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ISOLATION AND IDENTIFICATION OF HEAVY METAL- AND ANTIBIOTIC-RESISTANT BACTERIA FROM SEWAGE SLUDGE IN OFFA METROPOLIS, KWARA STATE

[1]Ajiboye A.E., *[2]Abdulsalam M.A., and [1]Jimoh F.A.

[1]Department of Microbiology, Faculty of Pure and Applied Sciences, Kwara State University, Malete
[2]Department of Biological Sciences, School of Applied Sciences, Federal Polytechnic Offa, Kwara State

*Corresponding Author Email Address: abdulsalammuinatayodele@gmail.com

ABSTRACT

Sewage sludge is a semi-solid by-product of wastewater treatment and a complex matrix of organic matter, inorganic compounds, and diverse microorganisms. Municipal sewage environments are recognized as critical reservoirs for the co-selection of antibiotic resistance and heavy metal tolerance due to persistent chemical pressures from domestic, industrial, and healthcare waste discharges. This study aimed to isolate and identify heavy metal-tolerant and antibiotic-resistant bacteria from sewage sludge collected within Offa Metropolis, Kwara State, Nigeria, and to determine their prevalence, resistance patterns, and associated public health and environmental implications. Sewage sludge was collected aseptically from four sites in Offa Metropolis. Bacteria were enumerated by serial dilution and the spread plate technique on selective and differential media (Nutrient Agar, MacConkey Agar, SS Agar, EMB Agar). Isolates were characterized by colonial morphology, Gram staining, and standard biochemical tests. Antibiotic susceptibility was determined by Kirby–Bauer disk diffusion and interpreted using CLSI M100 (34th edition, 2024) breakpoints; isolates were classified as Susceptible (S), Intermediate (I), or Resistant (R). Multidrug resistance (MDR) was defined as resistance to ≥3 antibiotic classes. Heavy metal tolerance was assessed using a modified disk diffusion assay with metal salt solutions at 20 mg/L; resistance was defined as a zone of inhibition ≤1 mm. Total bacterial counts ranged from 5.50×10^6 to 11.43×10^6 CFU/g. Sixteen isolates belonging to nine genera were recovered: *Escherichia coli, Staphylococcus* sp., *Bacillus, Pseudomonas, Enterobacter, Shigella, Streptococcus, Proteus,* and *Citrobacter* sp. All 16 isolates were classified as MDR, with resistance across β-lactam, macrolide, aminoglycoside, fluoroquinolone, and glycopeptide classes. All isolates were tolerant to mercury (Hg) at 20 mg/L; 8 of 9 genera were also tolerant to cobalt (Co), yielding a Metal Resistance Index (MRI) of 0.40. The universal co-occurrence of MDR and heavy metal tolerance is consistent with co-selection driven by shared mobile genetic elements. Municipal sewage sludge in Offa Metropolis harbours heavy metal-tolerant, multidrug-resistant bacteria driven by synergistic interactions between heavy metals and antibiotics. Improved waste management, regulatory control of pollutants, and integrated environmental surveillance that simultaneously monitors chemical contaminants and markers of microbial resistance are urgently recommended.

Keywords: Antibiotic resistance; Sewage sludge; Heavy metal tolerance; Multidrug resistance; Co-selection; Offa Metropolis

INTRODUCTION

The rapid emergence and global spread of antimicrobial resistance (AMR) represent one of the foremost public health challenges of the 21st century, driven by both the misuse of antibiotics and environmental factors that facilitate the persistence and dissemination of resistant microorganisms (Vats *et al.*, 2022; Mazhar *et al.*, 2021). Among environmental reservoirs, municipal sewage sludge plays a significant role due to its accumulation of diverse contaminants, including antibiotics and heavy metals originating from domestic, industrial, and healthcare activities. These complex environments create continuous selective pressure that promotes microbial adaptation and enrichment of antibiotic resistance genes (ARGs) within bacterial populations.

Recent studies have demonstrated that heavy metals and antibiotics interact synergistically in environmental systems, contributing to the co-selection of resistance traits. Co-selection occurs when resistance genes conferring resistance to antibiotics and heavy metals are co-located on mobile genetic elements such as plasmids, integrons, and transposons, enabling simultaneous horizontal transfer among bacteria (Gillieatt & Coleman, 2024; Joseph *et al.*, 2023). Bacterial exposure to heavy metals alone can thus select for antibiotic-resistant strains even without direct antibiotic pressure, significantly enhancing the persistence and spread of MDR organisms in environmental reservoirs (Heydari *et al.*, 2023; Wang *et al.*, 2021).

The persistence and non-biodegradable nature of heavy metals intensify this problem, as they continuously exert long-term selective pressure on microbial communities (Dongdem *et al.*, 2025). Heavy metal contamination alters microbial community structure and increases both the abundance and diversity of ARGs in soils and aquatic environments (Li *et al.*, 2024; Heydari *et al.*, 2022). The co-occurrence of metal resistance genes (MRGs) and ARGs highlights a strong evolutionary linkage that facilitates the maintenance and dissemination of resistance determinants in bacterial populations (Alabi *et al.*, 2024; Gillieatt & Coleman, 2024).

Despite growing global awareness, a significant gap exists in localized studies, particularly in developing countries such as Nigeria. In Offa Metropolis, Kwara State, limited information is available on the prevalence and characteristics of bacteria that exhibit both heavy metal tolerance and antibiotic resistance in sewage sludge environments. This lack of context-specific data restricts effective environmental risk assessment and undermines targeted AMR control strategies.

This study, therefore, aims to isolate and identify heavy metal-tolerant and antibiotic-resistant bacteria from sewage sludge in Offa Metropolis, Kwara State, to determine their prevalence, resistance patterns, and associated public health implications. The findings will contribute to understanding the synergistic interactions between heavy metals and antibiotics in driving AMR and provide evidence to inform effective prevention and control strategies.

MATERIALS AND METHODS

Study Area

This study was conducted in Offa Metropolis, Kwara State, Nigeria (approximately at latitude 8.15° N and longitude 4.72° E). Offa is a rapidly growing urban centre characterized by residential settlements, small-scale industries, healthcare facilities, and commercial establishments, all of which generate sewage sludge. The climate is tropical, with distinct wet (April–October) and dry (November–March) seasons. Sewage systems in the area are largely decentralized, with waste discharged into open drains, septic systems, and sludge accumulation sites, creating reservoirs for antibiotic-resistant and heavy-metal-tolerant bacteria.

Sample Collection

Sewage sludge samples were collected aseptically from four sites (A–D) within Offa Metropolis, representing major sources of municipal waste discharge (drainage channels, septic tank outlets, and sludge deposits associated with the Owode abattoir). Samples were collected using sterile spatulas into sterile, labelled polypropylene containers. Approximately 300 g of sludge was obtained from each site at a depth of 5–10 cm. Samples were transported immediately in insulated ice packs (approximately 4 °C) to the Microbiology Laboratory at Kwara State University and processed within 24 h of collection. Each sample was homogenized under aseptic conditions before analysis. All procedures followed standard microbiological protocols (APHA, 2017) to prevent contamination.

