and potential public health impact of these findings. These findings underscore the urgent need to integrate comprehensive surveillance systems and implement multifaceted antimicrobial strategies to curb the spread of multidrug-resistant bacterial strains across environmental, healthcare, and aquatic ecosystems. |

*Keywords: Antibiotics, Antibiotic resistance, Antimicrobial resistance, Bacteria isolate, Genetic diversity, Industrial effluents, Multidrug-resistant bacteria, Resistance genes*

1. INTRODUCTION

Antibiotic resistance has emerged as a major global health threat driven by the widespread dissemination of antibiotic-resistant bacteria and antibiotic resistant genes across human, animal, and environment interfaces. This complex phenomenon is best understood through the One Health approach which emphasizes the interconnectedness of human, animal, and environmental health (Badau, 2021; Mackenzie & Jeggo, 2019). The persistence use and misuse of antibiotics in clinical, agricultural, and industrial settings have accelerated the development of resistance undermining the efficacy of many antimicrobial agents. Beyond compromising treatment outcomes, antimicrobial resistance (AMR) imposes a significant economic burden on healthcare systems and is associated with increased morbidity and mortality particularly among immunocompromised individuals, children under five years of age, and the elderly (Murray et al., 2022). Increased human encroachment into diverse ecosystems including both terrestrial and aquatic environments has contributed to the rising emergence and re-emergence of antibiotic resistance genes (ARGs) (Ahmad et al., 2023; Milaković et al., 2020). Rapid industrialization driven by growing household demands and rural-to-urban migration has further intensified this trend. Populations relocating to industrial regions may carry resistant bacterial strains contributing to the environmental dissemination of antimicrobial resistance (AMR). Contaminated industrial zones have been hypothesized to harbor significantly higher concentrations of resistant organisms (Kamatham et al., 2024; Su et al., 2023). Antibiotic use in both humans and animals exerts selective pressure eliminating susceptible bacteria and enabling the survival of resistant strains. These multidrug-resistant bacteria can transfer resistance genes horizontally to otherwise susceptible bacteria complicating efforts to control AMR (Mutuku et al., 2022; Ngigi et al., 2020). The resulting infections are often more difficult to treat, associated with elevated mortality rates and present fewer therapeutic options (Atlaw et al., 2022). Industrial effluents in particular serve as significant reservoirs of resistance determinants and thus represent a growing public health concern. **Industrial effluents are not limited to wastewater by-products of diverse manufacturing processes; they often contain hazardous contaminants including antibiotic-resistant bacteria (ARB), heavy metals, and other pathogenic microorganisms. These effluents are frequently discharged either untreated or inadequately treated contributing significantly to environmental pollution. Contamination of surface and groundwater by such waste streams has been shown to elevate ecological and public health risks in surrounding communities (Inya et al., 2022; Shukla & Sahu, 2021a). The persistent release of antibiotic resistance genes (ARGs) into aquatic systems underscores the urgent need for stricter wastewater management and regulatory frameworks to mitigate the spread of resistance determinants from industrial sources (Mahmud et al., 2023; Wu et al., 2023). Without adequate intervention, ARGs present in effluents can persist and proliferate posing a growing global threat to both environmental and human health. This study aimed to identify and characterize antibiotic-resistant bacteria (ARB) isolated from industrial effluents in Nairobi County, Kenya. By examining both genotypic and phenotypic resistance profiles, the study sought to elucidate resistance mechanisms prevalent in industrial environments and to contribute to understanding the dissemination dynamics of ARGs. The findings are intended to inform targeted interventions and policy development to curb the environmental spread of resistance and protect public health.**

2. material and methods

**2.1 Study Area and Study Design**

This study investigated the role of industrial effluents in the dissemination of antibiotic-resistant bacteria (ARB) in industrial zones within Nairobi County, Kenya. A cross-sectional study design was employed. Industrial wastewater samples were systematically collected from multiple sites across the study area.

