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Surveillance of Antimicrobial-Resistant *Escherichia coli* in Abattoir Effluent and its Receiving River in Zaria, Nigeria

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Abstract

Abattoir effluent is an important environmental reservoir for antimicrobial-resistant bacteria due to the continuous discharge of untreated or inadequately treated waste containing antimicrobial residues into surrounding water bodies. This study conducted surveillance of antimicrobial-resistant Escherichia coli in abattoir effluent from Zangon Shanu and in River Koraye, the receiving water body, in Zaria, Nigeria. A total of 156 abattoir effluent and 156 river water samples were collected for the isolation of E. coli. Physicochemical analysis was carried out to assess pollution levels. Isolation was conducted using filtration for water samples and dilution for effluent samples, followed by biochemical confirmation tests, including Gram staining and biochemical tests, and antibiotic susceptibility testing using the Kirby-Bauer disk diffusion method. The results revealed elevated physicochemical parameters with Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Dissolved Solids (TDS) reaching up 1140mg/L, 5500mg/L and 3980mg/L respectively suggestive of high contamination, as low as 1.10mg/L of Dissolved Oxygen (DO) was recorded suggestive of high microbial activity, pH ranged between 6.59-8.51 which is favourable for the growth and proliferation of E.coli, 132 (84.62%) and 61 (39.10%) E. coli were isolated from abattoir effluent and River Koraye water samples respectively. The isolates demonstrated very high resistance to penicillin, amoxicillin, and 3rd*-generation cephalosporins (Cefotaxime and Ceftazidime) in both samples, with 97.7% resistance to amoxicillin and cefotaxime, and 95.5% resistance to ceftazidime in abattoir effluent. In the Koraye River water samples, 93.4%, 98.4%, and 96.7% resistance to amoxicillin, cefotaxime, and ceftazidime, respectively, was observed. This study demonstrates that Zangon Shanu abattoir effluent and the receiving water body, the River Koraye, serve as significant reservoirs for antimicrobial-resistant E. coli in Zaria, Nigeria. High levels of contamination were evident from both physicochemical parameters and bacterial isolation rates. The isolates also demonstrated multidrug resistance. This study also recommends implementing antibiotic stewardship programs in animal husbandry, effective abattoir waste treatment before discharge, and sensitisation of communities using the river.*

Keywords: Abattoir effluent; E coli; Antimicrobial resistance; Multidrug resistance; Physicochemical parameters

INTRODUCTION

Antimicrobial resistance (AMR) is a growing global crisis, driven by the widespread misuse of antibiotics in humans, animals and agriculture, affecting human, animal, and environmental health (Uddin *et al.*, 2021; Igbinosa *et al.*, 2023). The environment plays a critical role in the persistence and dissemination of resistant bacterial strains, serving as a conduit through which resistant bacteria and resistance genes circulate between humans, animals, and ecological systems (Cho *et al.*, 2023). In low- and middle-income countries, inadequate wastewater treatment, poor sanitation infrastructure, and unregulated discharge of animal wastes significantly contribute to the environmental AMR burden (Uddin *et al.*, 2021; Cho *et al.*, 2023).

Abattoirs are recognised as hotspots for the emergence and spread of antimicrobial-resistant bacteria. During slaughter, the gastrointestinal contents of animals, including commensal and pathogenic microorganisms, are released into wastewater streams (Olawale *et al.*, 2020). Many of these animals may have been exposed to antibiotics for therapeutic, prophylactic, or growth-promotion purposes. Consequently, abattoir effluents often contain high loads of faecal bacteria, antimicrobial residues, organic nutrients and suspended solids (Nigussie *et al.*, 2025). When these effluents are discharged untreated or only partially treated, they contaminate receiving water bodies, enabling the persistent environmental dissemination of AMR determinants (Olawale *et al.*, 2020).

Escherichia coli, a common indicator organism for faecal contamination, is frequently used in environmental surveillance of AMR because of its ubiquity in animal and human gut flora and its ability to acquire and disseminate multidrug resistance genes makes it a key pathogen of One Health significance (Willey *et al.*, 2013; Martinson and Walk, 2020). More importantly, *E. coli* readily acquires mobile genetic elements, such as plasmids, transposons, and integrons, that encode antimicrobial resistance genes (Martinson and Walk, 2020). Rivers receiving effluents from abattoirs can serve as reservoirs for resistant strains, creating pathways for human and animal exposure through irrigation, domestic use and recreational activities (Taylor *et al.*, 2025).