Bacteriological Enumeration

Culture media were prepared according to the manufacturer's specifications and sterilized by autoclaving at 121 °C for 15 min. One gram of homogenized sludge was dissolved in 9 mL sterile physiological saline (0.85% NaCl) to achieve a 10^{-1} dilution, then serially diluted to 10^{-5}. Aliquots of 0.1 mL from dilutions 10^{-3} to 10^{-5} were spread-plated in triplicate on: (i) Nutrient Agar (NA) and Tryptic Soy Agar (TSA) for total bacterial count (TBC); (ii) MacConkey Agar for total coliform count (TCC); (iii) Eosin Methylene Blue (EMB) Agar for faecal coliform count (TFC) and presumptive *E. coli* identification via metallic green sheen; (iv) Salmonella–Shigella (SS) Agar and Xylose Lysine Deoxycholate (XLD) Agar for Salmonella–Shigella count (TSC) (Iyevhobu *et al.*, 2024). Plates were incubated aerobically at 37 °C for 24 h. Colonies were counted and results reported as CFU/g. 'ND' (Not Detected) in Table 1 indicates the target organism was absent, not that the analysis was omitted. Superscript letters (a, b, c) indicate groupings from one-way ANOVA with Tukey's HSD post hoc test ($p < 0.05$); means sharing the same letter are not significantly different.

Isolation and Purification

Morphologically distinct colonies were subcultured onto fresh Nutrient Agar and incubated at 37 °C for 24 h to obtain pure cultures. Isolates were maintained on Nutrient Agar slants at 4 °C and assigned unique codes (A1–D4) based on the sampling site of origin.

Cultural Morphology and Gram Staining

Pure isolates were characterized by colonial morphology (size, pigmentation, shape, elevation, margin, surface texture, consistency, and optical properties). Gram staining was performed by heat-fixing smears on glass slides and staining sequentially with crystal violet (60 s), Lugol's iodine (30 s), 95% ethanol decolorizer (5 s), and safranin counterstain (60 s). Slides were examined under oil-immersion microscopy (×100 objective). Gram-positive organisms appeared purple; Gram-negative organisms appeared pink/red (Tripathi *et al.*, 2025).

Biochemical Characterization

A standard battery of biochemical tests was performed in triplicate to confirm identification (Holt *et al.*, 1994; Reiner, 2010; Dheyab *et al.*, 2018): Catalase (3% H_2O_2); Oxidase (TMPD reagent); Coagulase (slide agglutination with rabbit plasma, for Gram-positive cocci); Citrate Utilization (Simmons Citrate Agar; blue = positive); Triple Sugar Iron (TSI) Agar (butt-stab and slant-streak; observing glucose/lactose/sucrose fermentation, gas, H_2S production); Sugar Fermentation (phenol red broth + 1% glucose, lactose, or mannitol; Durham tube for gas); Indole (Kovacs' reagent); and Methyl Red. Results were compared against Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994).

Antibiotic Susceptibility Testing

Antibiotic susceptibility was determined using the Kirby–Bauer disk diffusion method in accordance with CLSI guidelines (CLSI, 2024). Overnight cultures were adjusted to a 0.5 McFarland turbidity (~1.5×10^8 CFU/mL) and inoculated onto Mueller–Hinton Agar (MHA) plates using the lawn technique. Commercial antibiotic discs (Oxoid, UK) were placed on the agar surface. Plates were incubated at 37 °C for 18–24 h, and inhibition zones were measured to the nearest millimetre. Interpretations (S, I, R) were based on CLSI M100, 34th Edition (2024) zone diameter breakpoints specific to each organism–antibiotic combination. MDR was defined as acquired non-susceptibility to ≥3 antibiotic classes (Magiorakos *et al.*, 2012).

Antibiotics tested: Azithromycin (AZM, 15 µg), Erythromycin (ERY, 15 µg), Ceftriaxone (CTR, 30 µg), Cotrimoxazole (COT, 25 µg), Gentamicin (GEN, 10 µg), Ciprofloxacin (CIP, 5 µg), Augmentin (AUG, 30 µg), Vancomycin (VAN, 30 µg), Chloramphenicol (CHL, 30 µg), Levofloxacin (LEV, 5 µg), Nitrofurantoin (NIT, 30 µg), Amikacin (AMK, 30 µg), and Meropenem (MEM, 10 µg). Clotrimazole was excluded from the antibacterial susceptibility analysis as it is an antifungal agent with no established antibacterial breakpoints (CLSI, 2024).

Heavy Metal Tolerance Assay

Heavy metal tolerance was assessed by a modified disk diffusion method (Jardine *et al.*, 2019; Tomova *et al.*, 2015). Standardized bacterial lawns were prepared on MHA as described in Section 2.7. Sterile filter paper discs (6 mm) were impregnated with aqueous metal salt solutions at 20 mg/L for: Mercury (Hg, as $HgCl_2$), Lead (Pb, as $Pb(NO_3)_2$), Cobalt (Co, as $CoCl_2$), Copper (Cu, as $CuSO_4$), and Nickel (Ni, as $NiSO_4$). The 20 mg/L concentration simulates elevated bioavailable metal levels in heavily contaminated sludge (Hechmi *et al.*, 2020). Discs were placed on inoculated plates and incubated at 37 °C for 24 h. A strain was classified as metal-resistant (MRB) when the zone of inhibition was ≤1 mm; susceptible when >1 mm. The Metal Resistance Index (MRI) was calculated as: MRI = number of metals resisted ÷ total metals tested (n = 5). This assay assessed the biological tolerance profile of isolates; quantitative chemical determination of metal concentrations in the sludge matrix was beyond the scope of this study, but is recommended for future work.

Statistical Analysis

Bacterial count data were expressed as mean ± standard deviation (SD). One-way ANOVA with post hoc Tukey's Honestly Significant Difference (HSD) test was used to compare counts across sites ($p < 0.05$). Co-occurrence of antibiotic resistance and heavy metal tolerance was assessed descriptively using frequency distributions and resistance indices. Statistical analyses were performed using SPSS version 26.0 (IBM, USA).

RESULTS AND

Bacteriological Load of Sewage Sludge Samples

To characterize the microbiological quality of sewage sludge across the four sampling sites in Offa Metropolis, total bacterial counts (TBC), total coliform counts (TCC), faecal coliform counts (TFC), and total Salmonella–Shigella counts (TSC) were determined by serial dilution and spread-plate enumeration. Results are presented in Table 1.

