Original Article

Characterization of Enterobacteriaceae from the Soil and Water of selected Abattoirs in Osun State Nigeria

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Abstract

Background: Environmental reservoirs of extended-spectrum β-lactamase (ESBL) producing Enterobacteriaceae are poorly documented in Nigeria despite increasing concerns about antimicrobial resistance. **Objective:** To characterize Enterobacteriaceae isolated from soil and water in selected abattoirs in Osun State, Southwestern Nigeria, and determine their antibiotic susceptibility patterns and the presence of β-lactam resistance genes (blaTEM, blaSHV, blaCTXM, and blaOXA48). **Methods:** A cross-sectional study was conducted in Osogbo, Ede, and Sekona abattoirs. Soil and water samples were collected during the dry season over a three-month period. Isolation and identification were performed using standard microbiological and biochemical methods. Antibiotic susceptibility was determined using the Kirby Bauer disk diffusion method, while polymerase chain reaction (PCR) was used to detect ESBL genes. **Results:** A total of 101 Enterobacteriaceae isolates were recovered (soil: 52; water: 49). Predominant soil isolates were *Salmonella* spp. (36.5%), *Enterobacter* spp. (32.7%), *Escherichia coli* (19.3%), and *Klebsiella* spp. (11.5%). Water samples were dominated by *E. coli* (48.9%). Multidrug resistance was observed in 82% of isolates. ESBL genes detected included blaOXA-48 (40%) and blaSHV (35%), while blaTEM and blaCTX-M were detected in single isolates. Molecular confirmation using 16S rRNA sequencing identified representative isolates as *E. coli* and *Enterobacter cloacae*. **Conclusion:** Improved abattoir waste management, environmental monitoring, and antimicrobial stewardship are essential to limit the spread of resistant bacteria and protect public health within a One Health framework.

Keywords: Enterobacteriaceae, Abattoir, Soil, Water, Antimicrobial resistance, One Health

Introduction

An abattoir (or slaughterhouse) is a facility that has been authorized and licensed by the regulatory body to perform clean slaughter and examination of animals as well as to carry out processing, proper preservation, and storage of meat products for human consumption. Therefore, it is of utmost importance to carry out good hygiene practices in slaughterhouses to be able to improve the quality of meat and protect public health [1]. Slaughterhouses produce massive amounts of organic waste (blood, feces, fat, bones, and water) that need to be adequately treated and disposed of. Improper disposal of waste results in the pollution of soil and water bodies in many low- and middle-income countries, and Nigeria is no exception.

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The contaminated water and waste lead to the growth of disease-causing and antimicrobial-resistant organisms in the environment [2]. Among the most dangerous bacteria are Enterobacteriaceae family members who, in particular, are extended-spectrum β-lactamase (ESBL) producing strains, like *Escherichia coli, Klebsiella pneumoniae,* and *Enterobacter spp.* These Gram-negative bacteria have the ability to break the structure of a large spectrum of β-lactam antibiotics, which leads to the deactivation of the treatment [3].

Abattoir environments are mostly the soil and wastewater, which are the reservoirs of the resistant bacteria. The waste from animals that is contaminated with resistant bacteria is the primary source of contamination of the environment, which in turn is affected beyond the local ecosystem, including agricultural lands, water sources, and the community. The soil, an important ecological factor, is the main character in nutrient cycling and provides life to plants, animals, and microbes. On the other hand, the introduction of bacteria that are ESBL producers comes along with the reduction of the microbial diversity of the soil, the decrease of soil fertility, and the occurrence of pathogens in the food chain due to the vegetables that are watered or grown in such contaminated soil [4]. Water is very much used in slaughterhouses for cleaning carcasses, equipment and facilities. Polluted effluents from killing houses, if allowed to run untreated into streams, or if they are used for irrigation, will bring resistant bacteria into the water ecosystem. This will be the result of the bacteria thereby endangering the aquatic life as well as the public health [5]. Great quantities of coliforms, *Salmonella spp*., and harmful *E. coli* have been registered in abattoir discharges in Nigeria [6], [7]. In such polluted sources, resistance may be passed along as genes from the environment to pathogenic bacteria, thus compounding the AMR crisis.