In Nigeria, the increasing use of antibiotics in livestock production, combined with the absence of effective wastewater treatment facilities, has intensified the risk of AMR transmission through environmental pathways (Gali *et al.*, 2020). Several studies have reported a high prevalence of multidrug-resistant *E. coli* in animal farms, slaughter slabs, and rivers receiving slaughterhouse waste (Aworh et al., 2022; Igbinosa *et al.*, 2023; Onuoha *et al.*, 2023; Beshiru *et al.*, 2024). However, despite continuous discharge of untreated effluent from Zangon Shanu abattoir into River Koraye in Zaria, there is limited documented surveillance of antimicrobial-resistant bacteria in this water body. River Koraye serves as the receiving river for a major abattoir in Zaria and is used by surrounding communities for irrigation, domestic activities and sometimes recreational purposes. Continuous exposure of this river to slaughterhouse wastewater may facilitate the proliferation of resistant *E. coli* strains and their spread within the environment. This creates potential public health risks, especially in communities that rely on river water for daily use. Therefore, surveillance of antimicrobial-resistant *E. coli* in abattoir effluent and in the receiving river is essential.

MATERIAL AND METHOD

Collection of Samples and Transportation

The effluent from the abattoir and the water from the River Koraye were sampled at three different points. Two samples were taken at each sampling point once a week for a 26-week period making a total of 156 effluent samples and 156 water samples. The samples were transported immediately to the laboratory in an ice box for microbiological analyses.

Determination of the Physicochemical Properties of the Samples

Physicochemical properties were determined for two seasons (wet and dry). The parameters analysed were pH, biochemical oxygen demand (BOD), chemical oxygen demand (COD), dissolved oxygen (DO) and total dissolved solids (TDS) using standard methods as described by American Public Health Association (APHA) (1998) and Patil *et al.* (2012).

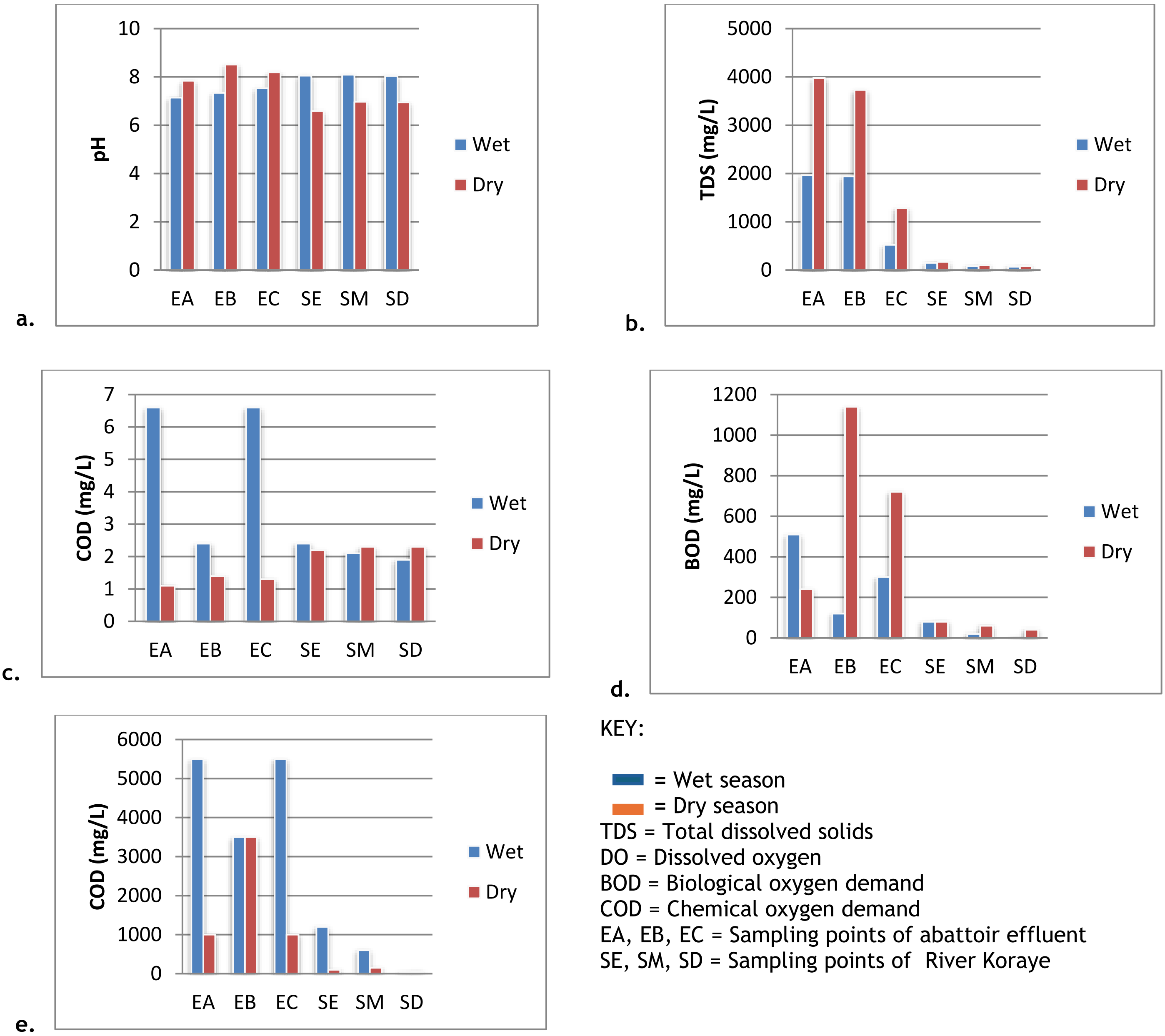
Isolation and Identification of *Escherichia coli* from water and effluent samples