Table 1. Bacteriological counts of sewage sludge samples from Offa Metropolis (CFU/g)

Sample	TBC (CFU/g)	TCC (CFU/g)	TFC (CFU/g)	TSC (CFU/g)
A	$8.50 \pm 5.21 \times 10^{6}$ ^{a}	ND	ND	$3.83 \pm 2.75 \times 10^{6}$ a
B	$8.13 \pm 4.00 \times 10^{6}$ ^{a}	$6.43 \pm 4.50 \times 10^{6}$ ^{ab}	$0.57 \pm 0.32 \times 10^{6}$ ^{b}	ND
C	$5.50 \pm 5.01 \times 10^{6}$ ^{a}	$1.23 \pm 1.25 \times 10^{6}$ ^{bc}	ND	ND
D	$11.43 \pm 2.26 \times 10^{6}$ ^{a}	$8.20 \pm 4.51 \times 10^{6}$ ^{a}	$3.97 \pm 2.83 \times 10^{6}$ ^{a}	ND

ND = Not Detected. Superscripts (a, b, c) denote Tukey's HSD groupings (one-way ANOVA, $p < 0.05$); means sharing the same letter do not differ significantly. TBC = Total Bacterial Count; TCC = Total Coliform Count; TFC = Faêcal Coliform Count; TSC = Total Salmonella–Shigella Count.

Table 1 reveals consistently high microbial burdens across all four sampling sites. Total bacterial counts ranged from $5.50 \pm 5.01 \times 10^{6}$ CFU/g (Site C) to $11.43 \pm 2.26 \times 10^{6}$ CFU/g (Site D), with no statistically significant inter-site differences observed (all sites share Tukey's HSD superscript 'a'; $p > 0.05$). Site D recorded the highest TBC, TCC ($8.20 \pm 4.51 \times 10^{6}$ CFU/g), and TFC ($3.97 \pm 2.83 \times 10^{6}$ CFU/g) simultaneously, representing the most heavily contaminated site. Site B showed the second-highest TCC ($6.43 \pm 4.50 \times 10^{6}$ CFU/g) and a low TFC ($0.57 \pm 0.32 \times 10^{6}$ CFU/g). Salmonella–Shigella organisms were detected exclusively at Site A ($3.83 \pm 2.75 \times 10^{6}$ CFU/g), whereas Site C yielded the lowest TCC ($1.23 \pm 1.25 \times 10^{6}$ CFU/g) and no detectable TFC or TSC. Notably, neither TCC nor TFC was detected at Site A, despite its non-zero TSC, reflecting differential contamination profiles associated with specific upstream sources at each site. All 'ND' entries confirm the absence of detectable counts rather than the omission of analysis.

The bacterial counts recorded in this study ($5.50–11.43 \times 10^{6}$ CFU/g) are consistent with values reported in comparative studies from West African urban environments (Alabi *et al*., 2024; Dongdem *et al*., 2025), confirming that municipal sewage sludge in Offa Metropolis harbours substantial microbial loads characteristic of poorly managed waste systems. The uniformly high TBC across all four sites, irrespective of source type, underscores the ubiquitous colonization of sludge matrices by environmental bacteria, a pattern consistent with the organic-rich, nutrient-laden composition of municipal sludge that supports dense and diverse microbial communities (Bouchaala *et al*., 2024; Popovici *et al*., 2024).

The co-detection of faecal coliforms and Salmonella–Shigella organisms across different sites is a particularly important public health finding. Faecal coliforms, as indicator organisms of enteric contamination, signal the presence of faecal material of human or animal origin, indicating inadequate treatment of domestic sewage and abattoir waste (APHA, 2017). The preferential detection of Salmonella–Shigella at Site A, a sludge deposit associated with the Owode abattoir drainage, suggests localized enrichment of enteric pathogens through animal waste inputs. This pattern is well-documented in slaughterhouse-adjacent environments throughout sub-Saharan Africa (Alabi *et al*., 2024). The statistical non-significance of inter-site TBC differences, despite considerable absolute variation, likely reflects high within-site heterogeneity inherent to sludge sampling, as previously described by Hechmi *et al.* (2020). Collectively, these counts establish that all four sites constitute microbiologically hazardous environments with potential for pathogen dissemination into surrounding soil and water resources.

Identification and Taxonomic Distribution of Bacterial Isolates

Following enumeration, morphologically distinct colonies were selected from each site, purified, and subjected to Gram staining and biochemical characterization. Table 2 presents the frequency and distribution of bacterial genera recovered across the four sampling sites.

Table 2. Frequency and distribution of bacteria isolated from sewage sludge samples, Offa Metropolis (n = 16)

Bacterial Genus	Isolate Codes	n	(%)
Escherichia coli	B1, B3, D2	3	18.75
Staphylococcus sp.	A1, C3, D1	3	18.75
Bacillus sp.	A2, B4	2	12.50
Shigella sp.	C1, D4	2	12.50
Streptococcus sp.	C4, D3	2	12.50
Pseudomonas sp.	A3	1	6.25
Enterobacter sp.	A4	1	6.25
Proteus sp.	B2	1	6.25
Citrobacter sp.	C2	1	6.25
Total	**—**	**16**	**100.00**

Isolate key: A1 = Staphylococcus sp.; A2 = Bacillus sp.; A3 = Pseudomonas sp.; A4 = Enterobacter sp.; B1 = E. coli; B2 = Proteus sp.; B3 = E. coli; B4 = Bacillus sp.; C1 = Shigella sp.; C2 = Citrobacter sp.; C3 = Staphylococcus sp.; C4 = Streptococcus sp.; D1 = Staphylococcus aureus; D2 = E. coli; D3 = Streptococcus sp.; D4 = Shigella sp.

A total of 16 bacterial isolates belonging to nine genera were recovered across the four sites (Table 2). *Escherichia coli* and *Staphylococcus* sp. were co-dominant, each accounting for 18.75% of all isolates (n = 3 each). *Bacillus* sp., *Shigella* sp., and *Streptococcus* sp. were each represented by two isolates (12.50% each). The remaining four genera — *Pseudomonas* sp., *Enterobacter* sp., *Proteus* sp., and *Citrobacter* sp. each contributed a single isolate (6.25% each). The distribution of isolates across sites was as follows: Site A yielded four isolates (A1–A4), Site B yielded four isolates (B1–B4), Site C yielded four isolates (C1–C4), and Site D yielded four isolates (D1–D4). Both Gram-positive genera (Staphylococcus, Bacillus, Streptococcus) and Gram-negative genera (*E. coli*, Shigella, Pseudomonas, Enterobacter, Proteus, Citrobacter) were represented, indicating a diverse phylogenetic composition of the sludge microbial community.

The diversity of nine genera recovered from a single urban sewage sludge matrix reflects the well-characterized role of such environments as microbiological confluences of domestic, clinical, and industrial waste streams (Vats *et al*., 2022; Guo *et al*., 2023). The co-dominance of *E. coli* and *Staphylococcus* sp., each at 18.75%, is ecologically and epidemiologically significant. *E. coli* is the definitive indicator of faecal contamination and a principal vehicle for the dissemination of mobile resistance determinants through horizontal gene transfer in aquatic and soil environments (Zhang *et al*., 2021; Gillings *et al*., 2021). Its high prevalence across multiple sites corroborates enteric contamination driven by inadequate sewage management, consistent with findings from comparable Nigerian studies (Akinyemi **et al**., 2006; Joseph **et al**., 2023).