Bacterial resistance mechanisms are such as enzyme production (for example, β-lactamases), changed membrane permeability, efflux pump overexpression, and target site mutations [8]. Resistance is thus complemented by excessive and wrong use of antibiotics in veterinary and human medicine. This creates selective pressure which in turn favours the survival of resistant strains. Multidrug-resistant (MDR) Enterobacteriaceae have been the most common in animal, environmental, and clinical isolates in Nigeria and other developing countries [9]. Most antibiotics utilized worldwide are betalactams such as cephalosporins, carbapenems, monobactams, and penicillin, which can be deactivated by ESBL enzymes. The rise in the identification of β-lactamase resistance genes like blaTEM, blaSHV, blaCTX-M and blaOXA-48 in environmental isolates shows that the environment is no longer just a garbage place for resistant bacteria but also a place where resistance determinants are generated. The genes mentioned above are typically located on mobile genetic elements such as plasmids and transposons which allow for fast spreading over different species and ecosystems [10].

With an emphasis on β-lactamase resistance genes, this study focuses on the molecular traits and prevalence of Enterobacteriaceae in soil and water samples from selected slaughterhouses in Ede, Osogbo, and Sekona, Osun State, Nigeria. Through the identification of bacterial species and the detection of important resistance genes like blaTEM, blaSHV, blaCTX-M, and blaOXA-48, the study seeks to clarify how abattoir settings contribute to the environmental spread of β-lactam resistance. The findings are meant to improve AMR surveillance systems, encourage efficient waste management and hygiene procedures, and influence policy choices to reduce the spread of resistance genes in the environment.

Materials and Methods

Ethical Review

Ethical approval was obtained from Osun State Health Research Ethical Committee (OSHREC) with approval number OSHREC/PRS/569T/524 dated 27th February, 2024. Formal permission to collect samples was obtained from the management of each abattoir (Osogbo, Ede and Sekona). No personal identifiers, biological specimens, or observational data involving humans or animals were collected. Although the study did not involve handling live animals, sampling locations were chosen to avoid interference with slaughtering processes or animal holding areas.

Sample Site

Osun State serves as the study area, with emphasis on slaughterhouses in Ede, Osogbo, and Sekona. The State is situated in the tropical rain forest zone and it covers an area of approximately 14,875 sq. km and lies between latitude 7° 30′ 0″ N and longitude 4° 30′ 0″ E. Though a landlocked state, it is blessed with many rivers and streams that serve the state's water needs. Located at the west side of Nigeria, it is bounded by Ogun State to the south, Kwara State to the north, Oyo State to the west and Ekiti and Ondo State to the east

Sample Collection

A sterile spatula was used to collect the top 5-10 cm of soil samples from four (4) sampling points aseptically within the abattoir premises which includes slaughter points, waste disposal sites, drainage systems, and areas used for animal holding from the abattoir in Ede, Osogbo and Sekona in Osun, State, Nigeria. The soil was placed into sterile, labeled sterile sample bottles. Each container was properly sealed to prevent contamination and place in a zip lock bag and transported to the laboratory.

For Water, 5ml was obtained from four (4) sampling points aseptically within the abattoir premises which includes slaughter points, waste disposal sites, drainage systems, and areas used for animal holding from the abattoir in Ede, Osogbo and Sekona in Osun, State, Nigeria. To maintain sample integrity, they were stored in a cooler with ice packs to preserve at low temperatures during transport. As WHO (2017) recommended, "Maintaining the cold chain from collection to laboratory analysis is critical for preserving the microbial quality of environmental samples." All collected samples were transported to the Microbiology Laboratory, Department of Biological Sciences, Redeemer's University, for microbiological analysis.

Sample Analysis

Serial dilution of the soil samples was first carried out. 1 g of soil sample was weighed and placed in 9 ml of sterile distilled water in a test tube. The water sample was shaken, and one ml of the water samples was taken from the sample bottles into test tubes using a pipette, and serial dilution was done using standard procedure. Approximately 1 ml of the diluted soil and water samples was poured inside different sterile plates and labelled accordingly, molten nutrient agar was added and gently swirled to guarantee even distribution, using the pour plate technique. They were incubated at 37 °C for 24 to 48 hours using an incubator. The resulting colonies were counted, and values were expressed as colony-forming units per milliliter (CFU/mL) [1]. Also, 1ml of the diluted samples was plated on MacConkey agar for isolation of Enterobacteriaceae, using pour plate method. To promote microbial growth, the culture plates were incubated for 24 hours at 37 °C. Subsequently, distinct colonies were sub-cultured to obtain pure isolates, followed by incubation at 37 °C for 24 to 48 hours. According to Cheesbrough (2010), "isolates should be sub-cultured onto fresh media to ensure that pure colonies are obtained for accurate identification and further testing." The Isolates were kept at 4°C for further use.

