

# PHENOTYPIC CHARACTERIZATION AND ANTIMICROBIAL RESISTANCE OF PRESUMPTIVE *ESCHERICHIA COLI* O157:H7 IN ABATTOIR EFFLUENTS IN JOS, NIGERIA

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## ABSTRACT

Discharge of untreated abattoir wastewater into urban ecosystems facilitates the dissemination of Shiga toxin-producing *Escherichia coli* (STEC), posing a severe zoonotic risk. This study characterized presumptive *E. coli* O157:H7 isolates from abattoir effluents in Jos, Nigeria, assessing their environmental distribution and antimicrobial resistance profiles. A total of 120 samples (wastewater and process water) were collected from slaughterhouses and associated drainage systems. Physicochemical parameters, including Biological Oxygen Demand (BOD), turbidity, and conductivity, were analyzed using standard titrimetric and electrometric methods. *E. coli* was isolated via enrichment and selective plating on Sorbitol MacConkey (SMAC) agar. Presumptive O157:H7 strains, identified as Non-Sorbitol Fermenters (NSF), were characterized using biochemical methods. Antibiotic susceptibility testing (AST) was conducted using the Kirby-Bauer disk diffusion method, and the results were interpreted using CLSI guidelines. Physicochemical analysis indicated that temperature, pH, and conductivity remained within regulatory limits; however, BOD and turbidity levels showed marked fluctuations. Drainage water demonstrated the highest mean microbial density ( $1.18 \times 10^{12}$  CFU/ml), though no statistically significant correlation ( $p > 0.05$ ) was established between bacterial load and water source. Of the 115 *E. coli* isolates, 13 (11.3%) were identified as NSF, with a notable cluster identified in the Vom Pig slaughterhouse. Resistance was most prevalent against tetracycline (76.9%), cefixime (69.2%), and ampicillin (61.5%). Conversely, high susceptibility was maintained for colistin sulphate (69.2%), amoxicillin (69.2%), and imipenem (61.5%). There is an urgent need for stricter enforcement of Hazard Analysis and Critical Control Points in Jos abattoirs to mitigate the risk of foodborne outbreaks.

**Keywords:** Characterization, Resistance, *E. coli* O157:H7, Jos.

## INTRODUCTION

The last four decades have seen a significant intensification of environmental degradation, largely due to ineffective environmental management practices, resulting in widespread aquatic pollution and a growing burden of waterborne diseases, notably typhoid fever, cholera, diarrhea, and dysentery, at the human-environment interface (Emran *et al.*, 2024). Developing countries are facing serious problems in wastewater management due to a demographic explosion and poor urban planning. In

Nigeria, particularly, the lack of a good abattoir waste management system has a negative influence on the environment and contributes to the increasing burden of foodborne diseases, thereby raising major public health concerns (Ajuwon *et al.*, 2021). Enterohaemorrhagic *Escherichia coli* O157:H7 is one of the most important foodborne pathogens.

*Escherichia coli* O157:H7 is one of the most important food-borne pathogens that causes significant losses among the human population in the past two decades. More than 75,000 cases of foodborne illness attributed to *E. coli* O157:H7 occur annually (Hassein *et al.*, 2015). The Center for Disease Control (CDC) estimates that each year, shiga toxin producing *E. coli* (STEC) causes 265,000 illnesses, 3,600 hospitalizations, and 30 deaths in the United States. About 5–10% of people diagnosed with *E. coli* O157 infection develop a potentially life-threatening complication known as a type of kidney failure. (CDC, 2019). Most people with HUS recover within a few weeks, but some suffer permanent health problems or die. (CDC, 2019). *E. coli* O157: H7 causes an estimated 63,000 hemorrhagic colitis cases annually in the United States. While *E. coli* O157: H7 causes diarrhoeal illness in both children and adults, systemic complications occur more frequently in children. In adults, *E. coli* O157: H7 colitis can occasionally cause HUS and thrombotic thrombocytopenic purpura. Outbreaks of *E. coli* O157: H7 typically occur sporadically in outbreaks with exposure to contaminated food sources.