The recovery of *Staphylococcus* sp., including the coagulase-positive *S. aureus* at Site D, indicates the influx of skin-associated and nosocomial organisms into the sludge matrix, consistent with contributions from healthcare and domestic wastewater. The isolation of *Shigella* sp., *Proteus* sp., and *Citrobacter* sp., all recognized enteric opportunists, reinforces the potential for waterborne pathogen transmission from inadequately treated sludge to human populations via direct contact or agricultural reuse. The recovery of environmental genera such as *Bacillus* sp. and *Pseudomonas* sp. indicates that sludge also harbours inherently resistant environmental bacteria that can serve as donors of resistance genes during horizontal gene transfer (Kiama *et al*., 2021; Bunduruş *et al*., 2023). Taken together, the genus-level diversity recorded here establishes the study sites as reservoirs of both pathogenic and environmental bacterial species, all of which contribute to the AMR burden within the sludge ecosystem.

Biochemical Characterization and Presumptive Identification

All 16 isolates were subjected to a standardized battery of biochemical tests to obtain presumptive identifications. Results of catalase, oxidase, coagulase, citrate utilization, indole production, methyl red, and Triple Sugar Iron (TSI) reactions are summarized in Table 3.

Table 3. Biochemical profile and presumptive identification of bacterial isolates

Isolate(s)	Cat	Ox	Coag	Cit	Ind	MR	TSI (Slant/Butt)	Probable Organism
A1, C3, D1	+	−	+	+	−	+	N/A	*Staphylococcus* sp.
A2, B4	+	−	−	+	−	−	N/A	*Bacillus* sp.
A3	+	+	−	+	−	−	K/K (Alk/Alk)	*Pseudomonas* sp.
B1, B3, D2	+	−	−	−	+	+	A/A (Acid/Acid)	*Escherichia coli*
C1, D4	+	−	−	−	−	+	K/A (Alk/Acid)	*Shigella* sp.
C4, D3	−	−	−	−	−	−	N/A	*Streptococcus* sp.
B2	+	−	−	+	−	+	K/A, H_2S+	*Proteus* sp.
A4	+	−	−	+	−	−	A/A (Acid/Acid)	*Enterobacter* sp.

C2	+	−	−	+	−	+	A/A, H_2S+	*Citrobacter* sp.

(+) Positive; (−) Negative; Cat = Catalase; Ox = Oxidase; Coag = Coagulase; Cit = Citrate; Ind = Indole; MR = Methyl Red; A/A = Acid/Acid TSI; K/A = Alkaline slant/Acid butt; K/K = Alkaline/Alkaline TSI; H_2S *= hydrogen sulfide production; N/A = not applicable.*

Table 3 demonstrates clearly differentiated biochemical profiles consistent with the presumptive identifications assigned to each isolate group. *Staphylococcus* sp. (A1, C3, D1) were catalase-positive, oxidase-negative, and coagulase-positive, with citrate utilization positive and methyl red positive profiles consistent with their genus assignment; D1 was further designated *S. aureus* based on positive coagulase reaction. *Bacillus* sp. (A2, B4) were catalase-positive, coagulase-negative, citrate-positive, with no indole or TSI gas production applicable. *Pseudomonas* sp. (A3) was the only oxidase-positive isolate in the collection with an alkaline/alkaline (K/K) TSI profile, characteristic of non-fermenting Gram-negative bacilli. *E. coli* (B1, B3, D2) were confirmed by positive indole, positive methyl red, and an acid/acid (A/A) TSI reaction without H_2S production. Shigella sp. (C1, D4) showed a K/A TSI pattern with no H_2S, consistent with their non-lactose-fermenting, anaerogenic profile. *Streptococcus* sp. (C4, D3) were catalase-negative and oxidase-negative, conforming to Gram-positive cocci profiles for which TSI is not applicable. *Proteus* sp. (B2) and *Citrobacter* sp. (C2) both displayed H_2S production on TSI, with citrate-positive and methyl red-positive reactions, distinguishing them from the other enteric genera.

The biochemical profiles obtained in this study are internally consistent and align with established reference profiles from Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994) and contemporary diagnostic protocols (Dheyab *et al*., 2018; Roy *et al*., 2023). The utility of the TSI agar reaction, particularly in distinguishing lactose/sucrose-fermenting Enterobacteriaceae from non-fermenters such as Pseudomonas, was clearly demonstrated in this study, with the alkaline/alkaline TSI pattern of A3 providing unambiguous differentiation from the acid-producing Enterobacteriaceae isolates. The H_2S production observed in *Proteus* sp. (B2) and *Citrobacter* sp. (C2) reflects their cysteine desulfurase activity, consistent with their ecological roles as secondary decomposers in organic-rich environments such as sewage sludge (Reiner, 2010).

The coagulase-positive designation of D1 (S. aureus) carries particular clinical significance, given the known pathogenic virulence of this organism and its capacity for methicillin resistance and toxin production in healthcare and community settings (Akinyemi *et al*., 2006; Walusansa *et al*., 2022). The oxidase-positive result for *Pseudomonas* sp. (A3), confirmed by K/K TSI and lack of glucose fermentation, is biochemically robust and consistent with reference profiles for this genus (Cheesbrough, 2010). Collectively, the biochemical differentiation achieved in this study establishes a reliable identification framework for downstream antibiotic susceptibility and heavy metal tolerance analyses, and confirms the taxonomic diversity of the bacterial population inhabiting the study's sludge matrix.

Antibiotic Susceptibility of Representative Isolates

Antibiotic susceptibility was assessed using the Kirby–Bauer disk diffusion method against 10 antibiotics spanning six major classes, with interpretations based on CLSI M100 breakpoints (34th edition, 2024). Table 4 presents the susceptibility profiles of six representative isolates alongside CLSI susceptibility thresholds.

Table 4. Antibiotic susceptibility patterns of representative isolates with CLSI M100 (2024) breakpoints.

Antibiotic	Disc (µg)	A1 (Staph.)	A2 (Bacillus)	B4 (Bacillus)	C3 (Staph.)	D2 (*E. coli*)	D3 (Strep.)	CLSI S ≥ (mm)
Azithromycin	15	R	R	R	R	I	R	≥18
Erythromycin	15	R	R	R	R	R	R	≥23
Ceftriaxone	30	R	R	R	I	R	R	≥23
Cotrimoxazole	25	R	R	R	R	R	R	≥16
Gentamicin	10	R	R	R	R	R	R	≥15
Ciprofloxacin	5	I	S	R	R	S	R	≥21
Augmentin	30	R	R	R	R	R	R	≥18
Vancomycin	30	R	R	R	R	R	R	≥17
Chloramphenicol	30	R	R	R	R	R	R	≥18
Levofloxacin	5	R	R	R	R	R	R	≥17

S = Susceptible; I = Intermediate; R = Resistant. CLSI M100, 34th Edition (2024). 'CLSI S ≥' = minimum zone diameter (mm) for Susceptible classification.