Water samples from the river were analysed using membrane filtration. Single sterile 0.45µm pore filter disks (Pall Corporation) were placed in a filtration unit to filter each water sample. The filter membranes were placed on Eosin Methylene Blue (EMB) agar plates and incubated at 37°C for 24 hours (Cheesbrough, 2006), while the effluent samples were diluted ten-fold to 10^{-3}. The spread plate method was used to inoculate dilution 10^{-3} on the surface of freshly prepared EMB agar. All plates will be incubated at 37°C for 24 hours (Atta *et al.*, 2021).

Blue-black colonies with a greenish metallic sheen on the EMB agar plate were subcultured, and the set of biochemical tests used for the characterisation of the isolate were Indole, Methyl Red, Voges-Proskauer (MR-VP), and Citrate utilisation test (IMViC) (Cheesbrough, 2006).

Antimicrobial Susceptibility Testing

The Kirby-Bauer disc diffusion method, based on the Clinical and Laboratory Standards Institute (CLSI) guideline (2023), was employed for antimicrobial susceptibility testing. Pure colonies were suspended in 2ml sterile normal saline. The turbidity was adjusted to match with 0.5 MacFarland pacity standards (Cheesbrough, 2006).



KEY:

🟦 = Wet season
🟧 = Dry season
TDS = Total dissolved solids
DO = Dissolved oxygen
BOD = Biological oxygen demand
COD = Chemical oxygen demand
EA, EB, EC = Sampling points of abattoir effluent
SE, SM, SD = Sampling points of River Koraye

Figure 1: Physicochemical Properties of Abattoir Effluent and River Koraye Samples during Wet and Dry Season (a) pH (b) Total dissolved solids (TDS) (c) Dissolved oxygen (DO) (d) Biological oxygen demand (BOD) (e) Chemical oxygen demand (COD)

Bacterial suspensions were spread evenly on Mueller-Hinton agar. Antibiotic discs, including Amoxicillin (10 µg), Cefotaxime (30 µg), Ceftazidime (30 µg), Ciprofloxacin (5 µg), Gentamicin (10 µg) and Trimethoprim-sulfamethoxazole (25 µg), were acceptically placed on the agar surface (CLSI, 2023). Plates were incubated aerobically at 37˚C for 18 hours (Cheesbrough, 2006). Inhibition zones were measured in millimetres, and susceptibility was interpreted according to the CLSI breakpoints (CLSI, 2023). The multiple antibiotic resistance (MAR) index was calculated for each isolate as described by Olonitola *et al*. (2007).

$$MAR\ index = \frac{number\ of\ antibiotics\ to\ which\ resistant}{total\ number\ of\ antibiotics\ tested}$$