## MATERIALS AND METHODS

### Study Area

This study was conducted across eight selected Abattoirs (the Jos main Abattoir along Dogon Karfe Junction, Jos South, the slaughterhouse at Yan shanu, Jos North, Yanganda Jos North, the slaughterhouse at Kwata, Bukuru Jos South, Gyel Bukuru Jos South, Gacen slaughterhouse Jos South, Dakyen pig slaughterhouse at trade center along Vom Road, and the pig slaughterhouse in Vom Jos South) all in Plateau State. Plateau State is located in the middle belt area of Nigeria with an area of 26,899 Km<sup>2</sup> and an estimated population of 3,000,000 people (National Population Census, NPC, 2006). It is located between latitude 08°24'N and Longitude 008°32' and 010°38' East. Plateau state shares boundaries with Kaduna state to the North West, Bauchi state to the North East, Nasarawa state to the South West, and Taraba state to the East.

### Sample Collection

Ethical approval for this study and permission to collect samples from the abattoirs were obtained from the University of Jos Veterinary Teaching Hospital, Jos, Plateau State, Nigeria, and appropriate managers and staff of the abattoirs in Jos. A total of 120 samples were collected in sterile 500ml glass bottles, process water samples (40), waste water samples from the slaughter slabs (40), and waste water samples from the drainages around the abattoir (40). The samples were transported to the Bacterial Research Laboratory, National Veterinary Research Institute, Vom, Plateau State, using an ice box in a cold chain for microbiological analysis. Upon arrival, the samples were stored in a refrigerator at 4°C for 24 hours until being processed for isolation.

### Physicochemical Analysis of Waste Water

The wastewater samples were analyzed for the parameters: temperature, pH at 25°C, conductivity ( $\mu\text{s}/\text{cm}$ ), biological oxygen demand (Mg/L), turbidity (NTU), and total dissolved solids (PPM mg/L). All parameters were determined using the standard protocol described by the [American Public Health Association \(APHA, 1998\)](#).

### Standard Plate Count of the Water Sample

A stock sample of the wastewater collected was prepared by adding 10ml of the sample in 90ml of sterile distilled water. This was serially diluted to  $10^{-9}$  and 0.1ml of the last two dilutions ( $10^{-8}$  and  $10^{-9}$ ) were inoculated on Plate count agar (PCA) using the spread plate method. All the plates were incubated at 37°C for 24 hours and colonies counted. Results were expressed as colony forming unit per ml (cfu/ml).

### Isolation and Identification of *E. coli*

Upon arrival in the laboratory, a portion of the sample 1ml was inoculated in the *E. coli* medium (EC Broth) for enrichment and incubated at 37°C for 24 hours. All pre-enriched water samples were subsequently subcultured on Eosin methylene blue (EMB) agar and incubated at 37°C for 24 hours. Purified suspected *E. coli*-like colonies were identified by examining the morphology and biochemical properties of growing colonies. Colonies showing typical dark red to purple red with metallic sheen were taken as *E. coli* isolates (Holt *et al.*, 1994). After selective enrichment was made in modified Tryptone Soya Broth supplemented with Novobiocin, the isolates were cultured on 1% Sorbitol MacConkey agar and incubated at 37°C for 24 hours. The Non-Sorbitol-Fermenting (NSF) *E. coli* (colorless or pale colonies) were considered as *E. coli* O157:H7 strains, whereas pinkish colored colonies (Sorbitol-Fermenters) were considered as non-O157:H7 *E. coli* strains.

### Antimicrobial Susceptibility Testing

The antimicrobial susceptibility testing was performed following the standard agar disk diffusion method according to CLSI (2012) using commercially available antimicrobial disks. Each isolated bacterial colony from pure fresh culture was transferred into a test tube of 5 ml Tryptone Soya Broth (TSB) (Oxoid, England) and