Table 4 reveals a striking pattern of broad-spectrum antibiotic resistance among the six representative isolates tested. All six isolates — A1 (*Staphylococcus* sp.), A2 (*Bacillus* sp.), B4 (*Bacillus* sp.), C3 (*Staphylococcus* sp.), D2 (*E. coli*), and D3 (*Streptococcus* sp.) — were uniformly resistant to Erythromycin (ERY), Cotrimoxazole (COT), Gentamicin (GEN), Augmentin (AUG), Vancomycin (VAN), Chloramphenicol (CHL), and Levofloxacin (LEV). Resistance to Ceftriaxone (CTR) was recorded in five of the six isolates; C3 (*Staphylococcus* sp.) showed an intermediate CTR profile. Resistance to Azithromycin (AZM) was recorded in five isolates, with D2 (*E. coli*) displaying an intermediate response. Regarding Ciprofloxacin (CIP), A2 (*Bacillus* sp.) and D2 (*E. coli*) were susceptible, while A1 showed an intermediate result, and the remaining three isolates were resistant. All six isolates were therefore resistant to ≥7 of 10 antibiotics tested, with measured inhibition zones consistently below CLSI susceptibility thresholds.

The near-universal resistance pattern documented in Table 4 is alarming and represents a significant departure from anticipated susceptibility profiles for environmental bacterial isolates. Resistance to glycopeptides (Vancomycin), carbapenems-adjacent agents, fluoroquinolones (Levofloxacin), and aminoglycosides (Gentamicin) occurring simultaneously within a single environmental isolate collection reflects the polyclonal co-selection of diverse resistance mechanisms within the sludge matrix. Vancomycin resistance in Gram-positive organisms — particularly *Staphylococcus* sp. is of grave clinical significance, as vancomycin represents a critical last-resort option for MRSA and other resistant Gram-positive pathogens; environmental Vancomycin-Resistant Staphylococcus (VRS) constitutes an escalating public health threat (WHO, 2023; Bunduruş *et al*., 2023).

The broad resistance to beta-lactam/beta-lactamase inhibitor combinations (Augmentin) and third-generation cephalosporins (Ceftriaxone) in both Gram-positive and Gram-negative organisms suggests the presence of extended-spectrum beta-lactamases (ESBLs) or chromosomally encoded beta-lactamase mechanisms consistent with those documented in sewage sludge isolates from comparable African environments (Joseph *et al*., 2023; Guo *et al*., 2023). The retention of susceptibility to Ciprofloxacin in A2 and D2 offers a narrow therapeutic window; however, the intermediate CIP profile of A1 and the observation that CIP zones across the extended panel (Table 6) fall well below CLSI susceptibility thresholds suggest that fluoroquinolone resistance is advancing within this bacterial community. These findings highlight the urgent need to monitor clinically relevant resistance phenotypes in environmental bacterial reservoirs that serve as potential sources of resistance gene transfer to pathogenic organisms.

Multidrug Resistance Profile Across All Bacterial Genera

To provide a comprehensive resistance overview at the genus level, Table 5 consolidates the MDR profiles of all nine bacterial genera recovered in this study, including the percentage frequency of each genus and its resistance patterns to seven key antibiotics across multiple classes.

Table 5. Combined frequency and multidrug resistance (MDR) profile of all bacterial genera isolated from sewage sludge

Organism	Freq (%)	NIT	GEN	AUG	CTR	CIP	AMK	MEM	MDR
E. coli (B1,B3,D2)	18.75	R	R	R	R	I	R	R	Yes
Staph. sp. (A1,C3,D1)	18.75	R	R	R	R	I	R	R	Yes
Bacillus sp. (A2,B4)	12.50	R	R	R	R	S	R	R	Yes
Shigella sp. (C1,D4)	12.50	I	I	R	R	R	R	R	Yes
Streptococcus sp. (C4,D3)	12.50	R	R	R	R	R	R	R	Yes
Pseudomonas sp. (A3)	6.25	R	R	R	I	S	R	R	Yes
Enterobacter sp. (A4)	6.25	R	I	R	R	S	R	R	Yes
Proteus sp. (B2)	6.25	R	R	I	R	I	I	R	Yes
Citrobacter sp. (C2)	6.25	R	R	R	R	R	R	R	Yes

S = Susceptible; I = Intermediate; R = Resistant. NIT = Nitrofurantoin; GEN = Gentamicin; AUG = Augmentin; CTR = Ceftriaxone; CIP = Ciprofloxacin; AMK = Amikacin; MEM = Meropenem. MDR = resistance to ≥3 antibiotic classes (Magiorakos et al., 2012). Interpretations per CLSI M100, 34th Edition (2024).

Table 5 confirms that all nine bacterial genera (100% of the isolate collection) met the internationally accepted definition of multidrug resistance (MDR: non-susceptibility to ≥3 antibiotic classes; Magiorakos *et al*., 2012). Resistance to Augmentin (AUG), Ceftriaxone (CTR), and Amikacin (AMK) was recorded across all nine genera. Nitrofurantoin (NIT) resistance was observed in seven genera, with *Shigella* sp. displaying an intermediate response. Gentamicin (GEN) resistance was recorded in seven genera; *Shigella* sp. and *Enterobacter* sp. showed intermediate responses. For Ciprofloxacin (CIP), *Bacillus* sp. retained susceptibility, while *Pseudomonas* sp. and *Enterobacter* sp. also remained susceptible, representing the only three genera with any non-resistant CIP classification. Resistance to Meropenem (MEM), a carbapenem antibiotic of last resort, was recorded universally across all nine genera, with no inhibition zones detected in any isolate. This 100% MDR rate is the most significant finding in this study's resistance profile.

The 100% MDR rate across all nine genera is among the most critical findings of this study and warrants careful epidemiological interpretation. The universal resistance to Meropenem, a broad-spectrum carbapenem routinely reserved for the most severe multidrug-resistant infections, is particularly alarming, as carbapenem resistance in environmental bacteria is among the most serious AMR threats globally (WHO, 2023; UNEP, 2023). While the modified disk diffusion method used here does not allow direct inference of specific carbapenem resistance mechanisms, the observed phenotype is consistent with the production of carbapenemases (e.g., NDM, OXA-type enzymes) or porin-loss mechanisms increasingly reported in environmental Enterobacteriaceae from wastewater systems (Guo *et al*., 2023; Chen *et al*., 2022).

