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Research Article

Detection of Multi- Antibiotic and Metallo- Resistant Genes in *Escherichia coli* and *Pseudomonas aeruginosa* from Contaminated Soil and Wastewater of Some Abattoirs in Adamawa State, Nigeria

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Abstract

Abattoir effluents constitute significant environmental reservoirs of antibiotic-resistant bacteria and heavy metals, posing substantial public health risks through co-selection mechanisms. This study investigated the occurrence and molecular characterization of multi-antibiotic and metallo-resistant genes in Escherichia coli and Pseudomonas aeruginosa isolated from abattoir wastewater and contaminated soils in Adamawa State, Nigeria. A total of nine samples were collected from three major abattoirs representing the senatorial districts of the State. Bacterial isolates were identified using standard microbiological techniques and confirmed by 16S rRNA gene sequencing. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method, while heavy metal tolerance was assessed through the broth dilution method at concentrations of 50, 150, and 300 ppm. Molecular detection of resistance genes (blaTEM-1, copA, and zntA) was conducted via PCR amplification. Results revealed nine bacterial isolates comprising three E. coli and six P. aeruginosa. Two isolates (E. coli JWW2 and P. aeruginosa JS7) exhibited remarkable multi-antibiotic resistance with MAR indices of 0.83 and 0.75, respectively, and demonstrated tolerance to copper, zinc, iron, lead, and cobalt at varying concentrations. Molecular analysis confirmed the presence of blaTEM-1 in both isolates and copA exclusively in E. coli JWW2, while zntA was not detected in either isolate. The co-occurrence of antibiotic and heavy metal resistance genes in these environmental isolates highlights the potential for co-selection and horizontal gene transfer, underscoring the need for improved abattoir waste management practices and continuous surveillance to mitigate the spread of multidrug-resistant pathogens in contaminated environments.

Keywords: *Abattoir wastewater, Escherichia coli, Pseudomonas aeruginosa, metallo-resistance genes, antibiotic resistance genes, heavy metal tolerance, Nigeria.*

INTRODUCTION

The coexistence of antibiotic resistance and metal tolerance within environmental bacterial communities constitutes a pressing ecological and public health hazard. Abattoir effluents represent particularly critical reservoirs, as they are enriched with organic debris, enteric pathogens, and notably high concentrations of heavy metals, including Cu, Zn, Fe, Pb, and Mn, which, when present at elevated thresholds, become toxic and induce selective microbial adaptation (Ja'afaru *et al.,* 2025; Sarker *et al.,* 2024; Abulreesh *et al.,* 2016). Among the ubiquitous Gram-negative bacilli inhabiting these niches are *Pseudomonas aeruginosa* and *Escherichia coli*, organisms distinguished both by their bioremediation capabilities and their well-documented capacity to acquire determinants conferring resistance to a broad spectrum of antibiotics and metallic compounds (Zoghi and Reihani, 2025; Mercimek Takci *et al.,* 2021).

The heavy metal burden in abattoir wastewater and adjacent soils is fundamentally attributed to bioaccumulation within slaughtered livestock. Animals typically incorporate these elements via the ingestion of contaminated feeds and drinking water, alongside occasional inhalation of polluted air (Akawu *et al.,* 2020). Owing to inherently poor excretion rates, these metals progressively deposit across various tissues and organs, although partial elimination does occur through urine, faeces, and other biological secretions (Ali *et al.,* 2019). During slaughtering operations, metal-laden bodily fluids, excreta, and organ contents are liberated directly into the surrounding environment. Additionally, the deterioration or inadequate upkeep of metal-based processing equipment, such as ferrous knives, tables, and hooks, can further augment the metallic load in effluent through active leaching (Ogbonna *et al.,* 2020). Consequently, the progressive accretion of these contaminants in abattoir discharge and soils poses a substantial threat to neighboring ecosystems (Lawrence *et al.,* 2020). This persistent metallic stress imposes a robust selective force on local microbial populations, actively fostering the emergence and dominance of both metal-tolerant and antibiotic-resistant strains (Pal *et al.,* 2015). Crucially, heavy metals are implicated in the co-selection of antimicrobial resistance; they facilitate the upkeep and intercellular transfer of resistance genes, predominantly via horizontal gene transfer, thereby accelerating the propagation of multidrug resistance across microbial communities inhabiting these polluted zones (Singh *et al.,* 2024).

Within the Nigerian context, a considerable number of abattoirs release untreated wastewater directly into terrestrial and aquatic systems, thereby introducing enteric pathogens and antibiotic-resistant bacteria carrying extended-spectrum β-lactamases (ESBLs) and metallo-β-lactamases (MBLs) (Ejikeugwu *et al.,* 2017; Ja'afaru *et al.,* 2025). Previous investigations have successfully isolated *Salmonella* spp., *Staphylococcus aureus*, *Klebsiella* spp., and *P. aeruginosa* from abattoir wastes and their surrounding environments (Adesomoye *et al.,* 2006; Ja'afaru *et al.,* 2021; Ja'afaru *et al.,* 2025). It is worth noting that the physicochemical composition of these effluents is subject to daily variation, dependent upon processing throughput and the species of livestock being handled (Ojegunle and Lateef, 2017).