incubated at 37°C for 6 hrs. The turbidity of the culture broth was adjusted using sterile saline solution and by inoculating more isolated colonies into the TSB to obtain turbidity usually comparable with that of 0.5 McFarland standards (approximately  $1.5 \times 10^8$  CFU per ml). Mueller-Hinton agar (Bacton Dickinson and Company, Cockeysville, USA). Was prepared according to the manufacturer's guidelines and poured into the plates. A sterile cotton swab was immersed in the suspension and rotated against the side of the tube to remove the excess fluid, and then swabbed in three directions uniformly on the surface of Mueller-Hinton agar plates. After the plates dried, antibiotic disks were placed on the inoculated plates using an antibiotic disc dispenser. The antibiotic disks were gently pressed onto the agar to ensure firm contact with the agar surface, and incubated at 37°C for 24 hours. After 24 hours of incubation, the diameter zone of inhibition formed around each disk was measured using a black surface, reflected light, and a transparent ruler by laying it over the plates. The results were classified as sensitive, intermediately resistant, and resistant according to the standardized table supplied by the manufacturer (CLSI, 2012).

### RESULTS

The physicochemical properties of the abattoir water samples were also determined, and the result is presented in Table 1. The mean pH ranged from 6.63 to 7.29, while temperature ranged from 26 to 27.5, turbidity from 145.5mg/L to 1586 5 mg/L, the Biological Oxygen Demand (BOD) ranged from 20mg/L to 710mg/L conductivity 1211 $\mu\text{s}/\text{cm}$  to 3814  $\mu\text{s}/\text{cm}$  while total dissolved solids (TDS) ranged from 777 to 3900.5 (PPM). Temperatures, pH and conductivity from the various locations were within the recommended abattoir effluent water discharge into the environment. However, wide variations were observed in turbidity and BOD, with Yan Shanu, Vom Pig Slaughter house, and Dakyen Pig slaughter house having turbidity over 1000NTU, and Main abattoir the only location with BOD less than 50mg/l. The total dissolved solids observed in this study also vary widely, with samples from Gyel Bukuru measuring 3900.5ppm, followed by Kwata 1906ppm, and the Main abattoir with the least total dissolved solids of 777ppm.

The results obtained show a total bacterial load of  $1.69 \times 10^{12}$ cfu/ml. On bacterial assessment of sources of samples, drainage water had the highest bacterial load of  $1.18 \times 10^{12}$ cfu/ml, followed by slaughter slabs with  $5.15 \times 10^{11}$ cfu/ml and  $6.47 \times 10^8$ cfu/ml in water sources. On general considerations of bacterial load across different locations, Vom pig slaughterhouse wastewater had the highest bacterial count of  $3.58 \times 10^{11}$  cfu/ml, followed by Yan Shanu with  $3.45 \times 10^{11}$  cfu/ml. Samples from the Main abattoir, Yan ganda, Gacen slaughter house, Dakyen pig slaughter house, and Gyel bukuru with counts of  $3.93 \times 10^{11}$  cfu/ml,  $1.02 \times 10^{11}$  cfu/ml,  $9.16 \times 10^{10}$  cfu/ml,  $4.82 \times 10^{10}$  cfu/ml, and  $3.22 \times 10^{10}$  cfu/ml, respectively. However, samples from Kwata Bukuru had the least bacterial load of  $2.52 \times 10^{10}$  cfu/ml as shown in Table 2.

**Table 1:** Physicochemical Parameters of the Waste Water Sample from Selected Abattoirs in Jos, Nigeria

Location	Temperature (°C) (20-30°C)*	pH (6.0-9.0)*	Turbidity (NTU)(300)*	BOD(Mg/L) (50mg/l)*	Conductivity (µS/cm) (5.97-4448)*	Total Dissolved Solid (PPM)(200.22)*
YS	27.5	7.09	1586.5	70	2404	1201
YG	27.0	7.17	145.5	26	1211	1186
MA	27.5	6.63	441.5	20	1552	777
KB	27.5	7.22	12.32	120	3814	1906
GB	26.5	7.29	502.5	299	7800	3900.5
GS	27.5	6.57	602.5	505	1500	2310
DP	27.0	7.23	1089.5	271	3500	1749
VP	26.0	7.25	1438.5	710	1236	1048

**KEY:** YS=Yanshanu, YG= Yanganda, MA= Main abattoir, KB= Kwata Bukuru, GB= Gyel Bukuru, GS= Gacen Slaughterhouse, DP= Dakyen Pig Slaughterhouse, VP= Vom Pig Slaughterhouse.