The simultaneous resistance to aminoglycosides (Amikacin, Gentamicin), fluoroquinolones (Ciprofloxacin, Levofloxacin), and beta-lactams observed across phylogenetically diverse genera — spanning both Gram-positive and Gram-negative organisms — strongly implicates shared, mobile resistance platforms such as class 1 integrons and broad-host-range conjugative plasmids as the primary dissemination vehicles (Gillings *et al*., 2021; Mazhar *et al*., 2021). The sludge environment, characterized by high microbial density, nutrient availability, and persistent chemical selection pressure, represents an ideal ecological niche for horizontal gene transfer and the amplification of such mobile elements. The partial retention of Ciprofloxacin susceptibility in Bacillus, Pseudomonas, and *Enterobacter* sp. suggests that fluoroquinolone resistance may not yet be universally established in these environmental lineages; however, as demonstrated in Table 6, zone diameters for these genera remain well below therapeutic thresholds, indicating incipient or low-level resistance that warrants continued monitoring (Kumar *et al*., 2023).

Extended Antibiotic Susceptibility: Zone Diameter Analysis

To complement the categorical S/I/R interpretations presented in Tables 4 and 5, Table 6 provides the raw zone diameters (in millimetres) alongside their CLSI-based interpretations for 10 representative isolates (A3, A4, B1–B3, C1, C2, C4, D1, D4) tested against an extended antibiotic panel comprising 11 agents.

Table 6. Zone diameters (mm) and S/I/R interpretations for representative isolates — extended antibiotic panel (CLSI M100, 2024).

Antibiotic	µg	A3	A4	B2	B1	B3	C1	C2	C4	D1	D4
Nitrofurantoin	30	R (0)	R (0)	R (0)	R (0)	R (7)	R (5)	R (0)	R (0)	R (0)	R (8)
Gentamicin	10	R (0)	I (4)	R (0)	R (0)	R (0)	I (9)	R (0)	R (0)	R (0)	R (0)
Augmentin	30	R (0)	R (0)	R (5)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)
Levofloxacin	5	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)
Ceftriaxone	30	R (5)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	I (12)	R (0)
Cefepime	30	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)
Ciprofloxacin	5	R (15)	R (13)	R (10)	R (12)	R (10)	R (5)	R (8)	R (8)	R (0)	R (0)
Amikacin	30	R (0)	R (0)	I (9)	R (0)	I (6)	R (0)	I (5)	R (0)	R (0)	I (6)
Vancomycin	30	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)
Aztreonam	30	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)
Meropenem	10	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)

Format: S/I/R (zone diameter in mm). R (0) = no zone detected, classified as resistant per CLSI 2024. All zones are measured in millimeters. Clotrimazole excluded (antifungal; no antibacterial CLSI breakpoints). C4 = Streptococcus sp. (Gram-positive; included for comparative profile).

Table 6 provides quantitative inhibition zone data that corroborate and extend the categorical resistance patterns established in earlier tables. For five antibiotics — Levofloxacin (LEV), Cefepime (FEP), Vancomycin (VAN), Aztreonam (ATM), and Meropenem (MEM) — all 10 isolates produced zero inhibition zones (R; 0 mm), confirming complete, unequivocal resistance in the absence of any bacteriostatic activity. For Ciprofloxacin (CIP), measured zones ranged from 0 mm (D1, D4) to 15 mm (A3); the highest zone recorded (A3: 15 mm) remains substantially below the CLSI susceptibility threshold of ≥21 mm for Enterobacteriaceae, confirming resistant classification for all isolates. Augmentin (AUG) produced a maximum zone of 5 mm (B2); all other isolates recorded 0 mm, confirming universal resistance. For Amikacin (AMK), four isolates — B2 (9 mm), B3 (6 mm), C2 (5 mm), and D4 (6 mm) yielded intermediate zones below the susceptibility breakpoint; all other isolates recorded 0 mm. Gentamicin (GEN) produced intermediate responses only in A4 (4 mm) and C1 (9 mm). Ceftriaxone (CTR) produced a 5 mm zone in A3 and an intermediate zone of 12 mm in D1, with all other isolates showing 0 mm or full resistance.

The quantitative zone diameter data presented in Table 6 are particularly valuable in contextualizing the phenotypic severity of resistance in this isolate collection. The observation that five of eleven antibiotics spanning fluoroquinolones (LEV), monobactams (ATM), glycopeptides (VAN), fourth-generation cephalosporins (FEP), and carbapenems (MEM) produced no inhibition whatsoever in any tested isolate reflects a degree of phenotypic resistance consistent with advanced, multi-mechanism resistance. The absence of any zone for Meropenem is particularly significant, as carbapenems are generally bactericidal even against many resistant organisms; zero-zone carbapenem resistance in environmental isolates has been associated with the presence of metallo-beta-lactamases and OXA-type carbapenemases on transferable elements (Guo *et al*., 2023; UNEP, 2023).

The near-zero Ciprofloxacin zones (maximum of 15 mm against a susceptibility threshold of ≥21 mm) further indicate that, although some residual membrane permeability to fluoroquinolones may exist, particularly in Pseudomonas sp. and Enterobacter sp., the inhibitory effect is insufficient for therapeutic outcomes. This pattern is consistent with efflux pump overexpression or reduced outer membrane permeability, rather than with target-site mutation alone (Nguyen *et al*., 2023; Tomova *et al*., 2015). The intermediate Amikacin responses observed in four isolates suggest partial activity of aminoglycoside-modifying enzymes, representing a transitional resistance phenotype that may progress to full resistance under continued selective pressure. These quantitative data collectively reinforce the conclusion that the sewage sludge environment of Offa Metropolis serves as an active evolutionary arena for the escalation and diversification of antibiotic resistance phenotypes.

Heavy Metal Tolerance Profiles

All 16 isolates were assessed for their tolerance to five heavy metals: mercury (Hg), lead (Pb), cobalt (Co), copper (Cu), and nickel (Ni) at a standardized concentration of 20 mg/L using a modified disk diffusion assay. Results, including the calculated Metal Resistance Index (MRI) for each genus, are presented in Table 7.

Table 7. Heavy metal tolerance profiles of bacterial isolates at 20 mg/L (modified disk diffusion method)

Bacterial Isolate	Hg	Pb	Co	Cu	Ni	MRI
Staphylococcus sp.	R	S	R	S	S	0.40
Bacillus sp.	R	S	R	S	S	0.40
Pseudomonas sp.	R	S	R	S	S	0.40
Enterobacter sp.	R	S	R	S	S	0.40
Escherichia coli	R	S	R	S	S	0.40
Citrobacter sp.	R	S	S	S	S	0.20
Shigella sp.	R	S	R	S	S	0.40
Streptococcus sp.	R	S	R	S	S	0.40
Proteus sp.	R	S	R	S	S	0.40

R = Resistant (zone ≤1 mm); S = Susceptible (zone >1 mm). Hg = Mercury; Pb = Lead; Co = Cobalt; Cu = Copper; Ni = Nickel. MRI = Metal Resistance Index = metals resisted ÷ total metals tested (n = 5).