RESULT

The physicochemical properties of abattoir effluent and water samples from River Koraye collected in the wet and dry seasons showed pH values ranging from slightly acidic to slightly basic in both seasons. Total dissolved solids (TDS) were higher in effluent samples, particularly in the dry season, with 3980 mg/L at the drainage system sampling point within the abattoir (EA), the highest value. Dissolved oxygen (DO) was lower in effluent samples in the dry season, with 1.10mg/L being the lowest value at the sampling point at the drainage system within the abattoir (EA). Biochemical oxygen demand (BOD) and chemical oxygen demand (COD) were markedly elevated in the effluents, with the highest values recorded at 1140 mg/L and 5500 mg/L, respectively (Figure 1). The effluent samples had the highest occurrence of *E. coli*, with the drainage system within the abattoir (EA) recording the highest, with 51 (98.1%) isolates. Lower occurrence was observed in the water samples, with the sampling point at the middle of River Koraye (SM) being the lowest (Table 1). The overall occurrence of *E. coli* isolated from abattoir effluent and River Koraye was 132 (84.62%) and 61 (39.10%), respectively, giving a total occurrence of 193 (61.90%) (Table 2).

Table 1: Distribution and Occurrence of *E. coli* isolated from Abattoir Effluent and River Koraye.

Sampling point	Total number of samples	Number of *E. coli* isolated (%)
EA	52	51 (98.1)
EB	52	44 (84.6)
EC	52	37 (71.2)
SE	52	25 (48.1)
SM	52	17 (32.7)
SD	52	19 (36.5)
Total	**312**	**193 (61.9)**

KEY: EA = Sampling point at drainage system within the abattoir, EB = Sampling point at effluent, 100 m away from the abattoir, EC = Sampling point at the junction where effluent enters River Koraye, SE = Sampling point at river bank (effluent-stream conjunction), SM = Sampling point at the middle of River Koraye, SD = Sampling point 500m downstream near farm area.

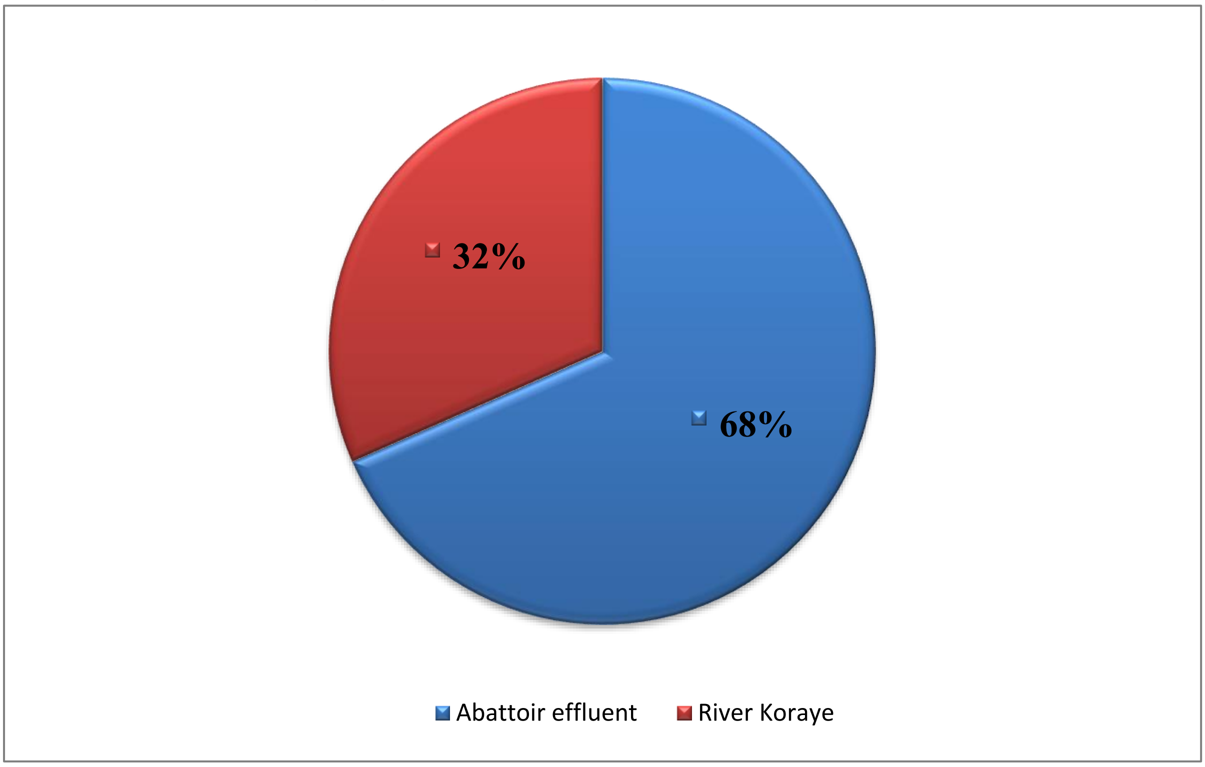


Figure 2: Percentages distribution of *E. coli* Recovered from Abattoir Effluent and River Koraye.

