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Assessment of Heavy Metal Co-Contamination and Antibiotic Resistance Genes in Hospital Wastewater Discharges in Southern Nigeria

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ABSTRACT

Hospital wastewater has emerged as an important environmental reservoir for heavy metals, multidrug-resistant bacteria, and antibiotic resistance genes (ARGs), posing significant ecological and public health concerns. This study assessed the occurrence of antibiotic resistance genes and heavy metal co-contamination in hospital wastewater discharges from selected healthcare facilities in Southern Nigeria. A cross-sectional analytical design was adopted, and 20 composite wastewater samples were collected for physicochemical, microbiological, heavy metal, and molecular analyses. Physicochemical parameters were determined using standard methods, while heavy metals (Fe, Zn, Cu, Pb, Cd, Cr, and Ni) were quantified using Atomic Absorption Spectrophotometry. Bacterial isolates were identified using conventional microbiological techniques, and antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion method.

Polymerase Chain Reaction (PCR) was employed to detect the presence of the blaTEM, blaCTX-M, blaNDM, mecA, sul1, tetA, qnrS, and intI1 genes. The Heavy Metal Pollution Index (HPI), Pearson correlation analysis, and Principal Component Analysis (PCA) were used to interpret the data. The results showed elevated Biological Oxygen Demand and Chemical Oxygen Demand, indicating high organic pollution. Lead, cadmium, chromium, and nickel exceeded recommended discharge limits in several samples, while 40% of the wastewater samples were classified as highly polluted based on HPI. Molecular analysis revealed a high prevalence of intI1 (90.0%), blaTEM (85.0%), sul1 (80.0%), and blaCTX-M (75.0%). Significant positive correlations were observed between heavy metals and ARGs, suggesting possible co-selection mechanisms. Principal Component Analysis explained 82.1% of the total variance, indicating that heavy metals, organic pollution, and hospital activities were the principal factors influencing wastewater quality. The study demonstrates that untreated hospital wastewater constitutes an important environmental source of heavy metals and antibiotic resistance genes, underscoring the urgent need for effective wastewater treatment, continuous environmental surveillance, and strengthened antimicrobial resistance control policies in Nigeria.

Keywords: *Antibiotic resistance genes, heavy metals, hospital wastewater, antimicrobial resistance, co-contamination, Southern Nigeria, public health.*

1.0 Introduction

Hospital wastewater is increasingly recognised as one of the most important environmental reservoirs of chemical and biological contaminants because it receives effluents from diverse healthcare activities, including patient care, surgical procedures, clinical laboratories, diagnostic units, pharmacies, laundries, and intensive care facilities. Unlike municipal wastewater, hospital effluents contain a complex mixture of pharmaceutical residues, disinfectants, pathogenic microorganisms, antibiotic-resistant bacteria, antibiotic resistance genes (ARGs), heavy metals, and other emerging contaminants that are continuously discharged into surrounding aquatic environments if not adequately treated [1–3]. The coexistence of these contaminants creates favourable conditions for environmental pollution, microbial adaptation, and the evolution of antimicrobial resistance, thereby posing significant ecological and public health concerns [4,5].

Antimicrobial resistance (AMR) has become one of the greatest global health challenges of the twenty-first century. The World Health Organization recognises AMR as a major threat capable of undermining decades of progress in infectious disease management. Healthcare facilities represent important hotspots for the emergence and dissemination of resistant microorganisms because of the extensive use of antibiotics for prophylaxis and treatment [6,7]. Consequently, hospital wastewater frequently contains antibiotic residues together with multidrug-resistant bacteria that are discharged into receiving water bodies, where they interact with indigenous microbial communities [8]. Such environments promote horizontal gene transfer through plasmids, integrons, transposons, and other mobile genetic elements, facilitating the dissemination of clinically important antibiotic resistance genes among environmental bacteria [9,10]. This environmental pathway contributes substantially to the persistence of resistant microorganisms and complicates efforts aimed at controlling antimicrobial resistance.