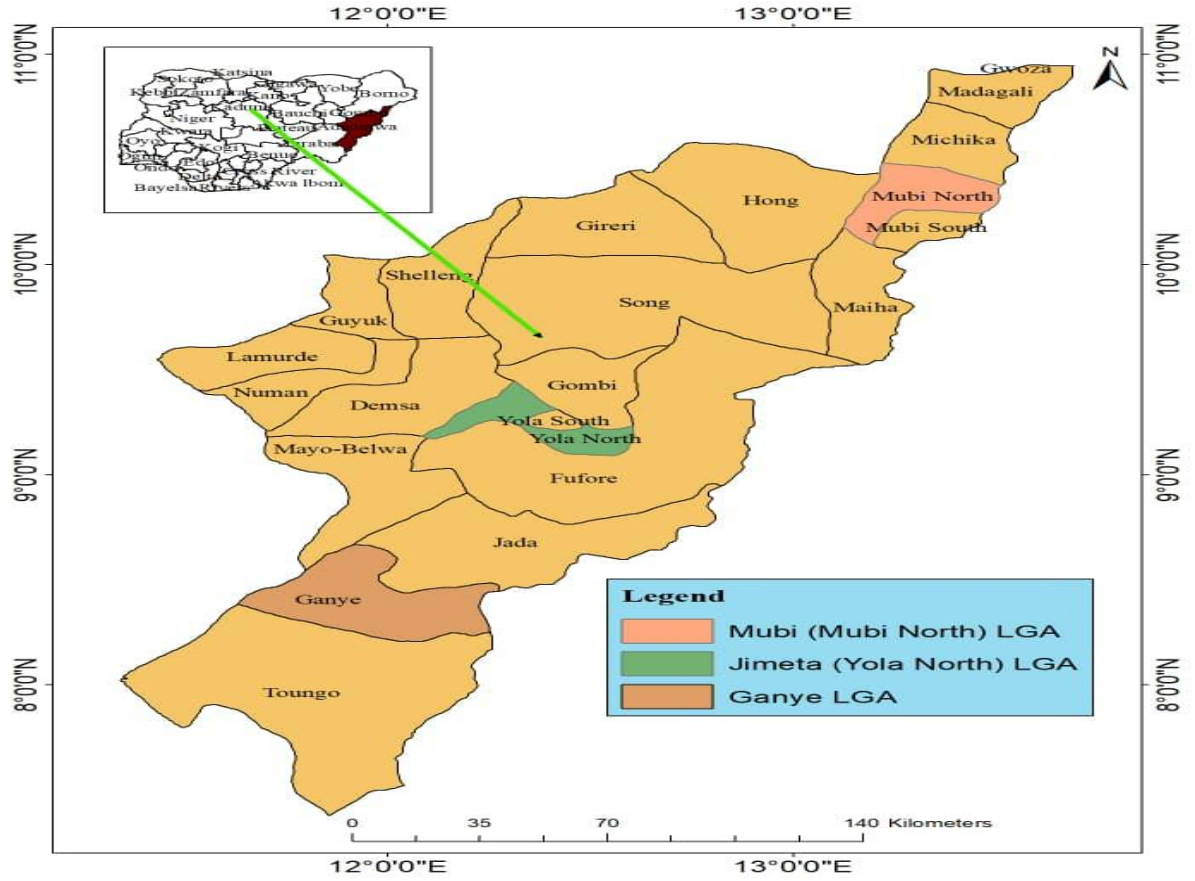
The ongoing accumulation of heavy metals fundamentally alters soil physicochemical characteristics, consequently selecting for robust, tolerant organisms that frequently exhibit multidrug resistance profiles (Adekanmbi and Falodun, 2015). Traditional phenotypic screening methods are inherently limited in their capacity to detect the full array of resistance determinants, thereby underscoring the urgent necessity for molecular characterization, which currently stands as the definitive benchmark for rigorous epidemiological surveillance, particularly within Adamawa State (Iroha *et al.,* 2016). Accordingly, this investigation is designed to examine the concurrent incidence of antibiotic and heavy metal resistance among *P. aeruginosa* and *E. coli* isolates derived from the Jimeta abattoir wastewater, with the overarching aim of furnishing empirical evidence to guide monitoring frameworks and mitigation efforts against the dispersal of resistant pathogens in contaminated environmental settings.

METHODOLOGY

Study Area

Situated within Nigeria's northeastern geopolitical zone, Adamawa State spans approximate latitudes of 7° to 11°N and longitudes of 11° to 14°E. Administratively, the state is subdivided into 21 Local Government Areas, which are further aggregated into three senatorial districts. The regional climate is defined by consistently high temperatures and low relative humidity, coupled with substantial evapotranspiration rates that become especially pronounced during the prolonged dry season (Adebayo, 1999). As the state capital and its primary urban hub, Yola accommodates an estimated population of roughly 200,000 residents (National Population Commission, as cited in Ajomale, 2007).

Adamawa holds a prominent position among Nigeria's leading livestock-producing states (Mahuta *et al.,* 2023). In tandem with this, ongoing demographic expansion has significantly intensified the regional demand for, and consumption of, meat products. To ensure comprehensive and representative sampling across the state's diverse ecological and operational settings, specimens for this investigation were systematically procured from the three foremost abattoirs, one strategically chosen from each senatorial district. A cartographic representation of Adamawa State is also included, explicitly delineating the three focal Local Government Areas covered in this study: Ganye, Jimeta (falling within Yola North), and Mubi (situated in Mubi North).