Abattoir effluent had the highest percentage of *E. coli* isolates (132, 68%), while River Koraye had the lowest (61, 32%), making a total of 193 (100%) (Figure 2). Most isolates were sensitive to Gentamicin and Trimethoprim-Sulfamethoxazole in both Abattoir effluent and River Koraye (Tables 3 and 4). The antibiotic resistance profile of *E. coli* isolated from abattoir effluents, exhibited multidrug resistance (MDR), with many resistant to 3-6 antibiotic classes simultaneously. High resistance was observed against Amoxicillin, Cefotaxime, and Ceftazidime in both abattoir effluent and River Koraye water samples (Tables

5 and 6). The interpretation of the isolates as resistant, intermediate, or susceptible to the antibiotic disc was performed according to the Clinical & Lab. Standards Institute (CLSI, 2023).

Table 2: Overall Occurrence of *E. coli* Isolated from Abattoir Effluent and River Koraye

Sample type	Total number of samples	*E. coli* isolated (%)
Abattoir effluent	156	132 (84.62%)
River Koraye	156	61 (39.10%)
Total	**312**	**193 (61.90%)**

Table 3: Antimicrobial susceptibility profile of *E. coli* isolated from Abattoir Effluent (n = 132)

Antimicrobial class	Antibiotics	Resistance n (%)	Intermediate n (%)	Susceptible n (%)
Penicillin	Amoxicillin (10µg)	129 (97.7)	1 (0.8)	2 (1.5)
Cephalosporin	Cefotaxime (30µg)	129 (97.7)	3 (2.3)	0 (0)
	Ceftazidime (30µg)	126 (95.5)	4 (3.0)	2 (1.5)
Aminoglycoside	Gentamicin (10µg)	2 (1.5)	10 (7.6)	120 (90.9)
Fluoroquinolones	Ciprofloxacin (5µg)	76 (57.6)	18 (13.6)	38 (28.8)
Sufonamide	Trimethoprim-Sulfamethoxazole, (25µg)	26 (19.7)	6 (4.6)	100 (75.7)

KEY: n = Total number of *E.coli* isolated from abattoir effluent

Table 4: Antimicrobial susceptibility profile of *E. coli* isolated from River Koraye (n = 61)

Antimicrobial class	Antibiotics	Resistance n (%)	Intermediate n (%)	Susceptible n (%)
Penicillin	Amoxicillin (10µg)	57 (93.4)	3 (5.0)	1 (1.6)
Cephalosporin	Cefotaxime (30µg)	60 (98.4)	1 (1.6)	0 (0)
	Ceftazidime (30µg)	59 (96.7)	2 (3.3)	0 (0)
Aminoglycoside	Gentamicin (10µg)	1 (1.6)	0 (0)	60 (98.4)
Fluoroquinolones	Ciprofloxacin (5µg)	20(32.8)	31(50.8)	10 (16.4)
Sufonamide	Trimethoprim-Sulfamethoxazole, (25µg)	16 (26.2)	15 (24.6)	30 (49.2)

KEY: n = Total number of *E.coli* isolated from River Koraye

Table 5: Antibiotic Resistance Profile of *E. coli* Isolated from Abattoir Effluent