\*Reference limits (FEPA 1999; WHO 2010)

**Table 2:** Comparison of the Mean Total Bacterial Count (cfu/ml) between water sources and Abattoirs

Locations	Sources						Total Count
	WS	P-value	SS	P-value	DR	P-value	
YS	5.35×10 <sup>7</sup>	0.678	1.75×10 <sup>11</sup>	0.707	1.70×10 <sup>11</sup>	0.411	3.45×10 <sup>11</sup>
YG	6.72×10 <sup>6</sup>		6.59×10 <sup>10</sup>		3.60×10 <sup>10</sup>		1.02×10 <sup>11</sup>
MA	3.20×10 <sup>5</sup>		4.33×10 <sup>10</sup>		3.50×10 <sup>11</sup>		3.93×10 <sup>11</sup>
KB	3.90×10 <sup>7</sup>		1.37×10 <sup>10</sup>		1.15×10 <sup>11</sup>		2.52×10 <sup>10</sup>
GB	9.70×10 <sup>5</sup>		2.12×10 <sup>10</sup>		1.10×10 <sup>10</sup>		3.22×10 <sup>10</sup>
GS	9.80×10 <sup>5</sup>		3.81×10 <sup>10</sup>		5.35×10 <sup>10</sup>		9.16×10 <sup>10</sup>
DP	1.62×10 <sup>8</sup>		1.95×10 <sup>10</sup>		1.25×10 <sup>11</sup>		4.82×10 <sup>10</sup>
VP	3.84×10 <sup>8</sup>		1.38×10 <sup>11</sup>		2.2×10 <sup>11</sup>		3.58×10 <sup>11</sup>
Total	6.47×10 <sup>8</sup>		5.15×10 <sup>11</sup>		1.18×10 <sup>12</sup>		1.69×10 <sup>12</sup>

**KEY:** WS= Water sources, SS=Slaughterslab wastewater, DR = Drainage wastewater, YS= Yanshanu, YG= Yanganda, MA= Main abattoir, KB= Kwata Bukuru, GB= Gyel Bukuru, GS= Gacen Slaughterhouse, DP= Dakyen Pig Slaughterhouse, VP= Vom Pig Slaughterhouse. F= F-value, P=P-value

A total of 115 suspected *E. coli* were isolated from 120 samples of wastewater and water source used in the slaughterhouses. Out of 115 isolates, 102(88.7%) were sorbitol fermenting (SF), and 13(11.3%) non-sorbitol fermenting (NSF). Of the 13 non-sorbitol fermenting (NSF) isolates, 3(20.0%) were isolated from Vom pig slaughterhouse, 2(13.3%) from Yanshanu slaughterhouse, Main abattoir, Kwata Bukuru slaughterhouse, and Dakyen Pig slaughterhouse (Trade centre) each, while Yanganda slaughterhouse and Gyel Bukuru slaughterhouse had 1(7.1%) and 1(7.7%) respectively. Gacen slaughterhouse had none during the period of this study (Table 3).

The result of the antibiotic susceptibility test on the 13 presumptive *Escherichia coli* O157:H7 isolates is presented in Table 4. Our results showed that most of the isolates were highly resistant to tetracycline (76.9%), cefixime (69.2%), and ampicillin (61.5%). Similarly, about 69.2%, 69.2%, 61.5% and 53.8% of the isolates, respectively, showed susceptibility to colistin sulphate, amoxicillin, imipenem, and chloramphenicol (Table 4).

**Table 3:** Frequency of Occurrence of Sorbitol Fermenting and Non-sorbitol Fermenting *E. coli* Isolated from Abattoir Effluent in Jos, Nigeria

Location	No. Isolated (%)	NSF (%)	SF (%)
YS	15(13.04)	2(13.30)	13(86.67)
YG	14(12.17)	1(7.10)	13(92.86)
MA	15(13.04)	2(13.30)	13(86.67)
KB	15(13.04)	2(13.30)	13(86.67)
GB	13(11.30)	1(7.70)	12(92.31)
GS	13(11.30)	0(0.00)	13(100.00)
DP	15(13.04)	2(13.30)	13(86.67)
VP	15(13.04)	3(20.00)	12(80.00)
<b>Total</b>	<b>115</b>	<b>13(11.3)</b>	<b>102</b>

**KEY:** YS= Yanshanu, YG= Yanganda, MA= Main abattoir, KB= Kwata Bukuru, GB= Gyel Bukuru, GS= Gacen Slaughterhouse, DP= Dakyen Pig Slaughterhouse, VP= Vom Pig Slaughterhouse. NSF- - non-sorbitol fermenting, SF- sorbitol fermenting.