Heavy metals constitute another important class of contaminants commonly detected in hospital wastewater. Metals including lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni), copper (Cu), iron (Fe), and zinc (Zn) originate from diagnostic reagents, laboratory chemicals, medical devices, pharmaceutical formulations, plumbing materials, and various hospital operations [11–13]. Unlike many organic pollutants, heavy metals are non-biodegradable, environmentally persistent, and capable of accumulating in sediments, aquatic organisms, and higher trophic levels. Continuous exposure to these toxic elements has been associated with neurological disorders, renal dysfunction, reproductive impairment, carcinogenesis, developmental abnormalities, and other adverse health outcomes [14]. Their persistence in aquatic ecosystems therefore represents a long-term environmental and public health challenge.

Recent evidence indicates that heavy metal contamination and antimicrobial resistance are closely interconnected through the process of co-selection. Resistance genes conferring tolerance to heavy metals and antibiotics are frequently located on the same plasmids or integrative mobile genetic elements [15]. Consequently, environmental exposure to heavy metals may indirectly select for antibiotic-resistant bacterial populations even in the absence of antibiotic pressure [16]. Hospital wastewater therefore provides an ideal ecological niche where heavy metals, pharmaceutical residues, disinfectants, and resistant microorganisms coexist, creating multiple selective pressures that enhance bacterial adaptation, genetic exchange, and the persistence of multidrug resistance [10,16]. This interaction has become an important focus of environmental microbiology because it extends antimicrobial resistance beyond healthcare settings into natural ecosystems.

In many developing countries, including Nigeria, hospital wastewater is often discharged with minimal or no treatment before entering municipal drainage systems, rivers, wetlands, or groundwater recharge zones [17]. Rapid urbanisation, inadequate wastewater treatment infrastructure, weak environmental regulation, and increasing healthcare activities have collectively contributed to the deterioration of water quality in many regions of the country [18,19]. Several investigations have documented elevated concentrations of heavy metals, pharmaceutical residues, pathogenic microorganisms, and emerging contaminants in surface water, groundwater, sediments, and hospital effluents across Nigeria [13,15,18,20]. These findings indicate that healthcare facilities contribute substantially to environmental contamination and may serve as important reservoirs for antibiotic resistance genes and other hazardous pollutants.

Studies conducted within the Niger Delta and other parts of Southern Nigeria have further demonstrated widespread contamination of aquatic ecosystems by petroleum hydrocarbons, volatile organic compounds, heavy metals, pharmaceutical residues, and pathogenic microorganisms arising from multiple anthropogenic activities [21–26]. While these investigations have improved understanding of environmental pollution, relatively few studies have simultaneously evaluated physicochemical characteristics, heavy metal contamination, bacterial diversity, antimicrobial susceptibility patterns, and the molecular occurrence of antibiotic resistance genes in hospital wastewater [10,15]. Integrated assessment of these parameters is essential for understanding the complex interactions between chemical contaminants and microbial resistance that influence environmental quality and public health.

Considering the increasing burden of antimicrobial resistance and the growing environmental impacts of untreated healthcare effluents, there is an urgent need for comprehensive investigations that integrate chemical, microbiological, and molecular approaches to wastewater assessment [6,15,18]. Such information is critical for strengthening wastewater management strategies, supporting antimicrobial stewardship programmes, guiding environmental monitoring, and informing public health policies aimed at reducing the dissemination of antimicrobial resistance [4,9,17].

Therefore, this study assessed the physicochemical characteristics, heavy metal concentrations, bacterial diversity, antimicrobial susceptibility profiles, antibiotic resistance genes, heavy metal pollution status, and the relationships between heavy metal contamination and antibiotic resistance genes in hospital wastewater discharged from selected healthcare facilities in Southern Nigeria. The findings provide valuable baseline information for environmental risk assessment and contribute to the growing body of evidence required to develop sustainable wastewater treatment and antimicrobial resistance control strategies in Nigeria [10,15,20,26].

2.0 Materials and Methods

2.1 Study Area

The study was conducted in selected tertiary and secondary healthcare facilities located across Southern Nigeria. The region lies between latitudes 4°30′N and 7°15′N and longitudes 3°00′E and 8°45′E, encompassing major urban centres within the South-South and South-East geopolitical zones. The climate is tropical, characterised by high annual rainfall (2,000–4,000 mm), relative humidity exceeding 75%, and mean annual temperatures ranging from 26 to 32 °C. Hospital wastewater generated within these facilities originates from medical wards, surgical units, laboratories, laundries, pharmacies, intensive care units, and diagnostic centres before being discharged into municipal drainage systems or receiving water bodies.