No. of isolates resistant (Sampling points)	Antibiotic resistance profile	No. of Antibiotic resistant isolates (%)	MAR index
6 (EA)	AMX, CTX, CAZ, CIP, STX	5 (83.33)	0.83
20 (EA)	AMX, CTX, CAZ, CIP	4 (66.67)	0.67
22 (EA)	AMX, CTX, CAZ	3 (50)	0.5
2 (EA)	AMX, CTX, CAZ, CIP, CN, STX	6 (100)	1
1 (EA)	AMX, CTX, CIP	3 (50)	0.5
21 (EB)	AMX, CTX, CAZ, CIP	4 (66.67)	0.67
18 (EB)	AMX, CTX, CAZ	3 (50)	0.5
3 (EB)	AMX, CTX, CAZ, CIP, STX	5 (83.33)	0.83
2 (EB)	AMX, CTX, CIP	3 (50)	0.5
13 (EC)	AMX, CTX, CAZ, CIP, STX	5 (83.33)	0.83
2 (EC)	AMX, CTX, CAZ, STX	4 (66.67)	0.67
10 (EC)	AMX, CTX, CAZ, CIP	4 (66.67)	0.67
12 (EC)	AMX, CTX, CAZ	3 (50)	0.5

KEY: MAR = Multiple antibiotic resistance, AMX = Amoxicillin, CTX = Cefotaxime, CAZ = Ceftazidime, CIP = Ciprofloxacin, CN = Gentamicin, STX = Trimethoprim-Sulfamethoxazole, n = Total number of antibiotics used, EA = Sampling point at drainage system within the abattoir, EB = Sampling point at effluent, 100m away from the abattoir, EC = Sampling point at the junction where effluent enters River Koraye, SE = Sampling point at river bank (effluent-stream conjunction), SM = Sampling point at the middle of River Koraye, SD = Sampling point 500m downstream near farm area.

Table 6: Antibiotic Resistance Profile of *E. coli* Isolated from River Koraye.

No. of isolates resistant (Sampling points)	Antibiotic resistance profile	No. of Antibiotic resistant isolates (%)	MAR Index
9 (SE3)	AMX, CTX, CAZ	3 (50)	0.5
7 (SE)	AMX, CTX, CAZ, CIP	4 (66.67)	0.67
1 (SE)	CTX, CAZ, CIP	3 (50)	0.5
1 (SE)	CTX, CAZ, STX	3 (50)	0.5
4 (SE)	AMX, CTX, CAZ, STX	4 (66.67)	0.67
3 (SE)	AMX, CTX, CAZ, CIP, STX	5 (83.33)	0.83
8 (SM)	AMX, CTX, CAZ	3 (50)	0.5
3 (SM)	AMX, CTX, CAZ, CIP, STX	5 (83.33)	0.83
2 (SM)	AMX, CTX, CAZ, STX	4 (66.67)	0.67
1 (SM)	AMX, CTX, CAZ, CN	4 (66.67)	0.67
1 (SM)	CTX, CAZ, CIP, STX	4 (66.67)	0.67
2 (SM)	AMX, CTX, CAZ, CIP	4 (66.67)	0.67
14 (SD)	AMX, CTX, CAZ	3 (50)	0.5
1 (SD)	AMX, CTX, CIP	3 (50)	0.5
1 (SD)	AMX, CTX, CAZ, STX	4 (66.67)	0.67
2 (SD)	AMX, CTX, CAZ, CIP	4 (66.67)	0.67
1 (SD)	AMX, CTX, CAZ, CIP, STX	5 (83.33)	0.83

KEY: MAR = Multiple antibiotic resistance, AMX = Amoxicillin, CTX = Cefotaxime, CAZ = Ceftazidime, CIP = Ciprofloxacin, CN = Gentamicin, STX = Trimethoprim-Sulfamethoxazole, n = Total number of antibiotics used, EA = Sampling point at drainage system within the abattoir, EB = Sampling point at effluent, 100m away from the abattoir, EC = Sampling point at the junction where effluent enters River Koraye, SE = Sampling point at river bank (effluent-stream conjunction), SM = Sampling point at the middle of River Koraye, SD = Sampling point 500m downstream near farm area.

DISCUSSION

The physicochemical analysis of abattoir effluent and River Koraye revealed seasonal fluctuations in pH, total dissolved solids (TDS), biological oxygen demand (BOD), chemical oxygen demand (COD) and dissolved oxygen (DO). Across the seasons sampled, pH values ranged from slightly acidic (6.59) to slightly alkaline (8.51) which favours the survival of enteric organisms such as *E. coli*, this could be as a result of breakdown of waste and release of substances like carbondioxide and ammonia for the abattoir effluent while for River Koraye it could be as a result of its natural buffering capacity. This is in line with the study of Chinakwe *et al*. (2022) and Agboola *et al*. (2025), who also recorded similar pH values of (6.3-6.5) and (7.55-8.31) from abattoir effluent and surface water, respectively. The elevated BOD (1140 mg/L), COD (5500 mg/L), and TDS (3980 mg/L), coupled with low DO (1.10 mg/L), clearly demonstrate heavy organic loading, high microbial activity, and poor abattoir effluent management. The high TDS, reaching up to 3980 mg/L, recorded especially during the dry season, could be due to the absence of rainfall, indicating a heavy mineral and organic load, which is typical of slaughterhouse wastewater, where faecal content, blood, indigestible food particles, and other animal wastes are discarded. Similar exceedances have been documented in Nigerian abattoirs in the study by Okundaye *et al*. (2024).