Biochemical Analysis

The preliminary identification of Enterobacteriaceae was done by conventional biochemical tests. Gram-negative, oxidase-negative bacteria were subjected to Triple Sugar Iron Agar, Indole, Citrate, Urease, and Methyl Red Voges-Proskauer tests, according to standard guidelines [11].

Antimicrobial Susceptibility Profile

Commercially available antimicrobial discs from (Biomark laboratories, India) were made use of to determine the sensitivity and resistance pattern of the isolates. The Disk Diffusion (Kirby- Bauer) method was used to carry out the Antibiotic Susceptibility test. Paper disks impregnated with specific concentrations of antibiotics are placed on an agar plate inoculated with the bacterial isolate. Using the Muller-Hinton agar, a 24-hour-old combination with saline solution, and standardizing its turbidity by comparing it to 0.5 McFarland solution, the mixture was evenly spread across each plate using a sterile swab stick and allowed to dry. Using a set of sterile forceps, the antimicrobial discs were placed on the streaked agar plate and incubated at 37°C for twenty-four hours.

Thirteen (13) antibiotics were tested, including Amikacin (30 µg), Cefotaxime (30 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg), Cefuroxime (30 µg), Chloramphenicol (10 µg), Ciprofloxacin (5 µg), Cotrimoxazole (25 µg), Gentamicin (10 µg), Meropenem (10 µg), Tetracycline (10 µg), Ciprofloxacin (5 µg), and Vancomycin (30 µg) (CLSI, 2023). After incubation for 18-24 hours, with the aid of a calibrated ruler, the diameter of the zone of inhibition that is, the area in which there is no growth was measured in millimeters to record the results. According to the Clinical and Laboratory Standards Institute (CLSI 2023), the results were categorized as sensitive (S), intermediate (I) and resistant (R).

Molecular Characterization

Extraction of Genomic DNA

Before their genomic DNA was extracted, the bacterial isolates were cultivated for 18 to 24 hours in 4 milliliters of peptone broth (Oxoid, England). The pellet was re-suspended in 100 µl of sterile distilled water after a 1 ml aliquot was centrifuged for two minutes at 10,000 rpm. After 15 minutes of boiling, the suspension was quickly cooled to -20 °C for 10 minutes, vortexed, and centrifuged for 10 minutes at 10,000 rpm. The crude DNA-containing supernatant was gathered and kept at 4°C so that it could be used as a template in PCR tests later on. Bebe *et al*., (2020) highlighted that "boiling lysis is a simple, rapid, and effective method for extracting DNA suitable for routine PCR based analyses.

Amplification of Beta-Lactam Resistance Genes

The isolates were screened for Enterobacteriaceae genes by amplifying CTM-X, blaSHV, blaTEM and OXA-48 genes. The procedure was carried out in a PCR thermal cycler using a 25µl reaction mixture containing 4µl of 2x master mix, 1µl each of the forward and reverse primers (Table 1), 14µl of nuclease-free water, and 5µl of the DNA template. The reaction consisted of an initial denaturation step at 94 °C for 3 minutes followed by 35 cycles of denaturation at 94 °C for 45 seconds, annealing at 60 °C for blaTEM, blaSHV, OXA-48 and 60 for blaCTX-M, extension at 72°C for 1minute, final extension at 72 °C for 3 minutes.

The amplified PCR products (10 µl) were evaluated on a 1.5% (w/v) agarose gel at 100 mV for 60 min using BIO-RAD Power Pac 3000 with a molecular weight marker (100 bp DNA Ladder). The DNA bands were then visualized and photographed under UV light after staining the gel with ethidium bromide. *Escherichia coli* ATCC 25922 was used as a negative control and *Klebsiella pneumoniae* ATCC 700603 as a positive control. Amplification products of 10 µl were electrophoresed on 1.5% agarose gels which were prepared in 1X TAE buffer for the separation of DNA fragments. All positive controls, using *Klebsiella pneumoniae* ATCC 700603, displayed the expected banding patterns, validating the PCR process. There was no amplification observed in negative control lanes containing *Escherichia coli* ATCC 25922, which confirms the specificity of the assay and rules out contamination or false-positive results

Table 1: Primers for Screening of Resistant Genes in Gram-negative Multiple Antibiotic-Resistant Isolates.