2.2 Research Design

A cross-sectional analytical study design was adopted to evaluate physicochemical characteristics, heavy metal contamination, bacterial diversity, antibiotic susceptibility profiles, antibiotic resistance genes (ARGs), and the relationship between heavy metal contamination and antimicrobial resistance in hospital wastewater discharged from selected healthcare facilities in Southern Nigeria.

2.3 Sample Collection

A total of 20 composite hospital wastewater samples were collected from wastewater discharge outlets of selected hospitals using sterile 1 L high-density polyethene bottles. Samples were obtained during peak hospital activity periods to ensure representative wastewater composition. Separate aliquots were collected for physicochemical analysis, heavy metal determination, microbiological examination, and molecular analysis.

Samples for heavy metal analysis were acidified with concentrated nitric acid to a pH below 2 immediately after collection, while microbiological samples were transported in ice-packed coolers at approximately 4 °C and analysed within 24 hours.

2.4 Determination of Physicochemical Parameters

Temperature, pH, electrical conductivity (EC), dissolved oxygen (DO), and total dissolved solids (TDS) were measured in situ using portable digital meters calibrated before each sampling exercise. Biological Oxygen Demand (BOD_5) was determined using the standard five-day incubation method, whereas Chemical Oxygen Demand (COD) was determined using the dichromate reflux method.

2.5 Heavy Metal Analysis

Hospital wastewater samples were digested using concentrated nitric acid and hydrogen peroxide digestion procedures before analysis. Concentrations of iron (Fe), zinc (Zn), copper (Cu), lead (Pb), cadmium (Cd), chromium (Cr), and nickel (Ni) were quantified using Atomic Absorption Spectrophotometry (AAS). Calibration standards, analytical blanks, and duplicate samples were analysed throughout the study to ensure analytical accuracy and quality control.

2.6 Isolation and Identification of Bacterial Isolates

Serial dilution techniques were employed for microbial enumeration, after which aliquots were cultured on selective and differential media using the spread plate technique. Pure bacterial colonies were obtained by repeated sub-culturing and identified using colony morphology, Gram staining, and standard biochemical tests. Representative isolates were further confirmed using commercially available bacterial identification systems where necessary.

2.7 Antibiotic Susceptibility Testing

The antimicrobial susceptibility of all bacterial isolates was determined using the Kirby–Bauer disc diffusion technique on Mueller–Hinton agar. Antibiotics evaluated included ampicillin, ceftriaxone, ceftazidime, ciprofloxacin, gentamicin, meropenem, and colistin. Zones of inhibition were measured after incubation and interpreted as susceptible, intermediate, or resistant according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

2.8 Molecular Detection of Antibiotic Resistance Genes

Genomic DNA was extracted from confirmed multidrug-resistant bacterial isolates using a commercial bacterial DNA extraction kit following the manufacturer's instructions. Polymerase Chain Reaction (PCR) amplification was performed to detect selected antibiotic resistance genes, including blaTEM, blaCTX-M, blaNDM, mecA, sul1, tetA, qnrS, and the class 1 integron gene (intI1). Amplified PCR products were separated by agarose gel electrophoresis, stained with ethidium bromide, visualised under ultraviolet illumination, and documented using a gel documentation system.

2.9 Heavy Metal Pollution Assessment

The level of heavy metal contamination in hospital wastewater was evaluated using the Heavy Metal Pollution Index (HPI). The index integrates individual heavy metal concentrations into a single pollution indicator to classify wastewater quality into low, moderate, and high pollution categories based on internationally accepted threshold values.

2.10 Statistical Analysis

Descriptive statistics, including means, standard deviations, frequencies, percentages, and ranges, were computed for all measured variables. Pearson correlation analysis was used to evaluate relationships between heavy metal concentrations and antibiotic resistance genes. Principal Component Analysis (PCA) was employed to identify the dominant factors influencing wastewater quality and antimicrobial resistance dissemination. Statistical significance was established at $p < 0.05$, and all analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 27.0.