(Source: Geography Department, MAU, YOLA (2025)

Sample Collection

Specimens, consisting of abattoir wastewater and wastewater-impacted soil, were obtained from three major slaughterhouses situated in Jimeta, Mubi, and Ganye, towns strategically selected to represent each of the three senatorial districts within Adamawa State, Nigeria. In total, nine discrete samples were collected per site, with specimens drawn from various spatial points to capture heterogeneity. Effluents were aseptically transferred into sterile glass bottles of 100 mL capacity, while soil cores were extracted at 0–20 cm depths using a soil auger, with collection performed at two-week intervals (Emmanuel *et al.,* 2018). For baseline reference, control samples of both wastewater and soil were concurrently gathered at each location, taken from points situated 300 to 400 meters distant from the principal slaughterhouse operations. All wastewater aliquots were promptly stored in chilled ice-boxes and transported to the laboratory under strict aseptic protocols, meticulously following the guidelines prescribed by Rabah et al. (2011).

Bacterial Culture and Identification

For the presumptive isolation of target organisms, selective media were employed: *E. coli* was provisionally identified on Eosin Methylene Blue (EMB) agar, whilst *P. aeruginosa* was similarly differentiated on Cetrimide agar. Definitive species confirmation was subsequently achieved through Gram staining, complemented by a comprehensive panel of standard biochemical assays, including catalase production, citrate utilization, oxidase activity, methyl red and Voges-Proskauer reactions, Triple Sugar Iron Agar (TSA) profiles, and indole tests. All identification procedures were rigorously executed in accordance with established microbiological protocols (Cowan and Steel, 1993; Seiyaboh *et al.,* 2013; Pola and Emmanuel, 2024).

Antimicrobial Susceptibility and Heavy Metal Tolerance Profiling

In vitro antimicrobial susceptibility testing was performed on all bacterial isolates using the Kirby-Bauer disk diffusion method, in strict accordance with the guidelines established by the Clinical and Laboratory Standards Institute (CLSI, 2020). The panel of antibiotics evaluated comprised: Amoxicillin-Clavulanate (AUG, 30 µg), Cefotaxime (CTX, 25 µg), Imipenem/Cilastatin (IMP, 10/10 µg), Nitrofurantoin (NF, 300 µg), Cefuroxime (CXM, 30 µg), Ceftriaxone-Sulbactam (CRO, 45 µg), Ofloxacin (OFX, 5 µg), Gentamicin (GN, 10 µg), Nalidixic Acid (NA, 30 µg), Ampiclox (ACX, 10 µg), Cefixime (ZEM, 5 µg), Levofloxacin (LBC, 5 µg), Ciprofloxacin (CIP, 5 µg), and Azithromycin (AZN, 15 µg).

Concurrently, the isolates were assessed for tolerance to a spectrum of heavy metals, specifically zinc, iron, copper, lead, and cobalt, across graded concentrations of 50, 150, and 300 ppm. This evaluation employed the broth dilution technique as outlined by Hossain et al. (2012) and Olowomofe et al. (2020). Metal-supplemented nutrient broths were prepared by filter-sterilizing appropriate concentrations, after which they were inoculated with a loopful of each selected bacterial isolate derived from the abattoir-contaminated specimens. A control set, consisting of inoculated broth devoid of any heavy metal supplementation, was maintained for comparative purposes. All cultures were incubated at 37°C for 48 hours. Post-incubation, bacterial growth was quantitatively assessed by measuring absorbance using a spectrophotometer, following the protocol detailed by Vashishth and Khanna (2015).

Molecular Characterization of Metal-Tolerant Isolates

From the pool of characterized organisms, isolates demonstrating the most pronounced profiles of multidrug resistance and heavy metal tolerance were selected for molecular identification. This was achieved through PCR amplification of the 16S rRNA gene region, employing universal primer sets. Genomic DNA extraction was accomplished using the ZR Bacterial DNA MiniPrep™ kit (Zymo Research), in accordance with the manufacturer's prescribed procedures. Briefly, bacterial cultures were grown overnight in Luria broth; subsequently, 1 mL of this culture was pelleted via centrifugation at 10,000 × g for one minute. The resultant pellet was resuspended in 200 µL of normal saline and thoroughly homogenized. This suspension was then introduced into a ZR BashingBead™ lysis tube, to which 750 µL of lysis solution was added, followed by vigorous vortexing for 14 minutes to ensure complete cellular disruption. The tube was then centrifuged at 10,000 × g for one minute, and 400 µL of the supernatant was carefully transferred to a Zymo-Spin™ IV Spin filter, which was subsequently centrifuged at 7,000 × g for one minute. A volume of 1,200 µL of DNA binding buffer was added to the filtrate, and the entire mixture was loaded onto a Zymo-Spin™ IIC column and centrifuged at 10,000 × g for one minute. Following this, 200 µL of DNA pre-wash buffer was applied to the column and centrifuged at 10,000 × g for one minute, after which 500 µL of DNA wash buffer was added and the column centrifuged again at 10,000 × g for one minute. Finally, the Zymo-Spin™ IIC column was transferred to a clean 1.5 mL microcentrifuge tube, and 70 µL of DNA elution buffer was added; the column was then centrifuged at 10,000 × g for 30 seconds to elute the purified DNA, which served as the template for subsequent PCR reactions.