Primer Name	Sequence 5' to 3'	Expected Band Size	Reference
BLA*SHV*	F: CGCCTGTGTATTATCTCCCT R: CGAGTAGTCCACCAGATCCT	293	Gholipour *et al.*, 2014.
Bla*CTX-M*	F: CGCTGTTGTTAGGAAGTGTG R: GGCTGGGTGAAGTAAGTGAC	569	Esraa *et al.*, 2013.
Bla*TEM*	F: ATCAGCAATAAACCAGC R: CCCCGAAGAACGTTTTC	403	Gholipour *et al.*, 2014.
Oxa-48	F: GCGTGGTTAAGGATGAACAC R: CATCAAGTTCAACCCAACCG	438	Elnaz *et al.*, 2023

Sanger Sequencing of the 16S rRNA Gene Amplicons

Genomic DNA amplification was carried out targeting the 16S rRNA gene with universal primers in the forward 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and reverse 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) sequence. The PCR mixture was prepared in a 25 µL reaction mixture containing 12.5 µL of 2x PCR master mix, 1 µL of each primer (10 pmol), 2 µL of template DNA, and water. The PCR amplification was performed under the following conditions: initial denaturation at 95 °C for 5 min; 35 cycles consisting of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 1 min; and final extension at 72 °C for 7 min [12]. Sequencing was performed using the dideoxy-chain termination method with fluorescent

dye-labeled dideoxynucleotides. The sequencing reaction was carried out on an ABI 3500 Genetic Analyzer (Applied Biosystems, USA).

Analysis of the 16S rRNA Gene Sequences

The obtained 16S rRNA sequences were edited and analysed using the Basic Local Alignment Search Tool (BLAST) available at the National Centre for Biotechnology Information (NCBI). Each sequence was compared with GenBank reference sequences for bacterial identification. Only matches exhibiting ≥97% sequence identity, 99–100% query coverage, and E-values of zero were accepted as valid identifications.

Statistical Analysis

All data were entered into Microsoft Excel spreadsheets for descriptive analysis. Bar charts were generated, and summary statistics, including 95% confidence intervals (CIs), were computed using Microsoft Excel (Microsoft Corporation, USA).

Results

Twenty-four samples (soil: 12; water: 12) collected yielded a total of one hundred and one (101) bacterial isolates, which included 49 isolates derived from water samples and 52 isolates sourced from soil samples across the three study sites.

Total Plate Count (TPC)

The TPC values for soil ranged from 82.86 × 10^3 to 48.27 × 10^5 CFU/g, whereas the water samples exhibited TPC values from 51.99 × 10^3 to 20.46 × 10^5 CFU/ml. The average TPC for soil samples fluctuated between 6.90 × 10^3 and 4.02× 10^5 CFU/g, while the water samples presented lower mean counts, ranging from 6.49 ×10^{-3} to 2.56 × 10^{-5} CFU/ml. as shown on Table 2 and 3 respectively.

Table 2: Total Plate Count (TPC) for Soil Samples

Isolate Code	Dilution 3 10-3	Percentage (%)	Dilution 5 10-5	Percentage (%)
Ede 1	7.12±0.00	8.95±0.00	5.21±0.00	9.10±0.00
Ede 2	5.08±0.00	6.13±0.00	2.03±0.00	3.54±0.00
Ede 3	10.48±0.00	12.65±0.00	6.52±0.00	11.39±0.00
Ede 4	4.41±0.00	5.32±0.00	3.94±0.00	6.88±0.00
Osogbo 1	8.48±0.00	10.23±0.00	5.76±0.00	10.06±0.00
Osogbo 2	7.44±0.00	8.98±0.00	6.33±0.00	11.05±0.00
Osogbo 3	11.82±0.00	14.27±0.00	4.47±0.00	7.81±0.00
Osogbo 4	4.13±0.00	4.98±0.00	3.01±0.00	5.25±0.00
Sekona 1	9.31±0.00	11.23±0.00	4.50±0.00	7.86±0.00
Sekona 2	2.42±0.00	2.92±0.00	1.01±0.00	1.76±0.00
Sekona 3	6.74±0.00	8.13±0.00	2.98±0.00	6.17±0.00
Sekona 4	5.43±0.00	6.55±0.00	2.51±0.00	4.38±0.00
Sum Total	**82.86**±0.00		**48.27**±0.00	
Mean of Total Plate Count	**6.90**±0.00		**4.02**±0.00	

