***Original Research Article***

**Environmental Surveillance of Antimicrobial Resistance Among Selected Gram-Negative Bacteria in Industrial Waste Water**

**ABSTRACT**

The environmental dispersal of antimicrobial resistant bacteria (ARB) and antimicrobial resistance genes (ARGs) represents an increasing global public health concern particularly in low and middle-income countries where industrial waste water management remains inadequate. Industrial waste water serves as a reservoir of antimicrobial resistant microorganisms facilitating the transfer of resistance traits within natural ecosystems and ultimately to human populations through direct contact, the food chain or through contaminated water sources. This study examined the occurrence of antimicrobial resistant bacteria in industrial waste water and evaluated their sensitivity profiles to commonly used antibiotics. A total of 101 bacterial isolates were recovered from the samples including *Klebsiella spp*. (37.6*%), Escherichia coli* (36.6%) and *Pseudomonas spp.* (25.7%). Antimicrobial sensitivity profiles were determined using the disc diffusion method against ten commonly used antibiotics including ampicillin, ciprofloxacin, ceftriaxone, levofloxacin and gentamicin. High resistance was observed to ampicillin (88.1%), trimethoprim/sulfamethoxazole (54.4%), and tetracycline (45.5%). Moderate resistance was observed to ceftriaxone (45.5%) and ciprofloxacin (22.7%) while the lowest resistance rates were recorded for levofloxacin (9.9%) and gentamicin (8.9%). *Pseudomonas spp.* exhibited 100% resistance to both trimethoprim/sulfamethoxazole and tetracycline. Multidrug resistance being defined as resistance to more than three classes of antibiotics was notably high among *Klebsiella spp.* (47.4%) and also *Escherichia coli* (29.7%). These findings are consistent with global reports that document perseverance of antimicrobial resistance bacteria in aquatic environments exposed to industrial contamination. This study highlights the role of industrial waste water as a significant reservoir of multidrug resistant bacteria and underscores the urgent need for waste water treatment and the integration of antimicrobial stewardship into both public health and environmental policy frameworks. The high resistance levels detected especially with the frequently dispensed antibiotics, highlights the probable health risks posed by environmental exposure to untreated or partially treated industrial discharges.

***Keywords:*** Antibiotics, *Antimicrobial resistant bacteria, antimicrobial resistance genes, environmental surveillance, industrial waste water,* *isolates*, *multidrug resistance, reservoir*

**1. INTRODUCTION**

Antimicrobial resistant bacteria (ARB) and antimicrobial resistance genes (ARGs) have emerged as significant environmental threats posing serious risks to both human health and ecological balance (Karkman et al., 2020; Peterson, E., & Kaur, P. 2018). Municipal and industrial waste water discharges play a critical role in disseminating resistance determinants within aquatic ecosystems. Waste water from chemical and pharmaceutical industries often contain elevated concentrations of antibiotics and resistance genes thereby intensifying selective pressure on environmental microbial communities (Larsson et al., 2022; Wang, et al., 2022). Aquatic systems contaminated with antimicrobials serves as reservoirs of antimicrobial resistance genes facilitating horizontal gene transfer and promoting the spread of resistance bacterial traits among both environmental and pathogenic bacteria (Kairigo, et al., 2020). The environment not only harbors naturally occurring resistance genes but also promotes their transmission to clinically relevant bacterial strains (Hendriksen et al., 2019 & Hanna, et al., 2023). The accumulation of antibiotics in the environment resulting from industrial discharges, inappropriate disposal and agricultural runoff drives the emergence of adaptive resistance mechanisms in previously antibiotic sensitive bacterial populations, frequently involving mobile genetic elements such as integrons and plasmids (Zhang et al., 2021). The detection of pharmaceutical compounds including antibiotics in water and soil samples has heightened global concern prompting intensified environmental surveillance. Recent studies have consistently reported the widespread presence of these compounds across diverse ecosystems (Wang, et al., 2021). Waste water treatment facilities particularly those associated with pharmaceutical manufacturing have been identified as hotspots for the emergence and dissemination of multidrug resistant bacteria (Shen, et al., 2023). Once released into terrestrial and aquatic ecosystems, these bacteria contribute to the expansion of the environmental resistome and facilitate the transfer of antimicrobial resistance genes to pathogenic bacterial species aggravating public health concerns (Peterson, E., & Kaur, P. 2018).