**2.2 Sample Collection**

Industrial effluent samples were aseptically collected using sterile 10 mL screw-cap bottles. The samples were immediately placed in cool boxes and transported to the microbiology laboratory for analysis. To ensure accurate microbiological assessment and minimize changes in bacterial populations, all samples were processed within 24 hours of collection.

**2.3 Bacterial Isolation and Identification**

Bacteria were isolated from the collected effluent samples using the serial dilution technique. Dilutions ranging from 10⁻¹ to 10⁻⁵ were prepared by mixing aliquots of each sample with sterile distilled water. Subsequently, 0.1 mL from each dilution was spread onto nutrient agar plates (Himedia Lab, India) which were incubated at 37°C for 18–24 hours following the procedure described by Shukla and Sahu (2021b). After incubation, distinct bacterial colonies were selected and sub-cultured onto MacConkey agar to facilitate isolation and differentiation of bacterial species. Initial morphological characterization of isolates was performed using Gram staining. For further identification, Gram-negative and Gram-positive bacteria were characterized using the API® 20E and API® 50 CHB identification system (BioMérieux, France) respectively as described by Logtong and Zakpaa, 2024 and Hong et al., 2020. Isolates were preserved in tryptic soy broth supplemented with 10% glycerol and stored at –80°C for subsequent analyses.

**2.4 Antimicrobial Susceptibility Testing**

The antibiotic susceptibility of bacterial isolates was determined using the disc diffusion method on Mueller-Hinton agar (Himedia Lab, India). The antibiotics tested included imipenem (10 µg), levofloxacin (30 µg), ciprofloxacin (25 µg), ampicillin (10 µg), tetracycline (30 µg), amoxicillin (30 µg), gentamicin (10 µg), sulfamethoxazole/trimethoprim (25 µg), cefepime (30 µg), and ceftriaxone (30 µg) (Oxoid Limited, United Kingdom). Pure bacterial colonies were evenly streaked onto Mueller-Hinton agar plates and antibiotic discs were subsequently placed on the agar surface. Plates were incubated at 37°C for 18–24 hours after which the diameters of inhibition zones were measured in millimeters. Results were interpreted according to the Clinical and Laboratory Standards Institute guidelines (Weinstein, 2021). Escherichia coli ATCC 25922 was used as a quality control strain to validate the performance of the agar medium and antibiotic discs.

**2.5 DNA Extraction, PCR Amplification of Resistance Genes and 16S rRNA Gene Detection**

Genomic DNA was extracted from antibiotic-resistant isolates using the Zyppy™ Plasmid Miniprep Kit (Zymo Research, USA) following the manufacturer’s instructions. The quality of the extracted DNA was evaluated by electrophoresis on a 1.5% agarose gel stained with SYBR Green dye and visualized under a UV transilluminator as described by Wang et al. (2020). PCR amplification targeted resistance genes associated with fluoroquinolones (ParC gene; primers: F 5’-CTATGCGATGTC-3’, R 5’-TAACAGCAGCTC-3’), third-generation cephalosporins (bla-TEM gene; F 5’-GGAGCTAACCGCTTTTTTGCA-3’, R 5’-CTAGAGTAAGTAGTTCGCCAG-3’; bla-OXA gene; F 5’-ATGAAAAACACA-3’, R 5’-GTGTGTTTAGAA-3’), and carbapenems (bla-KPC gene; F 5’-ACTGTATCGCCGTCTAGTTCT-3’, R 5’-TTTTTGCCGTAAGGATGG-3’; bla-NDM gene; F 5’-ACTGTATCGCCGTCTAGTTCT-3’, R 5’-TTTTTGCCGTAAGGATGG-3’). The 16S rRNA gene of bacterial isolates was amplified using universal primers (F 5’-AGAGTTTGATCCTGGCTCAG-3’, R 5’-GGTTACCTTGTTACGACTT-3’) according to Kabir and Tasmin (2019). The PCR reaction mixture (25 μL total volume) consisted of 12.5 μL OneTaq Quick-Load 2X Master Mix with standard buffer, 0.5 μL of each 10 μM primer, 2 μL DNA template, and 9.5 μL nuclease-free water. Amplification was performed under the following cycling conditions: initial denaturation at 94°C for 30 seconds; 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds; followed by a final extension at 72°C for 10 minutes (Robicsek et al., 2006). PCR products were resolved by electrophoresis on a 1.5% agarose gel at 80 V for 30 minutes and visualized using SYBR Green staining.