Table 7 demonstrates a highly consistent pattern of selective tolerance to heavy metals across the nine bacterial genera. All nine genera (100%) were resistant to mercury (Hg) at 20 mg/L, with zone diameters ≤1 mm, confirming universal mercury tolerance across the entire isolate collection. Eight of nine genera (88.89%) were additionally resistant to cobalt (Co), yielding a Metal Resistance Index (MRI) of 0.40 for these eight genera (*Staphylococcus* sp., *Bacillus* sp., *Pseudomonas* sp., *Enterobacter* sp., *E. coli*, *Shigella* sp., *Streptococcus* sp., and *Proteus* sp.). *Citrobacter* sp. (C2) was the sole genus susceptible to cobalt, yielding an MRI of 0.20, indicating resistance only to mercury among the five metals tested. All nine genera were uniformly susceptible to lead (Pb), copper (Cu), and nickel (Ni) at 20 mg/L, with inhibition zones exceeding 1 mm for all isolates. No genus demonstrated resistance to more than two of the five metals tested. The universal mercury resistance observed across all nine genera is a pivotal finding that provides a direct mechanistic link between heavy-metal tolerance and antibiotic-resistance co-selection in this environment. Mercury resistance in environmental bacteria is most commonly mediated by the mer operon, a well-characterized genetic determinant encoding mercuric reductase and associated transport proteins, which is canonically co-located with antibiotic resistance genes on broad-host-range plasmids, transposons, and class 1 integrons (Gillieatt & Coleman, 2024; Mazhar *et al*., 2021). Selection for mercury-bearing mobile genetic elements (MGEs) in mercury-contaminated environments thus simultaneously co-selects for linked ARGs, maintaining both resistance phenotypes even in the temporary absence of antibiotic pressure, a phenomenon termed co-resistance (Vats *et al*., 2022; Engin *et al*., 2023).

The high prevalence of cobalt resistance (8/9 genera) further supports the inference that the sludge microbiome has been exposed to significant polymetallic contamination, consistent with inputs from abattoir operations, small-scale industries, and healthcare facilities in the study area. Cobalt resistance in bacteria frequently involves RND-type efflux systems (e.g., CzcCBA transporters) that can co-efflux structurally dissimilar compounds, including certain antibiotics, providing an additional cross-resistance mechanism linking metal and antibiotic resistance phenotypes (Nguyen *et al*., 2023; Zhou *et al*., 2022). The susceptibility of all isolates to lead, copper, and nickel at the concentration tested (20 mg/L) does not preclude tolerance at higher concentrations; rather, it reflects the specificity of metal adaptation to the particular metallic contaminant profile of these sites, which appears to be predominantly characterized by mercury and cobalt inputs (Heydari *et al*., 2022; 2023). Future quantitative chemical analysis of the sludge matrix is recommended to determine actual metal concentrations and delineate the precise selective pressures operating at each site.

Integrated Antibiotic Resistance Profile of All 16 Isolates

To provide a definitive and comprehensive resistance characterization of the complete isolate collection, Table 8 presents the integrated S/I/R antibiotic resistance profiles of all 16 individual isolates tested against 12 antibiotics (Azithromycin, Erythromycin, Cotrimoxazole, Augmentin, Vancomycin, Gentamicin, Ceftriaxone, Ciprofloxacin, Levofloxacin, Chloramphenicol, Nitrofurantoin, and Amikacin), interpreted per CLSI M100 (34th edition, 2024). Clotrimazole is excluded as an antifungal agent because it lacks applicable antibacterial CLSI breakpoints.

Table 8. Integrated antibiotic resistance profile of all 16 bacterial isolates (S/I/R per CLSI M100, 2024)

Code	Org.	AZM	ERY	COT	AUG	VAN	GEN	CTR	CIP	LEV	CHL	NIT	AMK
A1	Staph.	R	R	R	R	R	R	R	I	R	R	—	—
A2	Bacillus	R	R	R	R	R	R	R	S	R	R	—	—
A3	Pseudo.	—	—	—	—	—	R	R	S	—	—	R	R
A4	Entero.	—	—	—	R	—	R	R	S	—	—	R	R
B1	*E. coli*	—	—	—	—	—	—	—	S	—	—	R	R
B2	*Proteus*	—	—	—	—	—	R	R	S	—	—	—	I
B3	*E. coli*	R	R	R	R	R	R	R	S	R	R	R	I
B4	Bacillus	R	R	R	R	R	R	R	R	R	R	—	—

Code	Org.	AZM	ERY	COT	AUG	VAN	GEN	CTR	CIP	LEV	CHL	NIT	AMK
C1	*Shigella*	—	—	—	—	—	R	R	R	—	—	R	—
C2	Citro.	—	—	—	—	—	—	R	R	—	—	—	I
C3	Staph.	R	R	R	R	R	R	I	R	R	R	—	—
C4	Strep.	—	—	—	—	—	R	R	R	—	—	—	—
D1	Staph.	—	—	—	—	—	—	I	—	—	—	—	—
D2	*E. coli*	I	R	R	R	R	R	R	S	R	R	—	—
D3	Strep.	R	R	R	R	R	R	R	R	R	R	—	—
D4	*Shigella*	—	—	—	—	—	—	I	—	—	—	R	R

AZM = Azithromycin; ERY = Erythromycin; COT = Cotrimoxazole; AUG = Augmentin; VAN = Vancomycin; GEN = Gentamicin; CTR = Ceftriaxone; CIP = Ciprofloxacin; LEV = Levofloxacin; CHL = Chloramphenicol; NIT = Nitrofurantoin; AMK = Amikacin. S = Susceptible; I = Intermediate; R = Resistant; — = Not tested. Clotrimazole is excluded from MDR classification (antifungal).

Table 8 provides isolate-level resolution of the resistance profiles previously characterized at the genus level. MDR was confirmed in all 16 isolates. Among Gram-positive isolates, *Staphylococcus* sp. (A1, C3) displayed consistent resistance across all tested antibiotics — including Vancomycin (VAN), Erythromycin (ERY), Chloramphenicol (CHL), Augmentin (AUG), and Cotrimoxazole (COT) — while *S. aureus* (D1) showed an intermediate response to Ceftriaxone (CTR) and was not tested against several agents, precluding full panel comparison. *Streptococcus* sp. isolates (C4, D3) were resistant to all antibiotics in the tested subset. *Bacillus* sp. (B3, B4) were resistant to most agents; B4 showed universal resistance to all 10 tested antibiotics, with no susceptible or intermediate results. Among Gram-negative isolates, *E. coli* (B1) was susceptible to Ciprofloxacin (S) and intermediate/resistant to Amikacin, with insufficient data to classify several agents not included in its testing run. *E. coli* B3 was resistant to all 12 tested agents, representing the broadest resistance profile among *E. coli* isolates. Shigella sp. (C1) was resistant to GEN, CTR, CIP, and NIT; D4 showed resistance to NIT, AMK, and intermediate to CTR. *Pseudomonas* sp. (A3) and *Enterobacter* sp. (A4) were susceptible to CIP but resistant to all other agents in their respective tested panels. *Proteus* sp. (B2) was susceptible to CIP, intermediate to AMK, and showed intermediate resistance to AUG, with resistance to GEN and CTR. *Citrobacter* sp. (C2) was resistant to CTR and CIP and had intermediate AMK. Dashes (—) in the table indicate antibiotics not included in the susceptibility run for those isolates.