Amplification of the 16S rRNA gene was performed using the forward primer 27F (MWG) (5'-GAGTTTGATCCTGGCTCAG-3') and the reverse primer 1492R (MWG) (5'-TACCTTGTTACGACTT-3'), as described by Rainey et al. (1996). Successful amplification was indicated by the production of an amplicon of approximately 1496 base pairs, consistent with findings reported by Garafutdinov et al. (2015). Purified PCR products were sequenced for the 16S rRNA gene using the BigDye Terminator sequencing methodology (IDT, Gemini Singapore), and sequencing reactions were resolved on an automated ABI 3700 DNA sequencer (Applied Biosystems, Foster City, California, USA), conducted at the Anchor University Biotechnology Laboratory in Ayopo Ipaja, Lagos State, Nigeria.

The resulting nucleotide sequences were subjected to homology searches against the GenBank database using the BLASTN tool (available at http://www.ncbi.nlm.nih.gov/BLAST). Sequences were compared to reference strains of well-characterized *E. coli* and *Pseudomonas aeruginosa* to ascertain sequence similarity. Homology assessment was based on two principal parameters: the E-value score and percentage sequence identity. Sequences demonstrating an E-value below the stringent threshold of 10^{-3} and exhibiting greater than 95% identity were considered to represent biologically significant homologous matches, in accordance with the criteria established by Nourouzi et al. (2023).

Molecular Screening for Antibiotic and Heavy Metal Resistance Determinants

The genetic basis of phenotypic resistance was further explored through PCR-based detection of selected antibiotic and heavy metal resistance genes. Specifically, the presence of the β-lactamase-encoding gene *blaTEM-1* (conferring antibiotic resistance) alongside the metal tolerance determinants *znt-A* and *cop-A* was examined. Each amplification reaction was prepared in a total volume comprising 12.5 µL of 5× Master Mix, 7.5 µL of nuclease-free water, 1.0 µL each of forward and reverse oligonucleotide primers, and 3.0 µL of extracted genomic DNA as the template. The target genes were amplified using locus-specific primer pairs with annealing temperatures carefully optimized to ensure primer specificity: 60.8°C for *znt-A*, 58°C for *cop-A*, and 50.8°C for *blaTEM-1*.

Following thermocycling, amplicons were resolved by electrophoresis on 1% agarose gels impregnated with ethidium bromide for visual detection under UV illumination. Successful gene amplification was confirmed by the visualization of discrete bands migrating at the expected molecular weights, corresponding to 2375 base pairs for *znt-A*, 796 base pairs for *cop-A*, and 500 base pairs for *blaTEM-1*. All positive PCR products were subsequently purified and subjected to Sanger sequencing at the Anchor University Biotechnology Laboratory in Ayopo Ipaja, Lagos State, following the procedures previously described.

Details of genes and primers used are given below

Gene	Function	Forward sequence	Reverse sequence	Target
Znt-A	Resistance to Zn/Cd/Pb	ATCGTCCGCTCGCTGTATCTCT	CCGCCTTTTCCCCTCACCCTA	2375 bp
Cop-A	Resistance to Cu	GGTGCTGATCATCGCCTG	GGGCGTCGTTGATACCGT	796bp
blaTem-1	Resistance to β-lactam	TCGGGGAAATGTGCG	GGAATAAGGGCGACA	500bp

RESULTS

Distribution of *Escherichia coli* and *Pseudomonas aeruginosa* Across Abattoir Wastewater and Contaminated Soil

From the total sample pool analyzed, three (3) distinct isolates of *Escherichia coli* were recovered across all specimen sources. Of these, two were obtained from soil samples contaminated with abattoir wastewater, while only a single isolate was derived directly from abattoir effluent. In contrast, *Pseudomonas aeruginosa* demonstrated a substantially higher recovery rate, with a total of six (6) isolates identified, three originating from abattoir wastewater and an equal number from associated contaminated soils (Table 1).

Geographical analysis of *P. aeruginosa* distribution revealed that Jimeta yielded the highest number of isolates (three), encompassing specimens from both wastewater and soil matrices. Mubi followed with two isolates, while Ganye recorded the lowest occurrence, with only one isolate recovered. Conversely, *E. coli* exhibited a uniform spatial distribution, with a single isolate detected from each of the three geographic locations, as illustrated in Figure 1.