**2.6 Sequencing and Sequence Analysis**

PCR products were purified and sequenced by Macrogen Europe (Amsterdam, The Netherlands). Sequence data were analyzed using BioEdit version 7.2.5 software (Li et al., 2023). The Basic Local Alignment Search Tool (BLAST) was employed to compare the sequences against the National Center for Biotechnology Information (NCBI) database for identification (Samal et al., 2021). Multiple sequence alignment was performed using MUSCLE software (Korak et al., 2021) to assess phylogenetic relationships among the bacterial isolates. Phylogenetic trees were constructed using MEGA version 11 (Tamura et al., 2021), employing the Maximum Likelihood method with 1,000 bootstrap replications. Evolutionary history was inferred using the Kimura 2-parameter model (Chi & Minin, 2023).

**2.7 Statistical Analysis**

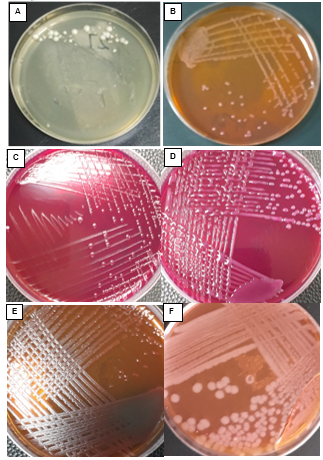
Data were documented using WHO-NET software and Microsoft Excel, both secured with password protection to ensure data integrity and confidentiality. Results were visualized using bar graphs. Phylogenetic relationships and genetic similarities among isolates were analyzed using the BioNumerics software.

3. results and discussion

3.1 Results

**3.1.1 Isolation and Identification**

The morphological characteristics of the bacterial isolates cultured on MacConkey agar are presented in Figure 1. Biochemical profiling of the isolates, performed using the API® 20E and API® 50 CHB identification system (BioMérieux, France), is summarized in Table 1.



**Fig. 1. Colony morphology of isolated bacteria**

A: Colonies of different isolated bacteria in Nutrient agar; B: *Aeromonas spp.*; C: *Escherichia coli*; D: *Klebsiella pneumoniae*; E: *Pseudomonas aeruginosa*; F: *Bacillus spp.*

**Table 1. Isolated bacteria identification, Gram reaction, colony morphology and biochemical characteristics**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Isolate** | **GR** | **Morph** | **Biochemical Characteristics** | | | | | | | | | | | | | | | | | | | |
|  |  |  | **ONPG** | **ADH** | **LDC** | **ODC** | **CIT** | **H2S** | **URE** | **TDA** | **IND** | **VP** | **GEL** | **GLU** | **MAN** | **INO** | **SOR** | **RHA** | **SAC** | **MEL** | **AMY** | **ARA** |
| *Escherichia coli* | -ve | Rod | + | - | + | + | - | - | - | - | + | - | - | + | +- | + | + | + | + | + | - | + |
| *Aeromonas spp.* | -ve | Rod | + | + | + | - | + | - | - | - | + | + | + | + | + | - | - | - | + | - | + | - |
| *Bacillus spp.* | +ve | Rod | + | + | - | - | + | - | - | - | - | + | + | + | + | - | + | - | + | + | + | - |
| *Pseudomonas aeruginosa* | -ve | Rod | - | + | - | - | - | - | + | + | + | + | + | + | + | + | + | + | - | + | + | + |
| *Klebsiella pneumoniae* | -ve | Rod | + | - | + | - | + | - | + | - | - | + | - | + | + | + | + | + | - | + | - | + |