3.0 Results

The physicochemical analysis revealed considerable variation in the quality of hospital wastewater across the sampled facilities. Temperature values ranged from 27.1 to 31.8 °C, with a mean of 29.3 ± 1.4 °C, remaining below the recommended discharge limit of 40 °C. The pH ranged between 6.21 and 8.46 (mean 7.32 ± 0.54), indicating that the wastewater was generally neutral to slightly alkaline and within the acceptable regulatory range. Electrical conductivity varied from 512 to 1,964 µS/cm, with a mean value of 1,228 ± 406 µS/cm, suggesting moderate to high concentrations of dissolved ions, while several samples exceeded the recommended discharge guideline. Total dissolved solids ranged from 318 to 1,278 mg/L, averaging 752 ± 281 mg/L, indicating appreciable mineral and dissolved solid content. Dissolved oxygen values were relatively low (1.4–5.8 mg/L; mean 3.2 ± 1.1 mg/L), reflecting poor oxygenation associated with organic pollution. Biological oxygen demand ranged from 68 to 286 mg/L (mean 148 ± 59 mg/L) and chemical oxygen demand ranged from 182 to 694 mg/L (mean 394 ± 126 mg/L), both substantially exceeding permissible limits, thereby indicating a high organic load and the presence of biodegradable and non-biodegradable pollutants requiring effective treatment before discharge, as shown in Table 3.1.

Table 3.1. Physicochemical characteristics of hospital wastewater samples (n = 20)

Parameter	Range	Mean ± SD	WHO/FMEnv Guideline
Temperature (°C)	27.1–31.8	29.3 ± 1.4	<40
pH	6.21–8.46	7.32 ± 0.54	6.0–9.0
Electrical Conductivity (µS/cm)	512–1,964	1,228 ± 406	1,500
Total Dissolved Solids (mg/L)	318–1,278	752 ± 281	2,000
Dissolved Oxygen (mg/L)	1.4–5.8	3.2 ± 1.1	≥5
Biological Oxygen Demand (mg/L)	68–286	148 ± 59	30
Chemical Oxygen Demand (mg/L)	182–694	394 ± 126	80

Heavy metal analysis demonstrated the presence of both essential and toxic metals in hospital wastewater. Iron exhibited the highest concentration, ranging from 0.62 to 3.81 mg/L with a mean of 1.92 ± 0.84 mg/L, approaching the recommended guideline in several samples. Zinc concentrations varied between 0.21 and 1.34 mg/L, while copper ranged from 0.04 to 0.63 mg/L, both remaining below the permissible limits. Conversely, toxic metals such as lead, cadmium, chromium, and nickel exceeded guideline values in several samples. Lead concentrations ranged from 0.02 to 0.19 mg/L, averaging 0.08 ± 0.05 mg/L, which is approximately eight times higher than the WHO permissible limit. Cadmium ranged from 0.003 to 0.041 mg/L (mean 0.018 ± 0.011 mg/L), chromium ranged from 0.02 to 0.27 mg/L (mean 0.11 ± 0.07 mg/L), and nickel ranged from 0.03 to 0.18 mg/L (mean 0.09 ± 0.04 mg/L), all exceeding recommended limits in numerous samples as shown in Table 3.2.

Table 3.2. Heavy metal concentrations in hospital wastewater samples (mg/L)

Heavy Metal	Range	Mean ± SD	WHO Limit
Fe	0.62–3.81	1.92 ± 0.84	2.00
Zn	0.21–1.34	0.68 ± 0.31	3.00
Cu	0.04–0.63	0.27 ± 0.15	2.00
Pb	0.02–0.19	0.08 ± 0.05	0.01
Cd	0.003–0.041	0.018 ± 0.011	0.003
Cr	0.02–0.27	0.11 ± 0.07	0.05
Ni	0.03–0.18	0.09 ± 0.04	0.02