Table 3: Total Plate Count (TPC) for Water Samples

Isolate Code	Dilution 3 10-3	Percentage (%)	Dilution 5 10-5	Percentage (%)
WWE 1	10.25±0.00	21.62±0.00	4.28±0.00	15.82±0.00
WWE 2	9.56±0.00	20.17±0.00	4.10±0.00	15.15±0.00
WWE 3	5.00±0.00	10.55±0.00	2.40±0.00	8.87±0.00
CWE 4	4.04±0.00	8.52±0.00	0±0.00	0±0.00
CWE 5	3.14±0.00	6.62±0.00	1.16±0.00	4.29±0.00
CWE 6	6.00±0.00	12.6±0.00	4.4±0.00	6.26±0.00
WWE 5	6.00±0.00	12.66±0.00	2.0±0.00	7.39±0.00
WWE 6	8.00±0.00	16.88±0.00	2.12±0.00	7.83±0.00
Sum Total	**51.99**		**20.46**	
Mean of Total Plate Count	**6.49**		**2.56**	

Table 4: Biochemical Properties of Bacterial Isolates from Water and Soil of Selected Abattoirs in Ede, Osogbo, and Sekona, Osun State, Nigeria

S/n	Probable identity	Gram staining	Catalase test	Oxidase test	Citrate test	MR	VP	Motility test	Urease test	Indole test
1	*Escherichia coli* (35)	-	+	-	-	+	-	+	-	+
2	*Klebsiella spp* (11)	-	+	-	+	+	+	+	-	-
3	*Enterobacter spp.* (24)	-	+	-	+	+	+	-	-	-
4	*Shigella spp.* (4)	-	+	-	+	+	-	-	-	-
5	*Salmonella spp.* (27)	-	+	+	-	+	-	+	-	+

Key: + Positive – Negative

Table 5: Genera of *Enterobacteriaceae* from the Three Location

Organisms	Soil					Water					Total
	Ede	Osogbo	Sekona	Total	Percentage (%)	Ede	Osogbo	Sekona	Total	Percentage (%)	
Enterobacter	10	3	4	17	33	7	0	0	7	14	24
Escherichia coli	6	5	0	11	21	13	6	4	23	49	34
Klebsiella	4	0	2	6	12	2	1	2	5	11	11
Salmonella	9	3	5	17	34	0	3	6	9	18	26
Shigella spp.	0	0	0	0	0	1	2	1	4	8	4
Sum Total	29	11	11	51		23	12	13	49		101

Table 6: Distribution of Bacterial Isolates from Soil and Water

Location	Soil			Water			TOTAL
	Total Isolates	Distribution (Count)	Most Common Genera	Total Isolates	Distribution (Count)	Most Common Genera	
Ede	29	*Enterobacter spp.* (10), *Salmonella spp.* (9), *E. Coli* (6), *Klebsiella spp.* (4), *Shigella spp.* (0)	*Enterobacter* (10)	24	*Enterobacter spp.*(7), *Salmonella spp.* (0), *E. Coli* (14), *Klebsiella spp.* (2), *Shigella spp.* (1)	*E. coli* (43)	53
Osogbo	12	*Enterobacter spp.* (3), *Salmonella spp.* (4), *E. Coli* (5), *Klebsiella spp.* (0), *Shigella spp.* (0),	*E. coli* (5).	12	*Enterobacter spp.*(0), *Salmonella spp.* (3), *E. Coli* (6), *Klebsiella spp.* (1), *Shigella spp.* (2)	*E. coli* (6)	24
Sekona	11	*Enterobacter spp.* (4), *Salmonella spp.* (5), *E. Coli* (0), *Klebsiella spp.* (2), *Shigella spp.* (0)	*Salmonella* (5).	13	*Enterobacter spp.*(0), *Salmonella spp.* (6), *E. Coli* (4), *Klebsiella spp.* (2), *Shigella spp.* (1)	*Salmonella* (6),	24

Figure 1: Antibiotic Resistance profiles of bacterial isolates from Soil. The bar chart represents the zone of resistance (ZOR) observed for different bacterial isolates against twelve antibiotics.