*Key: (ONPG) Ortho NitroPhenyl-ßD-Galactopyranosidase, (ADH) Arginine Di hydrolase, (LDC) Lysine Decarboxylase, (ODC) Ornithine Decarboxylase, (CIT) trisodium citrate, (H2S) sodium thiosulfate, (URE) urea, (TDA) Tryptophane Deaminase, (IND) Indole, (VP) Voges Proskauer, (GEL) Gelatin, (GLU) Glucose, (MAN) Mannitol, (INO) Inositol, (SOR) Sorbitol, (RHA) Rhamnose, (SAC) Saccharose, (MEL) Melibiose, (AMY) Amygdalin, (ARA) Arabinose, (GR) Gram Reaction, and (Morph) Morphology.*

**3.1.2 Multidrug Resistance Frequency of Different Classes of Antibiotics**

The multidrug resistance (MDR) profiles of the isolated bacterial strains against various classes of antibiotics were assessed and are presented in Figure 2. Notable differences in resistance patterns were observed among the five genera studied. *Escherichia coli* exhibited complete resistance (100%) to penicillin along with significant resistance to fluoroquinolones (50%) and cephalosporins (27.75%). Resistance to tetracycline (33.33%), aminoglycosides (33.33%), and sulfonamides (33.33%) was moderate while resistance to carbapenems was the lowest (16.6%). *Klebsiella pneumoniae* demonstrated high resistance to penicillin (100%) and sulfonamides (80%) with moderate resistance observed against cephalosporins and tetracycline (60% each). *Pseudomonas aeruginosa* showed complete resistance to both penicillin and tetracycline (100%), and a high resistance level to sulfonamides (80%). *Bacillus spp.* isolates displayed the highest resistance to penicillin and sulfonamides (100%). Cephalosporin resistance was moderate (57.16%) while tetracycline resistance was comparatively lower (17.43%). Resistance to carbapenems and fluoroquinolones was observed at 42.86% and aminoglycosides showed the lowest resistance (28.57%). *Aeromonas spp*. isolates were fully resistant (100%) to cephalosporins, sulfonamides, tetracycline, and penicillin. Remarkably, no resistance was observed against carbapenems, aminoglycosides, or fluoroquinolones indicating a more selective resistance profile.

**Fig. 2. Multidrug resistance frequency of different classes of antibiotics**

**3.1.3 Resistance genes, molecular identification and phylogenetic analysis**

Five antibiotic resistance genes associated with resistance to fluoroquinolones, aminoglycosides, and cephalosporins specifically *bla-TEM*, *bla-OXA*, *bla-KPC-1*, *bla-NDM*, and *ParC* were screened (Figures 3 and 4). The *bla-TEM* gene was the most frequently detected, present in nearly all tested isolates. The *bla-KPC-1* gene was the second most prevalent, identified in four isolates. Other resistance genes, including *bla-OXA*, *bla-NDM*, and *ParC* were less commonly detected, each appearing in only two isolates. Sequence similarity search was performed using BLAST analysis. Isolate S19 shared 56% sequence similarity with *Bacillus spp*. YVU (PP5756021), while S25 exhibited 100% similarity with *Aeromonas spp.* MG6 (MT393943.1). Furthermore, isolates S54 and S55 showed 100% sequence similarity with *Klebsiella pneumoniae* A15 (MW598404.1) and *Bacillus thuringiensis* MT-L36 (PQ283829.1) respectively. Phylogenetic analysis grouped these isolates into three major clades which included *Bacillus spp.*, *Aeromonas spp.*, and *Klebsiella pneumoniae* (Figure 5).