Molecular analysis revealed a widespread occurrence of antibiotic resistance genes (ARGs) within hospital wastewater. The intI1 gene showed the highest prevalence, occurring in 18 (90.0%) of the samples, indicating extensive dissemination of mobile genetic elements that facilitate horizontal gene transfer. The blaTEM gene was detected in 17 (85.0%) samples, followed closely by sul1 in 16 (80.0%) and blaCTX-M in 15 (75.0%), demonstrating widespread resistance to β-lactam and sulfonamide antibiotics. The tetA gene occurred in 14 (70.0%) samples, while qnrS and mecA were detected in 11 (55.0%) and 8 (40.0%) samples, respectively. The blaNDM carbapenem-resistance gene, although less prevalent, was identified in 6 (30.0%) samples, as shown in Table 3.4.

Table 3.3. Detection frequency of antibiotic resistance genes in hospital wastewater

Antibiotic Resistance Gene	Positive Samples (n)	Prevalence (%)
blaTEM	17	85.0
blaCTX-M	15	75.0
blaNDM	6	30.0
mecA	8	40.0
sul1	16	80.0
tetA	14	70.0
qnrS	11	55.0
intI1	18	90.0

A total of 65 bacterial isolates were recovered from hospital wastewater samples. Escherichia coli was the predominant organism, accounting for 18 isolates (27.7%), followed by Klebsiella pneumoniae with 14 isolates (21.5%) and Pseudomonas aeruginosa with 12 isolates (18.5%). Acinetobacter baumannii contributed 9 isolates (13.8%), while Enterococcus faecalis and Staphylococcus aureus accounted for 7 (10.8%) and 5 (7.7%) isolates, respectively, as shown in Table 3.4.

Table 3.4. Bacterial isolates recovered from hospital wastewater

Bacterial Isolate	Frequency (n)	Percentage (%)
Escherichia coli	18	27.7
Klebsiella pneumoniae	14	21.5
Pseudomonas aeruginosa	12	18.5
Acinetobacter baumannii	9	13.8
Enterococcus faecalis	7	10.8
Staphylococcus aureus	5	7.7
Total	**65**	**100.0**

The antibiotic susceptibility profile demonstrated widespread multidrug resistance among bacterial isolates. Ampicillin showed the highest resistance rate, with 60 isolates (92.3%)resistant and only 5 isolates (7.7%) susceptible. Resistance to ceftriaxone (80.0%) and ceftazidime (75.4%) was also high, indicating extensive resistance to third-generation cephalosporins. More than half of the isolates (58.5%) were resistant to ciprofloxacin, whereas resistance to gentamicin was comparatively lower at 43.1%. Carbapenem resistance remained relatively low, with only 18.5% of isolates resistant to meropenem, while colistin demonstrated the greatest effectiveness, with 93.8% of isolates remaining susceptible, as shown in Table 3.5.

Table 3.5. Antibiotic resistance pattern of bacterial isolates recovered from hospital wastewater

Antibiotic	Resistant n (%)	Susceptible n (%)
Ampicillin	60 (92.3)	5 (7.7)
Ceftriaxone	52 (80.0)	13 (20.0)
Ceftazidime	49 (75.4)	16 (24.6)
Ciprofloxacin	38 (58.5)	27 (41.5)
Gentamicin	28 (43.1)	37 (56.9)
Meropenem	12 (18.5)	53 (81.5)
Colistin	4 (6.2)	61 (93.8)

The Heavy Metal Pollution Index (HPI) revealed varying degrees of heavy metal contamination among hospital wastewater samples. Four samples (20.0%) were classified as having low pollution levels (HPI < 50), indicating relatively acceptable heavy metal concentrations. Eight samples (40.0%) exhibited moderate pollution (HPI = 50–100), suggesting increasing environmental contamination, while another eight samples (40.0%) recorded HPI values exceeding 100, indicating severe heavy metal pollution, as shown in Table 3.6.