The low DO (1.10 mg/L) recorded indicates high microbial respiration driven by abundant organic matter, creating a favourable environment for microbial survival and proliferation. This is also in line with the study of Amaechi Onyerimma (2025), who also recorded low DO values ranging (1.00-3.40mg/L) from abattoir effluent and groundwater. A high BOD of about 1140 mg/L indicates heavy organic loading, which rapidly depletes dissolved oxygen and supports the growth of microorganisms. Comparable findings have been reported by Ocheje *et al*. (2021), who recorded BOD levels of 5,009 mg/L in Nigerian abattoir effluent. The combination of high organic matter (BOD/COD), abundant suspended solids, low DO, and near-neutral pH creates an environment favourable to the growth and proliferation of *E. coli* and other enteric microorganisms. Chinakwe *et al*. (2022) and Okundaye *et al*. (2024) also observed similar results of low DO, high BOD, TDS and COD from abattoir effluent. Although all sampling periods demonstrated high values in BOD, COD, and TDS, a slight reduction was observed across all sampling points in the wet season, reflecting dilution due to rainfall.

The high occurrence of *E. coli* at point EA (98.1%) in abattoir effluent can be attributed to direct slaughterhouse activities, including intestinal evisceration, wash-down operations, and carcass dressing, which release faecal matter and intestinal contents rich in *E. coli* into the effluent. This highlights the abattoir effluent as primary reservoir of *E. coli* contamination. This is in line with the study by Igbinosa et al. (2023), who reported a high E. coli occurrence of 84% in abattoir environments.

The lowest occurrence at point SM (32.7%) could be due to the combined effects of dilution by river flow, natural die-off from ultraviolet radiation, predation by protozoa, and sedimentation of particulate matter. This is in line with the study by Beshiru *et al.* (2024), who also detected E. coli at a similar occurrence rate of 33.3% in surface water. The high resistance recorded against Amoxicillin, Cefotaxime, and Ceftazidime could be due to the presence of beta-lactamases, which disrupt the beta-lactam structure in this antibiotic group, rendering the active compound ineffective.

MAR indices of ≥0.5-1 observed could be due to the widespread use of antibiotics in livestock production, where mostly groups of beta-lactam antibiotics, especially amoxicillin, are frequently administered for prophylaxis, therapy, and growth promotion. This means bacteria in these animals can become resistant to many antibiotics, which can spread to humans and the environment and make infections harder to treat. This is in line with the findings of Onuoha *et al.* (2023), who reported high resistance, with MAR index values ranging from 0.4 to 0.9, in *E. coli* isolates from abattoir effluent and aquaculture environments, with resistance mostly to the beta-lactam antibiotic group. Igbinosa *et al.* (2023) also reported that 85.9% of *E. coli* isolates from agricultural environments were multidrug-resistant, with MAR indices exceeding 0.2, 100% resistance to cefotaxime and ceftazidime, and less resistance to non-beta-lactam antibiotics. This also supports the findings of this study. The high MAR indices observed among *E. coli* isolated from effluent and River Koraye reflect direct discharge of untreated abattoir waste containing blood and faecal material, with residual antimicrobials or even resistant bacteria, thereby exposing them to substantial antibiotic selection pressure, consistent with contamination from livestock production.

CONCLUSION

This study demonstrates the presence of *E. coli* in River Koraye (39.10%) and abattoir effluent (84.62%). Most of the isolates from both abattoir effluent and River Koraye were resistant to Amoxicillin, Cefotaxime and Ceftazidime antibiotic discs, with 97.7% resistance to Amoxicillin and Cefotaxime and 95.5% resistance to Ceftazidime in abattoir effluent and 93.4 %, 98.4% and 96.7% resistance to Amoxicillin, Cefotaxime and Ceftazidime in River Koraye water samples, respectively.

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