bla-NDM 630bp

bla- TEM 453bp

bla-KPC-1 575bp

2000

M 1 2 3 4 5 6 7 8 9 10 N

bla-ParC 267bp

100

300

500

400

1517

1000

**Fig. 3. Multiplex PCR gel image of resistance genes of Fluoroquinolones, Carbapenems and Cephalosporins classes of antibiotics. (M) 100 bp ladder (New England Biolabs). (N) Negative control**

M 11 12 13 1 4 15 16 17 18 19 20 N



bla-OXA 810BP

bla-KPC-1 575bp

bla-ParC 267bp

bla TEM 453bp

1000

400

300

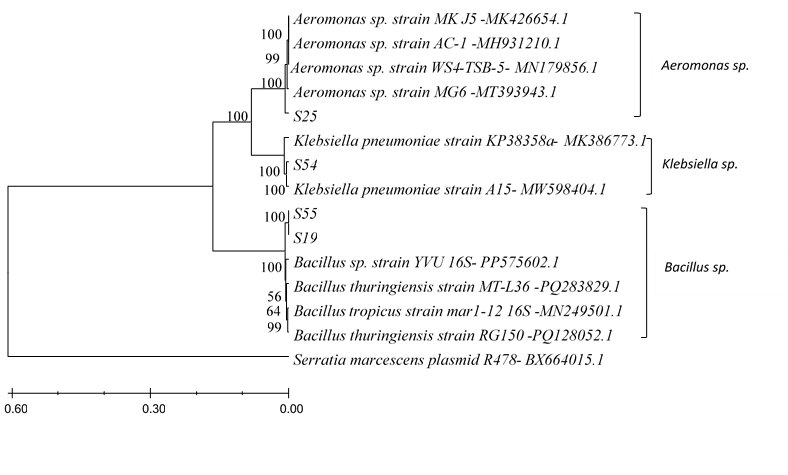
100

200

500

1517

**Fig. 4. Multiplex PCR gel image of resistance genes of Fluoroquinolones, Carbapenems and Cephalosporins classes of antibiotics. (M) 100 bp ladder (New England Biolabs). (N) Negative control**



**Fig. 5. Neibour-joining phylogenetic trees that shows the taxa correlated to the isolated *Klebsiella, Aeromonas and Bacillus species.* With *Serrati marcescens plasmid R478-BX664015.1* used as out group**

**3.2 DISCUSSION**

This study underscores the antibiotic resistance profiles and phylogenetic relationships of environmentally derived bacterial isolates with a particular focus on five key genera: *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Aeromonas spp.*, and *Bacillus spp.* These genera were selected due to their clinical relevance, environmental ubiquity, and documented potential for harboring multidrug-resistant traits (Moradi et al., 2025; Osei Sekyere & Reta, 2020; Mbhele et al., 2021). The findings of this study reveal significant multidrug resistance (MDR) patterns among environmental bacterial isolates underpinned by the presence of key resistance genes. These results carry critical implications for both public health and clinical therapy. Notably, *Escherichia coli* exhibited complete resistance (100%) to amoxicillin and penicillin, aligning with the widespread dissemination of β-lactamase-mediated resistance among Enterobacteriaceae as reported by Islam et al. (2023).

Fluoroquinolone resistance was observed in 50% of the *Escherichia coli* isolates likely reflecting increasing selective pressure resulting from their extensive clinical use. Resistance to aminoglycosides and tetracyclines was moderate (33.33%) while carbapenem resistance was comparatively low (16.6%), consistent with their classification as agents of last resort. The high prevalence of the *bla-TEM* genes among isolates supports previous reports (Z. Wang et al., 2022) and underscores its critical role in the dissemination of extended-spectrum β-lactamase (ESBL)-producing strains.