Table 3.6. Heavy Metal Pollution Index (HPI) classification of hospital wastewater samples

Pollution Category	Number of Samples	Percentage (%)
Low (<50)	4	20.0
Moderate (50–100)	8	40.0
High (>100)	8	40.0

Pearson correlation analysis revealed significant positive associations between heavy metals and antibiotic resistance genes. The strongest correlation was observed between nickel and intI1 ($r = 0.74$; $p < 0.001$), followed closely by lead and blaTEM ($r = 0.71$; $p < 0.001$). Cadmium showed a strong positive relationship with sul1 ($r = 0.66$; $p = 0.002$), while chromium correlated positively with tetA ($r = 0.61$; $p = 0.004$). Iron demonstrated a weaker but statistically significant association with qnrS ($r = 0.42$; $p = 0.048$), as shown in Table 3.7.

Table 3.7. Pearson correlation coefficients between heavy metals and antibiotic resistance genes

Variable Pair	Correlation Coefficient (r)	p-value
Pb vs blaTEM	0.71	<0.001
Cd vs sul1	0.66	0.002
Cr vs tetA	0.61	0.004
Ni vs intI1	0.74	<0.001
Fe vs qnrS	0.42	0.048

Principal Component Analysis identified three principal components that collectively explained 82.1% of the total variance in the dataset. The first principal component (PC1) had an eigenvalue of 5.82 and accounted for 48.5% of the total variance, indicating that it represented the dominant environmental processes influencing wastewater quality. The second component (PC2) explained an additional 20.6%, increasing the cumulative variance explained to 69.1%, while the third component (PC3) contributed 13.0%, resulting in a cumulative explained variance of 82.1%. The high cumulative variance indicates that the selected physicochemical parameters, heavy metals, and antibiotic resistance genes were largely controlled by a few dominant environmental factors, highlighting the combined influence of hospital activities, wastewater composition, and anthropogenic pollution on the overall quality of hospital wastewater, as shown in Table 3.8.

Table 3.8. Principal component analysis of physicochemical parameters, heavy metals, and antibiotic resistance genes

Principal Component	Eigenvalue	Variance Explained (%)	Cumulative Variance (%)
PC1	5.82	48.5	48.5
PC2	2.47	20.6	69.1
PC3	1.56	13.0	82.1

4.0 Discussion

The findings of this study demonstrate that hospital wastewater discharged from selected healthcare facilities in Southern Nigeria represents a significant environmental reservoir of organic pollutants, heavy metals, multidrug-resistant bacteria, and antibiotic resistance genes (ARGs). The simultaneous occurrence of elevated physicochemical pollutants, toxic metals, resistant bacterial isolates, and mobile genetic resistance determinants highlights the growing environmental and public health implications of untreated hospital effluents. Similar observations have been reported in previous investigations, which identified healthcare wastewater as an important source of emerging contaminants capable of accelerating antimicrobial resistance dissemination and environmental pollution [10,17,29]. These findings further reinforce the growing concern that hospital wastewater constitutes a critical interface linking healthcare activities with environmental contamination.

The physicochemical characteristics observed in this study indicate that the hospital wastewater possessed a considerable pollution load before discharge into receiving water bodies. The elevated electrical conductivity and total dissolved solids suggest high concentrations of dissolved inorganic ions originating from pharmaceuticals, laboratory reagents, disinfectants, diagnostic chemicals, and other hospital-derived wastes. Likewise, the high biological oxygen demand (BOD) and chemical oxygen demand (COD) values demonstrate substantial organic pollution requiring considerable oxygen for microbial degradation. Such elevated oxygen demand reduces the assimilative capacity of receiving aquatic systems and adversely affects aquatic biodiversity. Comparable hydrochemical deterioration has been documented in hospital-associated water sources and urban wastewater systems across Nigeria, where untreated healthcare effluents contributed significantly to declining water quality [6,13,14,17].

Although the measured pH values remained within acceptable discharge limits, the relatively low dissolved oxygen concentrations indicate active microbial degradation of organic matter within the wastewater. Reduced dissolved oxygen has important ecological implications because oxygen depletion limits aerobic biological processes, alters aquatic ecosystem functioning, and favours the proliferation of facultative anaerobic and pathogenic microorganisms. Previous investigations similarly reported that persistent wastewater discharge lowers dissolved oxygen concentrations and compromises the ecological integrity of receiving rivers and groundwater systems [3,17]. Consequently, the physicochemical characteristics obtained in the present study demonstrate the necessity for effective treatment of hospital wastewater before environmental discharge.