Table 1. Distribution of *E. coli* and *P. aeruginosa* across sample sources

Isolates	Sample Sources		Total
	Abattoir wastewater	Contaminated soil	
E. coli	1	2	3
P. aeruginosa	3	3	6
Total	4	5	9

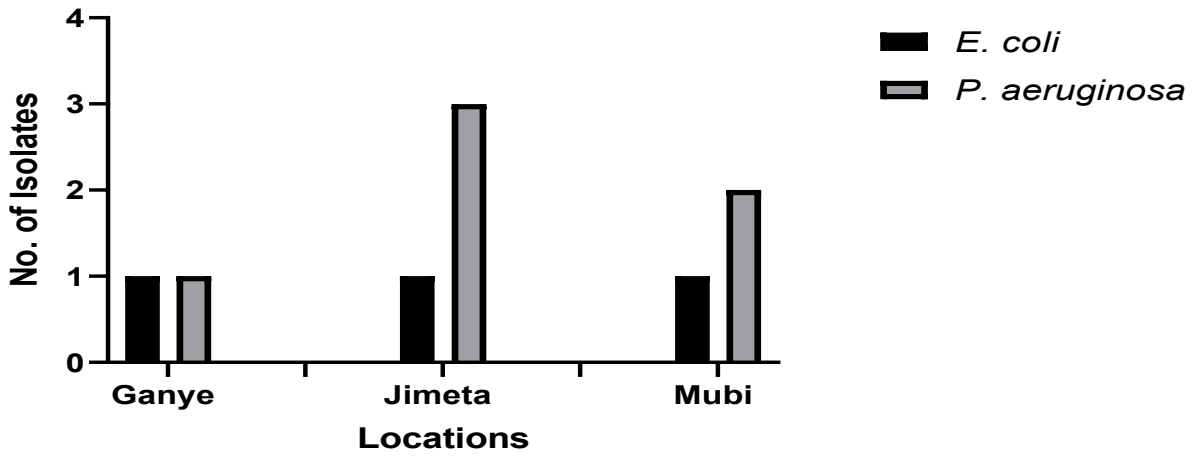


Fig.1. Distribution of isolates across the three geolocations.

Antibiogram of the isolates and heavy metal tolerance

The isolates were tested for resistance against 12 antibiotics as described earlier (Table 2). Two of the isolates, designated as *E. coli* JWW2 (isolated from Jimeta abattoir wastewater) and *P. aeruginosa* JS7 (isolated from Jimeta wastewater-contaminated soil sample), have demonstrated remarkable resistance to the tested antibiotics with antimicrobial resistance (AMR) indices of 0.83 and 0.75. Heavy metals tolerance test indicated that the two isolates were tolerant to at least one of the concentrations of the metals. The results showed that *Pseudomonas aeruginosa* (JS 7) and *Escherichia coli* (JWW2) tolerated all the heavy metals tested by showing moderate to luxuriant growth, except Zn at 300ppm (Table 3).

Table 2. Multiple- Antibiotic Resistance Index (MAR) of *E. coli* and *P. aeruginosa* from the Study Area

Isolates	No. of Antibiotics Tested	No. of Antibiotics Resistant	MAR Index
E. coli	12	10	0.83
P. aeruginosa	12	09	0.75

Table 3: Qualitative Estimates of *E. coli* and *P. aeruginosa* Tolerance to Heavy Metals Concentrations (ppm)

Bacterial Isolates	Heavy Metals Concs (ppm)														
	Cu			Zn			Fe			Pb			Co		
	50	150	300	50	150	300	50	150	300	50	150	300	50	150	300
E. coli	+++	++	++	+	-	-	++	++	++	++	++	+	++	++	+
P. aeruginosa	++	+	+	+	+	-	++	+	+	++	++	++	+++	++	+

KEY: + = Scanty growth, ++ = Moderate growth, +++ = Luxuriant growth, - = No visible growth, Absorbance ≤ 0.06 = (No growth), 0.08 – 0.16 (Scanty growth) = +, 0.11- 0.20 (Moderate growth) = ++, > 0.2= +++ (Luxuriant growth)

Molecular Confirmation and Genotypic Characterization of Selected Isolates

Based on their phenotypic resistance profiles and tolerance characteristics, two representative isolates, *Pseudomonas aeruginosa* (designated JS7) and *Escherichia coli* (designated JWW2), were selected for molecular identification and subsequent screening for multidrug resistance and metallo-tolerance determinants. Conventional biochemical identification of both isolates was unequivocally confirmed through PCR amplification of the 16S rRNA gene using universal primer pairs. Agarose gel electrophoresis revealed successful amplification of the expected ~1496 bp fragment for both organisms (Fig 2A), thereby validating their taxonomic assignment.

Antibiotic and Heavy Metal Resistance Gene Profiling

Screening for the extended-spectrum β-lactamase gene *blaTEM-1* demonstrated positive amplification, yielding a 500 bp amplicon in both isolates. Specifically, *E. coli* JWW2 exhibited a distinct 500 bp band corresponding to *blaTEM-1*, alongside a 1 Kbp molecular weight marker (300–10,000 bp range) and the positive control *E. coli* ATCC 25922 (Fig 2B). Similarly, *P. aeruginosa* JS7 also harbored the *blaTEM-1* gene, as evidenced by an identical 500 bp amplification product (Fig 2B, lane 4).

Regarding heavy metal resistance determinants, *E. coli* JWW2 demonstrated carriage of the *copA* gene, which encodes copper tolerance, with positive amplification of a 796 bp product confirmed against a 1 Kbp molecular weight marker and a positive control (*K. pneumoniae* ATCC 700603) (Fig 2C). Notably, this isolate concurrently possessed both the *blaTEM-1* antibiotic resistance gene and the *copA* metallo-resistance gene, consistent with its phenotypic expression of ESBL and metallo-β-lactamase activity at specific metal concentrations.