Comparative studies have shown significantly higher loads of antimicrobial resistance bacteria in polluted versus unpolluted environments highlighting anthropogenic activities as significant drivers of environmental antimicrobial resistance (Stanton, et al., 2022). Environmental bacteria adapted to diverse ecological conditions act as vectors for the dissemination of resistance genes either directly or through food chains. Existence of antimicrobial resistance genes in environmental bacteria intensifies the chances of gene exchange among clinically related pathogens thereby escalating public health risks (Sharma, et al., 2024). Improper discharge of untreated or inadequately treated industrial waste water contributes to environmental degradation leading to the contamination of ground and surface water and the introduction of pathogenic microorganisms into human populations (Lorenzo, M., & Pico, Y. 2019).Through bioaccumulation in the food chain, humans are increasingly exposed to resistance microorganisms and antimicrobial resistance bacteria resulting in infections that are more difficult to treat and control (Lee, et al., 2020). The rise of antimicrobial resistance pathogens particularly enteric bacteria in environmental samples from low-income countries is a growing concern often attributed to insufficient infrastructure and limited public awareness (Hanna et al., 2023). **The widespread and often indiscriminate use of antibiotics in both clinical and non-clinical settings has been strongly linked to the dissemination and persistence of resistance** (Mutua et al., 2023). **Chronic overexposure to antimicrobials fosters the selection of resistant bacterial strains, ultimately reducing treatment efficacy and contributing to increased morbidity, mortality, and healthcare costs** (Pormohammad et al., 2019).

**2. MATERIALS AND METHODS**

The study was conducted in Nairobi County, Kenya. Industrial waste water samples were collected using a cross-section study design employing a systematic sampling approach across selected sites within the study area.

**Sample Collection**

Waste water samples for this study were collected from the Industrial Area in Nairobi, Kenya. Sampling was performed aseptically using sterile 10 ml screw-cap bottles. The samples were immediately stored in an icebox and transported under cold chain conditions to the bacteriology laboratory for microbiological analysis.

**Bacteria Isolation from Waste Water**

Bacterial isolation was performed using the serial dilution technique with sterile distilled water. A sample suspension was prepared by mixing the wastewater effluent with sterile distilled water and subsequently diluted in tenfold series from 10⁻¹ to 10⁻⁵. From each dilution, 0.1 ml was aseptically pipetted and spread onto nutrient agar plates (HiMedia Laboratories, India) using a sterile glass spreader. The inoculated plates were incubated at 37 °C for 18–24 hours, following the method described by Shukla and Sahu (2021). After incubation, discrete bacterial colonies were selected and sub cultured onto MacConkey agar and Mannitol salt agar for further characterization.

**Identification of Isolated Bacteria Species**

Bacterial cell morphology was initially characterized using Gram staining. Subsequent identification of Gram-negative isolates was performed using the API® 20E identification system (BioMérieux, France) in accordance with the manufacturer's instructions. All isolates were preserved in tryptic soy broth supplemented with 10% glycerol and stored at −80 °C for further analysis.

**Antibiotic Susceptibility Testing**

Antibiotic susceptibility testing was carried out using the disk diffusion method on Mueller Hinton agar plates (HiMedia Laboratories, India) following standard protocols. The antibiotics tested included Ampicillin (10 mg), Tetracycline (30 mg), Amoxicillin (30 mg), Gentamicin (10 mg), Cefepime (30 mg), Ceftriaxone (30 mg), Imipenem (10 mg), Levofloxacin (30 mg), Ciprofloxacin (5 mg), and Sulfamethoxazole/Trimethoprim (25 mg) (Oxoid Ltd., United Kingdom). Pure bacterial colonies were inoculated onto Mueller–Hinton agar and antibiotic discs were aseptically placed on the surface. Plates were incubated at 37 °C for 18–24 hours. Zones of inhibition diameter were measured in millimeters and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. For quality assurance of media and antibiotic discs, Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 29213 (American Type Culture Collection) were used.

**Statistical Analysis**

Data were documented using WHO-NET software and Microsoft Excel, both secured with password protection to ensure confidentiality. Findings were presented using bar graphs.