Heavy metal analysis revealed appreciable concentrations of iron, zinc, copper, lead, cadmium, chromium, and nickel, with several toxic metals exceeding recommended discharge limits.

While iron occurred at the highest concentration, the environmental significance of lead, cadmium, chromium, and nickel is considerably greater because these metals are highly toxic, persistent, and capable of bioaccumulation within aquatic ecosystems. Their occurrence in hospital wastewater reflects contributions from diagnostic laboratories, pharmaceutical formulations, medical equipment, plumbing systems, and other healthcare-related activities. Similar findings have been reported in surface waters, groundwater, sediments, and estuarine environments across Nigeria, where anthropogenic activities significantly increased heavy metal contamination and ecological risks [8,12,19,23].

The elevated concentrations of lead and cadmium are particularly concerning because of their well-established toxicological effects on humans and aquatic organisms. Chronic exposure to lead has been associated with neurological impairment, cardiovascular disorders, and developmental abnormalities, whereas cadmium exposure has been linked to renal dysfunction, skeletal disorders, reproductive toxicity, and carcinogenesis. Likewise, chromium and nickel are recognised for their carcinogenic and mutagenic properties following prolonged exposure. The presence of these metals above regulatory thresholds therefore indicates that untreated hospital wastewater may contribute substantially to long-term environmental contamination and increased public health risks if discharged without adequate treatment [8,12,23,28].

One of the most important observations in this study is the coexistence of heavy metals and antibiotic resistance genes within the same wastewater environment. Current evidence indicates that heavy metals contribute to antimicrobial resistance through co-selection mechanisms whereby genes conferring resistance to metals and antibiotics are frequently located on the same mobile genetic elements. Consequently, continuous environmental exposure to heavy metals favours bacterial populations carrying both heavy metal resistance genes and antibiotic resistance genes, even when antibiotics are absent. Similar relationships have been documented in previous environmental studies, where heavy metal contamination was shown to promote the persistence and dissemination of multidrug-resistant bacteria through horizontal gene transfer [10,15]. This interaction significantly increases the environmental burden of antimicrobial resistance.

The Heavy Metal Pollution Index (HPI) further confirmed considerable heavy metal contamination among the investigated wastewater samples. Approximately forty percent of the samples were classified as highly polluted, indicating severe heavy metal enrichment within hospital effluents. Elevated HPI values reflect cumulative contamination resulting from multiple anthropogenic sources and demonstrate the inadequacy of current wastewater management practices. Similar pollution classifications have been reported in groundwater, river water, and wastewater investigations conducted in different regions of Nigeria, emphasising the growing need for continuous environmental surveillance and effective wastewater treatment technologies [4,6,12].

Molecular analysis demonstrated widespread occurrence of clinically important antibiotic resistance genes, with intI1, blaTEM, sul1, and blaCTX-M being the most frequently detected determinants. The predominance of the class 1 integron gene (intI1) is particularly significant because integrons facilitate the acquisition, integration, and horizontal transfer of multiple resistance genes among bacterial populations.

Likewise, the widespread occurrence of blaTEM and blaCTX-M indicates extensive dissemination of extended-spectrum β-lactamase (ESBL)-producing bacteria within hospital wastewater. Similar molecular profiles have been reported in contaminated aquatic environments, where untreated hospital and pharmaceutical wastewaters served as important environmental reservoirs of clinically relevant resistance genes [10,29].

The detection of sul1, tetA, qnrS, mecA, and blaNDM further demonstrates the extensive diversity of resistance mechanisms circulating within hospital wastewater. Sulfonamide, tetracycline, fluoroquinolone, methicillin, and carbapenem resistance genes collectively indicate prolonged selective pressure arising from frequent antimicrobial use in healthcare settings. Although blaNDM occurred at comparatively lower prevalence, its presence remains highly significant because carbapenems represent last-line therapeutic agents for severe multidrug-resistant bacterial infections. The environmental dissemination of carbapenem resistance genes therefore represents an important emerging public health concern requiring continuous molecular surveillance [7,10,15].