Conversely, *P. aeruginosa* JS7, within the scope of this investigation, carried only the antibiotic resistance determinant; it was positive exclusively for *blaTEM-1*, while no metal resistance genes were detected among those examined. A critical observation from this study is that neither *E. coli* JWW2 nor *P. aeruginosa* JS7 harbored the *znt-A* gene, which encodes zinc tolerance. Both isolates consistently exhibited negative amplification for this determinant across all reactions (Fig 2D).

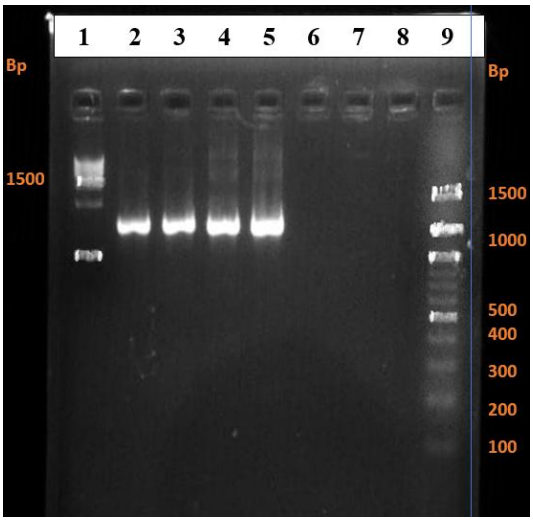
Sequence Homology Confirmation

The nucleotide sequences obtained from the amplified products were subjected to BLAST analysis against the NCBI nucleotide database. The resulting sequence homologies confirmed the identity of the *blaTEM-1* and *copA* resistance genes in the respective *E. coli* and *Pseudomonas* isolates, with the alignment data and homology parameters summarized in Table 2.

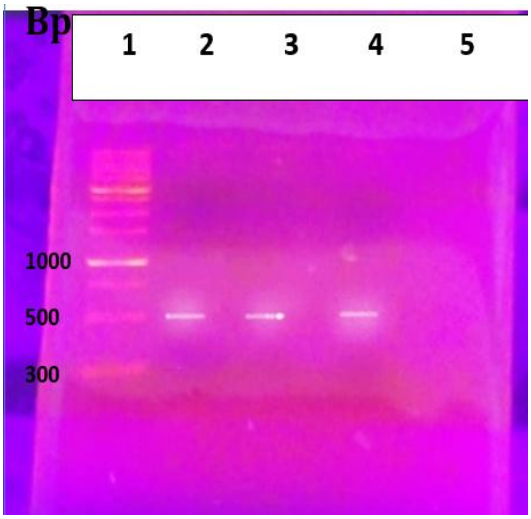
Table 2: NCBI NUCLEOTIDE SEQUENCE MEGA BLAST OF *blaTem-1* and *copA* RESISTANT *E. coli and Pseudomonas* ISOLATEs

NCBI NUCLEOTIDE SEQUENCE MEGA BLAST OF *blaTem-1* and *copA* RESISTANT *E. coli and Pseudomonas* ISOLATEs										
S/N	Nucleotide Sequence	Sample Identity	Accession	Max score	Total score	Query cover	E value	Per. Identity	Acc length	Description
1	TGAGACACGGTCCAGACTCCTACGG GAGGCAGCAGTGGGGAATATTGCAC AATGAGGCGCAAGCCTGATGCAGCC ATGCNGCGTGTATGAAGAAGGCCTTC GGGTTGTAAAGTACTTTCAGCGGGGA GGAAGGAGAGTAAAGTTAATACCTT TGCTCATTGACGTTACCCGCAGAAGA AGCACCGGCTAACTCCGTGCCAGCA GCCGCGGTAATACGGAGGGTGCAAG CGTTAATCGGAATTACTGGGCGTAAA GCGCACGCAGGCGGTTTGTTAAGTCA GATGTGAAATCCCCGGGCTCAACCTG GGAACTGCATCTGATACTGGCAATGC TTGAGTCTCGTAGAGGGGGGTAGAA TTCCAGGTGTAGCGGTGAAATGCGTA GAGATCTGGAGGAATACCGGTGGCA AGGCGGCCCCCTGGACGAAGACTGA CGCTCAGGTGCGAAAGCGTGGGGAG CAAACAGGATTAGATACCCTGGTAGT CCACGCCGTAAACGATGTCGACTTGG AGGTTGTGCCCTTGAGGCGTGGCTTC CGGANNTAACGCGTTAAGTCGACCG CCTGGGGAGTACGGCCGCAAGGTTA AAACTCAAATGAATTGACGGGGGCC GCACAAGCGGTGGAGCATGTGGTTT AATTCGATGCAACGCGAAGAACCTT ACCTGGTCTTGACATCCACGGAAGTT TTCAGAGATGAGAATGTGCCTTCGGG AACCGTGAGACAGGTGCTGCATGGC TGTCGTCAGCTCGTGTTGTGAAATGT T	Jww2	MT416449.1	1098	1098	100%	0.0	100%	1423	*Escherichia coli strain d5*
2	CGGATTGTAAAGCACTTTAAGTTGGA GAGGAAGGGCAGTAAGTTAATACCT TGCTGTTTTGACGTTACCGACAGAAT AAGCACCGGCTAACTCTGTGCCAGCA GCCGCGGTAATACAGAGGGTGCAAG CGTTAATCGGAATTACTGGGCGTAAA GCGCGCGTAGGTGGTTCGTTAAGTTG GATGTGAAAGCCCCGGGCTCAACCT GGGAACTGCATCCAAAATTTGGCGA GCTAGAGTATGGTAGAGGGTTGGTG GAATTTCCTGTGTAGCGGTGAAATGC GTAGATATAGGAAGGAACACCAGTG GCGAAGGCGACCACCTGGACTGATA CTGACACTGAGGTGCGAAAGCGTGG GGAGCAAACAGGATTAGATACCCTG GTAGTCCACGCCGTAAACGATGTCAA CTAGCCGTTGGAATCCTTGAGATTTT AGTGGCGCAGCTAACGCATTAAGTTG ACCGCCTGGGGAGTACGGCCGCAAG GTTAAAACTCAAATGAATTGACGGG GGCCCGCACAAGCGGTGGAGCATGT GGTTTAATTCGAAGCAACGCGAAGA ACCTTACCAGGCCTTGACATGCAGAG AACTTTCCAGAGATGGATTGGTGCCT TCGGGAACTCTGACACAGGTGCTGCA TGGCTGTCGTCAGCTCGTGTCGTGAG ATGTTGGGTTAAGTCCCGTAACGAGC GCAACCCTTGTCCTTAGTTACCAGCA CGTTATGGTGGGCACTCTAAGGAGAC TGCCGGTGACAAACCGGAGTAGGAA GGTGGGGATGACGTCAAGTCATC	JS7	MT584853.1		1109	1100%	0.0	100%	13301075	*Pseudomonas guariconensis strain LE3*