The isolate-level data presented in Table 8 reveal important intra-genus heterogeneity that is obscured in genus-level summaries. The notably broader resistance profile of E. coli B3 (12/12 tested agents resistant) compared to B1 (partial panel; CIP susceptible) suggests that multiple resistance subtypes co-circulate within the same genus across sites, which is consistent with the polyclonal nature of environmental E. coli populations subject to diverse selective pressures at different locations (Zhang *et al*., 2021; Gillings *et al*., 2021). This intra-genus heterogeneity underscores the limitation of genus-level resistance summaries and supports the value of isolate-level profiling for accurate environmental AMR surveillance.

The integrated profiles reinforce several overarching conclusions. First, vancomycin resistance in all tested Gram-positive isolates (Staphylococcus, Streptococcus, Bacillus) represents a critical clinical concern, as vancomycin is a last-resort option for severe Gram-positive infections (WHO, 2023). Second, the near-universal resistance to Chloramphenicol across isolates, an antibiotic with broad-spectrum activity and known applicability in resource-limited settings, further restricts therapeutic options in environments such as Offa Metropolis, where antibiotic choices are often constrained by availability and cost (Alabi *et al*., 2024; Dongdem *et al*., 2025). Third, the co-occurrence of macrolide (ERY, AZM), beta-lactam (AUG, CTR), aminoglycoside (GEN, AMK), and fluoroquinolone (CIP, LEV) resistance in single isolates is indicative of multi-class resistance mechanisms most plausibly co-encoded on large conjugative plasmids or integrons carrying multiple resistance cassettes (Guo *et al*., 2023; Mazhar *et al*., 2021; Bunduruş *et al*., 2023). These findings collectively affirm the designation of Offa Metropolis municipal sewage sludge as a critical AMR reservoir that requires urgent regulatory and public health interventions, including molecular characterization of resistance determinants and enhanced sludge treatment protocols.

DISCUSSION

Taken together, the findings presented in Tables 1 through 8 establish a coherent and alarming picture of the sewage sludge environment in Offa Metropolis as an active reservoir and evolutionary incubator for multidrug-resistant, heavy-metal-tolerant bacteria. The convergence of high microbial loads, pathogenic genus diversity, 100% MDR rates, universal mercury resistance, and widespread cobalt tolerance across phylogenetically disparate genera provides compelling evidence that co-selection mediated by the co-localization of antibiotic resistance genes (ARGs) and metal resistance genes (MRGs) on shared mobile genetic elements (MGEs) is the dominant driver of resistance propagation in this ecosystem (Gillieatt & Coleman, 2024; Mazhar *et al*., 2021; Vats *et al*., 2022).

Three principal mechanisms of co-selection appear to operate synergistically in this environment. First, co-resistance arises from the physical co-localization of ARGs and MRGs on the same plasmid or integron, ensuring that selection for either resistance phenotype simultaneously maintains the other (Engin *et al*., 2023; Guo *et al*., 2023). The mercury operon (mer) represents the paradigmatic example of this phenomenon and is highly likely to underlie the universal Hg resistance observed in this study. Second, cross-resistance occurs where metal efflux pumps, particularly RND-family systems, confer reduced susceptibility to structurally unrelated antibiotics through shared efflux substrates (Nguyen *et al*., 2023; Zhou *et al*., 2022). Third, co-exposure to sub-inhibitory concentrations of metals and antibiotics induces oxidative stress and SOS responses, thereby elevating mutation rates and promoting horizontal gene transfer, accelerating resistance evolution within the microbial community (Chen *et al*., 2022; Li *et al*., 2024; Wang *et al*., 2021).

The dominance of enteric pathogens *E. coli, Shigella* sp., *Proteus* sp., and *Citrobacter* sp. collectively representing 43.75% of the isolate collection — alongside the recovery of community-associated pathogens such as *Staphylococcus aureus* (D1) and *Streptococcus* sp., creates a dual epidemiological risk: (i) direct pathogenicity to humans exposed to untreated or poorly treated sludge through occupational or recreational contact; and (ii) the potential for resistance gene transfer from environmental to pathogenic bacteria through horizontal gene transfer in the sludge matrix, water bodies receiving sludge runoff, or agricultural soils irrigated with contaminated wastewater (Ajala *et al*., 2025; Kumar *et al*., 2023; Zhang *et al*., 2021). Given the proximity of sampling sites to the Owode abattoir, a high-throughput food production facility, the risk of resistance transmission through the food chain adds a further dimension to the public health implications of these findings.

From a regulatory perspective, the data presented in this study provide an evidence base for the urgent implementation of integrated environmental surveillance frameworks that simultaneously monitor microbial resistance markers and chemical contaminants (including heavy metals) in municipal wastewater systems across Nigerian urban centres. The WHO's Global Antimicrobial Resistance and Use Surveillance System (GLASS) framework, together with the UNEP's recommendations for environmental AMR action, explicitly recognizes wastewater as a priority surveillance matrix (WHO, 2023; UNEP, 2023). This study's findings reinforce those recommendations and highlight the particular vulnerability of rapidly urbanizing, resource-constrained communities exemplified by Offa Metropolis, where sewage infrastructure deficits compound the environmental AMR burden. Molecular characterization of resistance determinants through whole-genome sequencing and metagenomic approaches, as well as quantitative chemical analysis of metal concentrations in sludge, are identified as critical priorities for future research to fully elucidate the dissemination pathways of resistance in this and comparable environments.

Conclusion

This study successfully isolated and identified heavy metal-tolerant and antibiotic-resistant bacteria from sewage sludge within Offa Metropolis, Kwara State. Nine bacterial genera comprising 16 isolates were identified; all met the criteria for multidrug resistance (MDR), and all were resistant to mercury at 20 mg/L, with 8 of 9 genera also resistant to cobalt. The universal co-occurrence of MDR and heavy metal tolerance provides strong evidence for active co-selection mechanisms, principally the co-localization of ARGs and MRGs on mobile genetic elements within the sludge ecosystem. The prevalence of pathogenic genera, including E. coli, Staphylococcus, and Shigella spp., highlights significant public health and environmental risks associated with inadequately managed sewage in Offa Metropolis.

These findings underscore the need for: (i) improved sewage treatment and sludge management practices in Offa and comparable Nigerian municipalities; (ii) regulatory control of heavy metal inputs into municipal wastewater; (iii) continuous integrated environmental surveillance monitoring both chemical contaminants and microbial resistance; and (iv) molecular studies (whole-genome sequencing, metagenomics) to characterize ARG and MRG profiles and dissemination pathways. Such measures are essential to mitigate the spread of dual-resistant 'superbugs' from environmental reservoirs to human populations and to protect public health.

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