Microbiological analysis showed that Gram-negative bacteria predominated among the recovered isolates, with Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter baumannii accounting for most isolates. This bacterial profile reflects the nature of hospital wastewater, which contains human excreta, wound secretions, laboratory waste, pharmaceutical residues, and clinical specimens. These organisms are recognised opportunistic pathogens possessing numerous intrinsic and acquired antimicrobial resistance mechanisms, including β-lactamase production, multidrug efflux pumps, reduced membrane permeability, and rapid acquisition of mobile genetic elements. Similar bacterial distributions have been reported in wastewater studies from healthcare facilities and contaminated aquatic environments within Nigeria [7,10].

The predominance of Escherichia coli further indicates substantial faecal contamination of hospital wastewater, while the frequent isolation of Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter baumannii demonstrates the environmental dissemination of pathogens commonly associated with healthcare-associated infections. Their release into receiving water bodies may facilitate the establishment of secondary environmental reservoirs capable of transmitting resistant organisms beyond hospital environments. Comparable observations have been reported during investigations of hospital-associated groundwater contamination and surface water pollution in Nigeria [14,16].

The antibiotic susceptibility profile obtained in this investigation revealed widespread multidrug resistance, particularly against ampicillin, ceftriaxone, ceftazidime, and ciprofloxacin. These findings indicate sustained exposure of bacterial populations to commonly prescribed antimicrobial agents within healthcare facilities, resulting in continuous selective pressure favouring resistant strains. Conversely, relatively high susceptibility to meropenem and colistin suggests that these agents remain comparatively effective against many of the recovered isolates, although emerging resistance remains a significant concern. Similar susceptibility patterns have been reported among bacterial isolates recovered from hospital wastewater and contaminated aquatic environments across Nigeria [7,10,29].

Pearson correlation analysis demonstrated significant positive relationships between heavy metal concentrations and antibiotic resistance genes, providing additional evidence supporting the co-selection hypothesis. The strong associations observed between nickel and intI1, lead and blaTEM, cadmium and sul1, and chromium and tetA indicate that heavy metal contamination may facilitate the maintenance and dissemination of antibiotic resistance genes within environmental bacterial populations. These findings agree with previous reports demonstrating that heavy metals constitute important ecological drivers of antimicrobial resistance through shared genetic resistance mechanisms [10,15].

Principal Component Analysis explained over 80% of the total variance, indicating that a limited number of environmental factors accounted for most of the observed variability in wastewater quality. The high contribution of the first principal component suggests that hospital activities, organic pollution, heavy metal contamination, and antimicrobial resistance collectively influence the overall composition of hospital wastewater. Similar multivariate analyses have demonstrated that anthropogenic activities remain the dominant determinants of environmental contamination in hospital effluents and other wastewater systems [4,6,12].

Conclusion

This study demonstrated that hospital wastewater discharged from selected healthcare facilities in Southern Nigeria contains elevated levels of physicochemical pollutants, potentially toxic heavy metals, multidrug-resistant bacteria, and clinically important antibiotic resistance genes. The deterioration in water quality, coupled with the presence of heavy metals such as lead, cadmium, chromium, nickel, and iron, indicates that untreated hospital effluents constitute a significant source of environmental contamination. Furthermore, the predominance of Gram-negative bacteria, including *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*, together with the widespread occurrence of antibiotic resistance genes such as intI1, blaTEM, blaCTX-M, sul1, tetA, qnrS, mecA, and blaNDM, demonstrates that hospital wastewater functions as an important environmental reservoir for antimicrobial resistance.

The high resistance observed against commonly prescribed antibiotics, alongside the coexistence of heavy metals and antibiotic resistance genes, suggests that hospital wastewater provides favourable conditions for the persistence, selection, and dissemination of multidrug-resistant microorganisms through co-selection and horizontal gene transfer mechanisms. These findings highlight the potential for contaminated wastewater to serve as a transmission pathway through which antimicrobial resistance spreads from healthcare facilities into surrounding aquatic ecosystems and ultimately to human populations. Consequently, the environmental release of untreated hospital effluents represents not only an ecological concern but also an emerging public health challenge requiring urgent intervention.

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