A.



B.



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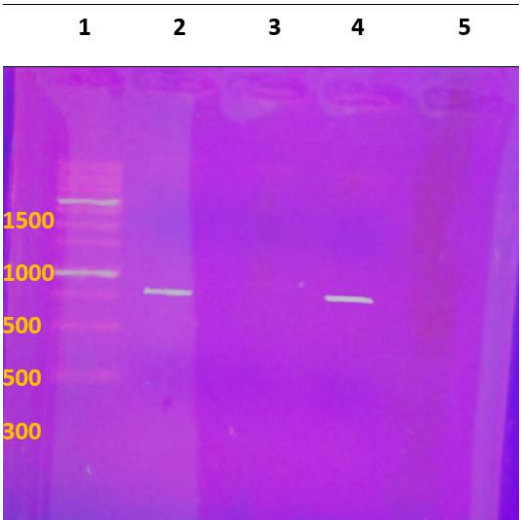

D.

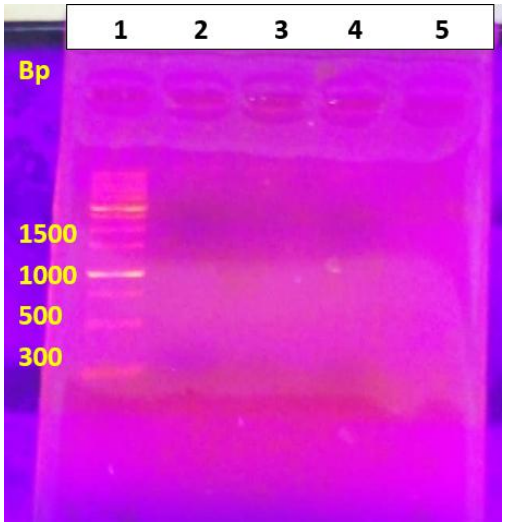

Fig. **2. (A**). Gel Electrophoresis showing positive amplification of a 1496 bp fragment of 16S rRNA. Lane 1; 1Kbp molecular weight marker (1000-10000 bp), lane 2: positive control *E. coli ATCC 25922*, lane 3: Jww2, lanes 4-5: JS7 samples, Lane 9: 100bp DNA ladder (100-3000 bp). (**B).** Gel Electrophoresis showed a positive amplification of a 500 bp product of the blaTem-1 gene. Lane 1: 1Kbp molecular weight marker (300-10000 bp), lane 2: positive control *E. coli* ATCC 25922, lane 3: Jww2, and lane 4: JS7 samples. (**C**). Gel Electrophoresis showed a positive amplification of a 796 bp product of the *copA* gene for *E. coli* and a negative amplification for *P. aeruginosa*. Lane 1; 1Kbp molecular weight marker (300-10000 bp), lane 2; positive control *K. pneumoniae ATCC 700603*, lane 3: JS7 (*P. aeruginosa*), and lane 4: Jww2 (*E. coli*) samples. (**D**). Gel Electrophoresis showed a negative amplification of the *zntA* gene.

Lane 1; 1Kbp molecular weight marker, lane 2and 3: Jww2 (*E. coli*)) and JS7 (*P.aeruginosa*) samples.