*Klebsiella pneumoniae* demonstrated elevated resistance levels to multiple antibiotic classes including cephalosporins (60%), sulfonamides (80%), fluoroquinolones (80%), and penicillin (100%). These findings are consistent with recent studies identifying *Klebsiella pneumoniae* as a major reservoir of extended-spectrum β-lactamase (ESBL) and carbapenemase genes (Raheem et al., 2021; Q. Wang et al., 2024). Notably, while carbapenems and aminoglycosides retained partial effectiveness, the detection of *bla-OXA*, *bla-KPC*, and *bla-NDM* genes in several isolates indicates the emergence of carbapenem producers. This trend mirrors the growing global concern reported in the 2023 WHO Antimicrobial Resistance Surveillance Update and highlights the expanding threat posed by carbapenemase-producing Enterobacteriaceae (CPE) in both clinical and environmental contexts.

*Pseudomonas aeruginosa* isolates exhibited high resistance to tetracyclines and penicillin alongside substantial resistance to sulfonamides (80%). Despite this, the isolates largely remained susceptible to cephalosporins, carbapenems, and aminoglycosides, suggesting continued efficacy of these drug classes. These findings are consistent with those of Oliver et al. (2024), who reported that aminoglycosides remain among the few antibiotic classes retaining activity against *Pseudomonas aeruginosa* because of the partial resistance advance mechanisms as related to beta lactams.

*Bacillus spp*., predominantly associated with environmental sources, exhibited complete resistance to both sulfonamides and penicillin (100%). Moderate resistance was observed for cephalosporins (57.14%) while susceptibility to fluoroquinolones and carbapenems was variable (42.86%). Notably, aminoglycosides demonstrated relatively high efficacy with 71.43% of isolates remaining sensitive. These findings are in agreement with prior studies by Adamski et al. (2023) and Wash et al. (2022), who reported similar resistance profiles in *Bacillus spp.* isolated from agricultural soils. The identification of *Bacillus thuringiensis* through 16S rRNA gene sequencing is particularly noteworthy as it highlights the potential clinical relevance of environmental *Bacillus* species. Although traditionally regarded as non-pathogenic or limited to biocontrol applications, certain *Bacillus* strains are increasingly implicated in opportunistic infections particularly in immunocompromised hosts. This underscores the importance of monitoring resistance patterns even in seemingly low-risk genera.

*Aeromonas spp.* isolates displayed the highest multidrug resistance (MDR) profiles among the genera studied exhibiting 100% resistance to sulfonamides, penicillin, tetracyclines, and cephalosporins. Despite this extensive resistance, all isolates remained susceptible to aminoglycosides, carbapenems, and fluoroquinolones indicating that these antibiotic classes may still provide effective treatment options. These findings align with those of Wu et al. (2023), who reported similar MDR patterns in *Aeromonas spp*. from aquatic and clinical environments. The high resistance burden observed in this genus often overlooked in routine clinical diagnostics underscores its emerging role as a potential reservoir of resistance genes and a contributor to the broader antimicrobial resistance crisis. Given its environmental ubiquity and increasing clinical relevance, *Aeromonas spp.* warrant closer surveillance and inclusion in diagnostic panels especially in cases of persistent or atypical infections.

Molecular analysis revealed a high prevalence of the *bla-TEM* gene indicating widespread β-lactam resistance among *Aeromonas spp*. and members of the Enterobacteriaceae family. The detection of *bla-KPC* in four isolates, alongside the presence of *bla-OXA*, *bla-NDM*, and *ParC* in one isolate each, underscores the diverse genetic basis underpinning resistance to fluoroquinolones and carbapenems. Particularly concerning, is the identification of *bla-NDM* in *Klebsiella pneumoniae* isolates as this gene is strongly associated with global outbreaks of carbapenem-resistant *Klebsiella pneumoniae* (Logan et al., 2021; Logan & Weinstein, 2017). Its presence in environmental isolates suggests a potential route for the environmental dissemination of high-risk resistance genes into clinical settings. Interestingly, the low prevalence of *ParC* mutations suggests that fluoroquinolone resistance in these isolates may primarily result from plasmid-mediated mechanisms or efflux pump activation rather than chromosomal mutations in topoisomerase IV or DNA gyrase. This highlights the need for further studies to elucidate non-mutational resistance pathways and their epidemiological significance.