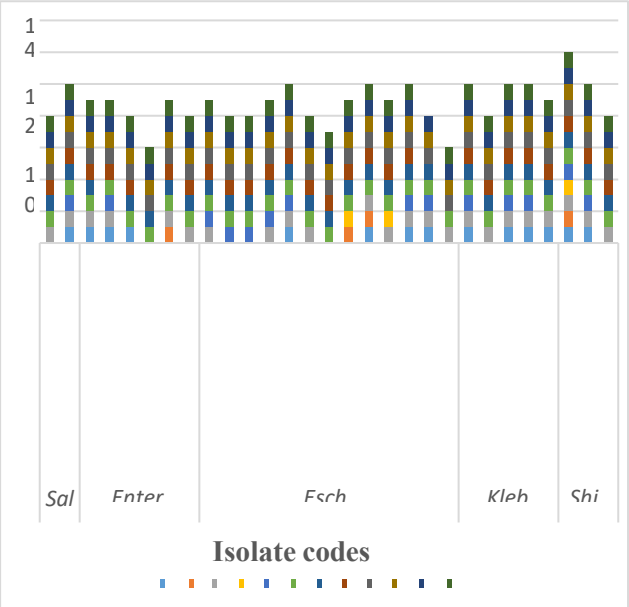

Figure 2: Antibiotic Resistance profiles of bacterial isolates from Water. The bar chart represents the zone of resistance (ZOR) observed for different bacterial isolates against twelve antibiotics.

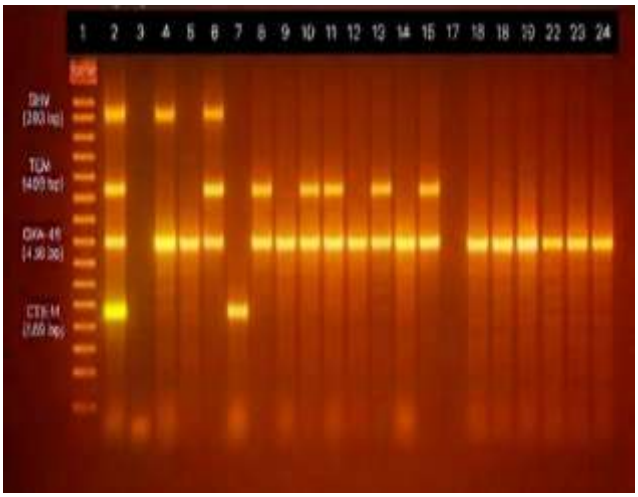

Figure 3: A gel picture with the bands of amplified ESBL genes (CTX-M, SHV, TEM and OXA-48)

Lane 1: 100kb Ladder

Lane 2: Klebsiella pneumoniae positive control

Lane 3: Escherichia coli 29522 negative control;

Table 7: Summary of Bacterial Isolates carrying Beta-Lactam Resistance Genes by PCR

Sample Type	ESBL n (%)	Non-ESBL	p-value
Soil	4 (7.7)	48	
Water	6 (12.2)	43	0.52

Table 8: Bacterial isolates identified by 16S rRNA gene amplicon Sanger Sequencing

Isolate Code	Organism	Sequence Length (bp)	Genebank Accession	NCBI % Similarity
OS4A	*Enterobacter cloacae*	1505	CP123598.1	100.00%
WWO2C	*Escherichia coli*	1505	CP195759.1	99.73%

Discussion

Abattoirs contribute substantially to environmental pollution through the discharge of animal blood, feces, intestinal contents, and other organic wastes. The accumulation of these materials creates nutrient-enriched environments that favor the growth, persistence, and dissemination of microorganisms, including antibiotic-resistant bacteria (ARB). Many of these organisms originate from the gastrointestinal tracts, mucosal surfaces, and skin of slaughtered animals such as cattle, goats, sheep, and pigs [13]. Such conditions position abattoir environments as important ecological niches for the maintenance and spread of antimicrobial resistance.