**3. RESULTS**

**3.1 Antimicrobial Patterns**

A total pf 101 bacterial isolates were recovered from waste water samples collected from industrial environments in Nairobi. Among these, *Escherichia coli* accounted for 37 isolates (36.6%), *Klebsiella spp.* for38 isolates (37.6%)*,* and *Pseudomonas spp*. 26 (25.7%)*.* All isolates were subjected to antimicrobial susceptibility testing using antibiotic-impregnated discs. The majority of isolates exhibited multidrug resistance (MDR), with the highest resistance observed against Ampicillin (89/101, 88.1%), followed by Cotrimoxazole (55/101). In contrast, Gentamicin demonstrated the lowest resistance rate (9/101) which may be attributed to its restricted availability over the counter reducing the likelihood of misuse compared to commonly accessible oral antimicrobials. Levofloxacin exhibited the highest susceptibility, with 82 of the 101 isolates (81.1%) remaining sensitive to this agent as shown in Table 1.

**Table 1. Frequency of Antimicrobial Susceptibility Levels in *Escherichia coli, Klebsiella* and *Pseudomonas* Bacterial Species**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Disc Potency** | **Resistant** |  | **Intermediate** |  | **Susceptible** |  |  |
| Antibiotics | **μg** | **n** | **%** | **n** | **%** | **n** | **%** | **Total**  |
| Ampicillin  | 10 | 89 | 88.1 | 3 | 2.9 | 9 | 8.9 | 101 |
| Cefuroxime  | 30 | 39 | 39 | 8 | 8 | 53 | 53 | 101 |
| Ceftriaxone  | 30 | 46 | 45.5 | 7 | 6.9 | 48 | 47.5 | 101 |
| Cefepime  | 30 | 20 | 19.8 | 19 | 18.8 | 62 | 61.3 | 101 |
| Imipenem | 10 | 13 | 12.8 | 20 | 19.8 | 68 | 67.3 | 101 |
| Gentamicin  | 10 | 9 | 8.9 | 25 | 24.7 | 67 | 66.3 | 101 |
| Ciprofloxacin  | 5 | 23 | 22.7 | 17 | 16.8 | 61 | 60.3 | 101 |
| Levofloxacin  | 5 | 10 | 9.9 | 9 | 8.9 | 82 | 81.1 | 101 |
| Trimethoprim/Sulfamethoxazole | 25 | 55 | 54.4 | 2 | 1.9 | 44 | 43.5 | 101 |
| Tetracycline  | 30 | 46 | 45.5 | 3 | 2.9 | 52 | 51.4 | 101 |

**3.2 Distribution of Antimicrobial Resistance in Different Bacterial Isolates**

Resistance to Ampicillin was predominantly observed in *Klebsiella* species followed by *Pseudomonas* species with *Escherichia coli* isolates exhibiting the lowest resistance rates. Notably, *Pseudomonas* species demonstrated resistance to all antibiotics tested. Overall, all the organisms tested showed moderate resistance to 3rd generation Cephalosporins (Cefuroxime, Ceftriaxone) compared to Fluoroquinolones (Ciprofloxacin). The least probable resistance to all the isolates was shown in Carbapenems (Imipenem) as shown in Figure 1.