DISCUSSION

Escherichia coli and *Pseudomonas aeruginosa* are ubiquitous Gram-negative bacilli widely distributed across diverse environmental niches, including wastewater and soil ecosystems. These organisms are predominantly recognized as opportunistic human pathogens, yet their ecological success is underpinned by remarkable physiological plasticity. They exhibit exceptional adaptability to fluctuating environmental conditions and demonstrate the metabolic versatility to utilize a broad spectrum of carbon substrates, including organic constituents of abattoir origin, such as blood components and other slaughterhouse by-products. Moreover, their intricate genetic architecture confers the capacity to detoxify or withstand both antimicrobial agents and elevated concentrations of heavy metals (Abulreesh *et al.,* 2016).

The findings of the present investigation carry profound implications for environmental integrity and, more critically, for the health of human populations residing in proximity to abattoir facilities. The identification of multidrug-resistant and metallo-tolerant *E. coli* and P. aeruginosa strains represents a significant public health concern, given the inherent pathogenic potential of these organisms and the resistance determinants they harbor. Such strains threaten adjacent ecosystems and simultaneously expose local communities to infections that may prove refractory to conventional therapeutic regimens, thereby contributing to a mounting global health crisis in infectious disease management (Ja'afaru *et al.,* 2025). Furthermore, the dissemination of these microbes through human movement and their persistence in peri-abattoir environments, which are often situated within residential areas, amplifies the risk of environmental transmission. This is particularly alarming given that both *E. coli* and P. aeruginosa isolated in this study were confirmed to carry genes encoding both metallo-tolerance and multidrug resistance, a combination that poses formidable challenges to clinical practice (Singh *et al.,* 2024).

Among the numerous bacterial species recovered during this investigation, two isolates, designated JWW2 (*E. coli*) and JS7 (P. aeruginosa), exhibited particularly pronounced tolerance to multiple antibiotics and varying concentrations of the heavy metals tested. Molecular confirmation via 16S rRNA gene sequencing (Figure A) validated their taxonomic identities. The primary objective of this research was to screen for specific antibiotic and metallo-resistance determinants in bacteria derived from abattoir wastewater and contaminated soils in Adamawa State. Targeted genes included the metal resistance determinants znt-A and cop-A, alongside the β-lactamase-encoding gene blaTEM-1. Notably, the znt-A gene was absent in both isolates, while cop-A was detected exclusively in *E. coli* JWW2. Conversely, the blaTEM-1 antibiotic resistance gene was present in both JWW2 and JS7, underscoring its widespread distribution among these environmental isolates.

These findings align with established molecular mechanisms in Gram-negative bacteria, wherein the transcription of ATPase-encoding genes is typically upregulated in response to metal exposure, including copper at varying concentrations (Adhikary *et al.,* 2024). The absence of cop-A in P. aeruginosa JS7 does not preclude the existence of alternative metal tolerance pathways; indeed, this species is known to harbor other genetic determinants, such as multi-copper oxidases (MCO) and pco-R genes encoding DNA-binding repressor proteins, which may compensate for the lack of cop-A (Elsen *et al.,* 2024). The detection of blaTEM-1 in both isolates, coupled with the presence of cop-A solely in *E. coli*, reinforces these established genetic paradigms and highlights the divergent evolutionary trajectories of metal and antibiotic resistance acquisition among co-occurring environmental bacteria.

CONCLUSION

This study has successfully demonstrated the co-occurrence of multi-antibiotic resistance and heavy metal tolerance in *Escherichia coli* and *Pseudomonas aeruginosa* isolated from abattoir wastewater and contaminated soils in Adamawa State, Nigeria. The phenotypic characterization revealed that both *E. coli* JWW2 and *P. aeruginosa* JS7 exhibited remarkable resistance to multiple antibiotics with MAR indices of 0.83 and 0.75, respectively, while simultaneously demonstrating tolerance to copper, zinc, iron, lead, and cobalt at varying concentrations. Molecular confirmation through 16S rRNA sequencing validated the identity of these isolates, and PCR amplification specifically detected the *blaTEM-1* antibiotic resistance gene in both organisms and the *copA* heavy metal resistance gene exclusively in *E. coli* JWW2. The absence of the *zntA* gene in both isolates, however, does not preclude the presence of other heavy metal resistance determinants, as *P. aeruginosa* is known to harbour alternative resistance mechanisms such as multi-copper oxidase and *pcoR* genes.

The detection of both antibiotic and heavy metal resistance genes in these environmental isolates has profound implications for public health and environmental management. The presence of these genetic determinants in bacteria from abattoir environments suggests that selective pressure from heavy metal contamination may be co-selecting for antibiotic resistance through mechanisms such as horizontal gene transfer, potentially compromising the effective treatment of infections caused by these pathogens. Given that abattoirs in Nigeria often discharge untreated wastewater directly into surrounding environments, and that many are situated within residential areas, the risk of exposure to these multidrug-resistant and metallo-tolerant organisms represents a serious public health concern. This study underscores the urgent need for improved abattoir waste management practices, regular environmental surveillance of resistance determinants, and the implementation of regulatory frameworks to mitigate the spread of antibiotic and heavy metal resistance genes in contaminated ecosystems. Further research is recommended to investigate the full spectrum of resistance genes harbored by these organisms and to assess the potential for horizontal gene transfer between environmental and clinical bacterial populations.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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