The total plate counts (TPCs) recorded in this study showed higher bacterial loads in soil samples compared with water samples, indicating that soil serves as a more favorable reservoir for bacterial persistence. This observation aligns with ecological principles, as soil provides a complex matrix rich in nutrients, organic matter, and stable microhabitats that support microbial colonization and proliferation. In contrast, water within abattoir settings is often subject to flow dynamics, runoff dilution, and intermittent treatment, which may limit sustained bacterial accumulation [14].

The morphological and cultural characteristics of the bacterial isolates from abattoir water reveal considerable levels of microbial contamination and unsanitary conditions in the study areas. Although colony morphology is a preliminary method of presumptive identification, it remains useful in environmental microbiology [11]. The predominance of circular, smooth colonies with flat to raised elevations is consistent with Enterobacteriaceae, which are commonly associated with fecal contamination [15]. The presence of these isolates reflects the impact of slaughtering activities, which generate organic-rich conditions that support bacterial persistence [16]. Variation in colony coloration, ranging from cream to pink, suggests differences in lactose fermentation patterns, indicating the presence of both lactose fermenters (e.g., presumptive Escherichia coli and Klebsiella spp.) and non-lactose fermenters (e.g., Salmonella spp., Shigella spp., and Enterobacter spp.). This observation is consistent with previous reports from abattoir effluents in Nigeria and similar settings [6]. The detection of potential pathogens such as Escherichia coli, Salmonella spp., and Shigella spp. highlights public health concerns and reflects inadequate hygiene practices and poor effluent management [17], [18].

The bacterial isolates were predominantly Gram-negative and exhibited biochemical characteristics consistent with members of the Enterobacteriaceae family, which are widely associated with fecal and environmental contamination in abattoir environments [18], [19]. Escherichia coli was the most frequently isolated organism, displaying characteristic biochemical reactions. Its predominance indicates significant fecal contamination and poor sanitary conditions [20], [21]. Klebsiella spp. and Enterobacter spp. were differentiated based on their biochemical profiles, including positive citrate and Voges–Proskauer tests and negative indole reactions. Their presence reflects their adaptability to nutrient-rich, contaminated environments [19], [22]. The detection of Shigella spp., although at low frequency, suggests possible human-derived contamination [23], while the presence of Salmonella spp. indicates zoonotic risk associated with abattoir effluents [24], [25].

Antimicrobial susceptibility testing revealed high resistance to tetracycline and cotrimoxazole, likely reflecting their widespread use and associated environmental selection pressure. Resistance to third-generation cephalosporins suggests the presence of ESBL-producing Enterobacteriaceae, particularly among Escherichia coli, Klebsiella, and Enterobacter species, consistent with global trends [26]. Similar resistance patterns have been reported in abattoir environments in Nigeria [4], [27]. Overall, these findings indicate the persistence of multidrug-resistant Enterobacteriaceae, particularly against commonly used antibiotics [28].

Molecular analysis provided insight into the genetic basis of resistance, with a substantial proportion of isolates harboring at least one β-lactamase gene [4], [29]. The detection of blaOXA-48 as the predominant gene, alongside blaSHV, indicates their widespread occurrence in the study environment. In contrast, blaTEM and blaCTX-M were detected at lower frequencies, which may reflect local antibiotic usage patterns or ecological factors influencing gene distribution. While ESBL genes are often associated with mobile genetic elements, this study did not include plasmid analysis; therefore, the mechanism of gene dissemination cannot be conclusively determined [31].

Sequence-based identification using 16S rRNA gene analysis confirmed the identities of representative isolates as Enterobacter cloacae and Escherichia coli, supporting the reliability of phenotypic and biochemical identification methods. However, phylogenetic relationships among isolates were not assessed in this study, and therefore genetic relatedness cannot be inferred [32].

These findings reinforce the role of abattoir environments as significant reservoirs of antimicrobial-resistant Enterobacteriaceae. Strengthening waste treatment systems, improving effluent management, enforcing hygiene practices, and providing regular WASH education for abattoir workers are critical interventions to reduce environmental dissemination and protect public health [33].

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None

Conflicts of interest
There are no conflicts of interest.

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