**Fig. 1. Percentage Resistance of Isolate Bacteria to Different Tested Antimicrobials**

**4. DISCUSSION**

Industries discharge of untreated or partially treated waste water remains a major source of environmental pollution and poses a significant public health threat. In this study, a concerning trend of antimicrobial resistance (AMR) was observed among selected Gram-negative bacterial isolates. Ampicillin exhibited the highest resistance rate with 88.1% of all tested isolates demonstrating non-sensitivity. This finding is consistent with a study conducted in North Kerala, India which reported 92.59% ampicillin resistance to *Klebsiella pneumoniae* (Kumara et al., 2022) and a similar study in Northeast Ethiopia that reported 93% Ampicillin resistance (Tilahun, M., Sharew, B., & Shibabaw, A. 2024). The high resistance rates may be attributed to the extensive production of β-lactamase enzymes particularly bla SHV and bla TEM genes which effectively hydrolyze ampicillin. Cefuroxime resistance was observed in 39% of the bacterial isolates with an additional 8% showing intermediate sensitivity. These results align with findings from a study in Democratic Republic of Congo where 50% resistance to Cefuroxime was reported among *Escherichia coli* and *Klebsiella spp.* (Mukubwa, et al., 2023). The finding demonstrates sensible effectiveness of Cefuroxime is compromised probably by ESBLs (Extended spectrum β-lactamases). Ceftriaxone resistance was observed in 45.5% of all isolates in this study. This contrasts with findings from a study in Ethiopia by Geleta, et al. (2024) which reported 100% resistance to ceftriaxone among *Klebsiella spp* isolates. Such discrepancies likely reflect differences in the prevalence of extended spectrum beta-lactamase (ESBL) producing organisms and variations in antibiotic usage patterns across regions. Cefepime demonstrated a susceptibility rate of 61.3% while 19.8% of isolates were resistant. However, a separate hospital-based study reported 100% resistance to cefepime among Pseudomonas spp. suggesting that nosocomial environments may exert greater selective pressure contributing to the emergence of highly resistant strains (Kemal et al., 2025). **This underscores the role of healthcare settings as reservoirs and amplifiers of resistant organisms.** Imipenem exhibited relatively low resistance at 12.8% with a sensitivity rate of 67.3%. These findings are consistent with a study by Mukubwa et al. (2023), who reported 13% imipenem resistance among Escherichia coli and Klebsiella spp. isolates. However, considerably higher resistance rates of 56.9% in Klebsiella pneumoniae and 15.6% in Escherichia coli were reported in a study from North India (Jaggi et al., 2019) likely due to the presence of carbapenem producing genes such as New Delhi metallo- β-lactamase (NDM) and Klebsiella pneumoniae carbapenemase (KPC). **These enzymes significantly compromise carbapenem efficacy and are a growing concern in the treatment of multidrug-resistant infections.** Gentamicin was the most effective antibiotic in this study demonstrating a low resistance rate of 8.9% among all the tested isolates. This corroborates findings from a study in Uganda which reported a similar gentamicin resistance rate of 9% (Abongomera, et al., 2021). In contrast, a study in Egypt observed a significantly higher resistance rate of 84.6% in *Klebsiella pneumoniae* isolates (Geleta, et al., 2024) highlighting regional disparity in antimicrobial resistance patterns and variations in aminoglycoside use. Ciprofloxacin showed susceptibility rate of 60.3% with a resistance rate of 22.7%. Higher resistance rates such as the 44% reported in a previous study in Uganda have been linked to the widespread availability and inappropriate use of ciprofloxacin particularly in settings where antibiotics can be obtained over the counter without prescription (Abongomera et al., 2021). In the present study, Levofloxacin resistance was observed at 9.9%, with the highest susceptibility recorded at 81.1%. These results highlight levofloxacin's ongoing effectiveness in environmental isolates and point to its possible use in treating Gram-negative bacterial infections in comparable environments. These findings contrast with those of Cheng et al. (2020) who reported a significantly higher levofloxacin resistance rate of 38.8%. In this study, resistance to tetracycline was observed in 45.5% of isolates which is lower than the 73.33% resistance reported in Escherichia coli isolates from Ghana (Adzitey, et al., 2020). The observed resistance may be attributed to intrinsic mechanisms such as reduced membrane permeability and the activity of efflux pumps which limit intracellular antibiotic accumulation. Resistance to trimethoprim/sulfamethoxazole was recorded in 54.4% of isolates. A recent study by Geleta et al. (2024) reported 100% resistance in Klebsiella pneumoniae isolates which was primarily attributed to the widespread use of this antibiotic in the general population and the horizontal gene transfer of sulfonamide resistance genes, including sul1 and sul2. These genes are frequently linked to mobile genetic elements, which speeds up their spread among pathogenic and environmental bacteria.

**5. CONCLUSION**

The detection of antimicrobial resistant bacteria including *Klebsiella spp.,* *Escherichia coli*, and *Pseudomonas spp.* in industrial waste water with varying degrees of resistance to commonly used antimicrobials poses a significant public health threat. The discovery of multidrug resistance bacteria strains mainly in *Pseudomonas spp* and *Klebsiella spp* highlights significant role of industrial waste in the development and dissemination of antimicrobial resistance in the environment. These findings emphasize crucial need for enhanced waste management strategies, routine monitoring and the implementation of stringent environmental regulations to mitigate the transmission of antibiotic resistance from industrial sources to the public. Without prompt and coordinated action, environmental reservoirs of resistance may substantially contribute to the escalating global burden of drug resistant infections.

**INFORMED CONSENT STATEMENT**

Not applicable.

**ETHICAL APPROVAL**

Ethical approval for the study was obtained from the Jomo Kenyatta University of Agriculture and Technology (JKUAT) Institutional Scientific and Ethical Review Committee under the approval number JKU/2/11/TM318-C003-1774/2016.

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