Phylogenetic clustering based on 16S rRNA gene sequences revealed three distinct clades corresponding to the genera *Klebsiella, Bacillus, and Aeromonas*. This taxonomic resolution underscores the reliability of 16S rRNA sequencing for species-level identification. Notably, *Bacillus* isolates of environmental origin formed a separate clade clearly divergent from the clinically associated *Klebsiella* and *Aeromonas* clusters. The close phylogenetic proximity observed between certain *Aeromonas* and *Klebsiella* isolates suggests potential horizontal gene transfer (HGT) events especially given the co-occurrence of carbapenemase genes across both genera. This supports growing evidence that environmental microbial communities may serve as reservoirs for antimicrobial resistance determinants with the capacity to transfer into clinically relevant pathogens (Mutuku et al., 2022). These findings reinforce the hypothesis that environmental niches contribute to the dissemination of multidrug resistance factors emphasizing the need for integrated surveillance approaches that bridge environmental, clinical, and molecular microbiology. The high prevalence of multidrug-resistant (MDR) bacteria across all genera investigated in this study suggests persistent antimicrobial pressure likely driven by the unregulated use of antibiotics in both clinical and agricultural settings. The detection of clinically significant resistance genes such as *bla-KPC*, *bla-NDM*, and *bla-TEM* underscores the urgent need for robust surveillance systems and the implementation of rapid diagnostic tools capable of detecting resistance determinants at the point of care. Moreover, the identification of MDR strains among *Aeromonas* and *Bacillus* genera traditionally considered environmental or non-pathogenic signals a shifting epidemiological landscape. This emerging threat highlights the importance of considering all microbial reservoirs in antimicrobial resistance (AMR) monitoring frameworks.

These findings align with the One Health approach emphasizing the interconnectedness of human, animal, and environmental health in the emergence and spread of AMR. Integrated efforts across these sectors are essential to contain the spread of resistance and safeguard the effectiveness of existing antimicrobials.

4. Conclusion

This study underscores the alarming diversity and prevalence of multidrug-resistant (MDR) bacteria in industrial effluent environments, highlighting their potential role as reservoirs and vectors in the dissemination of antimicrobial resistance (AMR). The isolation and identification of Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Aeromonas spp., and Bacillus spp. reflect a highly diverse microbial community as evidenced by distinct biochemical and phenotypic profiles. All isolates exhibited resistance to penicillin (100%) while elevated resistance to sulfonamides, tetracyclines, and cephalosporins was particularly pronounced in Klebsiella pneumoniae and Aeromonas spp. Molecular characterization revealed the widespread presence of clinically significant resistance genes including ***bla-TEM*, *bla-OXA*, *bla-KPC*, *bla-NDM*,** and ***ParC*** highlighting a strong genetic basis for the phenotypic resistance observed. Notably, ***bla-TEM*** emerged as the most frequently detected gene, consistent with global reports on extended-spectrum β-lactamase (ESBL)-mediated resistance. Phylogenetic analysis demonstrated high genetic similarity between sequenced isolates and globally reported clinical strains reinforcing the evolutionary linkage between environmental and clinical bacterial populations. This finding raises concern about the potential for horizontal gene transfer and gene flow between environmental reservoirs and human pathogens. The presence of multiple resistance determinants in environmental isolates from industrial effluents emphasizes the critical role of such ecosystems in the propagation of AMR. These results call for sustained environmental monitoring, enforcement of strict waste disposal regulations and the implementation of targeted intervention strategies. Moreover, evaluating the effectiveness of effluent treatment systems and exploring the mechanisms of resistance gene dissemination in aquatic ecosystems are essential steps toward mitigating the spread of AMR across ecological and clinical boundaries.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Consent

Not applicable.

Ethical approval

This study was reviewed and approved by the Jomo Kenyatta University of Agriculture and Technology (JKUAT) Institutional Scientific and Ethical Review Committee, under approval number JKU/2/11/TM318-C003-1774/2016.

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