**Table 4:** Antibiotic Susceptibility Profile of *E. coli* Isolated from Abattoir Effluent in Jos, Nigeria

Antibiotic	Disc Potency	<i>E. coli</i> O157:H7		
		S (%)	I (%)	R (%)
CFM	5µg	1(7.7)	3(23.1)	9(69.2)
AMP	10 µg	0(0.0)	5(38.5)	8(61.5)
CIP	5 µg	5(38.5)	4(30.8)	4(30.8)
CN	30 µg	5(38.5)	3(23.1)	5(38.5)
C	30 µg	7(53.8)	2(15.4)	4(30.8)
AMC	30 µg	9(69.2)	1(7.7)	3(23.1)
TE	30 µg	3(23.1)	0(0.0)	10(76.9)
CRO	30 µg	5(38.5)	5(38.5)	3(23.1)
IPM	10 µg	8(61.5)	2(15.4)	3(23.1)
CT	10 µg	9(69.2)	3(23.1)	1(7.7)

**Key:** S = Sensitive I = Intermediate R = Resistant CFM = Cefixime AMP = Ampicillin CIP = Ciprofloxacin CN = Gentamicin C = Chloramphenicol AMC = Amoxycillin TE = Tetracycline CRO = Ceftriaxone IMP = Imipenem CT = Colistin sulphates

## DISCUSSION

Physicochemical parameters of abattoir wastewater were assessed with temperature ranging from 26.0-27.5°C, pH; 6.57-7.29, turbidity; 145.5-1586.5NTU, BOD; 20-710mg/l, conductivity; 1211-7800µS/cm, and total dissolved solids; 777-3900.5PPM. The temperatures recorded in this study are within the recommended limit of <40°C for wastewater discharge and similar to temperatures of wastewater reported by Odeyemi *et al.* (2011) from Oyo state, and Dankaka *et al.* (2018) in Sokoto, Nigeria.

The near neutrality of pH (6.57-7.29) obtained from the present study plays a vital role in determining the type and abundance of bacteria in the different water sources sampled. Our finding agrees with the 6.92-8.18 pH obtained by Rout and Sahoo (2015) and Dankaka *et al.* (2018); however, Adesemoye *et al.* (2006) were of a contrary opinion. They documented an acidic pH from abattoir wastewater in Lagos, Nigeria.

The high BOD and total dissolved solids observed in our study could be attributable to the increase in microbial load and anaerobic degradation of organic compounds, which releases ammonia that reacts with carbon dioxide during the anaerobic process to produce ammonia bicarbonate, thereby also contributing to the increased pH. The temperatures and pH conditions obtained from this study favor the growth of enteric mesophilic bacteria that thrive best at pH near neutrality; hence, the 1.69×10<sup>12</sup>cfu/ml bacterial count obtained in this study is not surprising. Based on the findings of this study, the total bacterial load of the wastewater samples of 1.69×10<sup>12</sup>cfu/ml indicates very high microbial loads and exceeded the recommended unit for discharge of effluents into water bodies and land application in Nigeria (FEPA, 1999; WHO, 2010; Okoyomon *et al.*, 2021). This is similar to reports of Odeyemi *et al.* (2011) and Ijah *et al.* (2022). The bacterial counts observed in this study might be related to poor sanitary conditions of the abattoir, the poor state of health of the slaughtered animals, and faecal contamination from intestinal contents of the slaughtered animals. As observed from the study areas, untreated wastewater is washed into open drainages and streams around the slaughterhouses. The wastewater could eventually percolate into the surrounding surface and ground

waters, which poses a danger to those using the water for domestic cooking and irrigation purposes. Added to this danger is the possibility of recontamination of meat products when such water is used for washing and cleaning the same abattoir.

The water used for cleaning procedures and meat processing in the slaughterhouses must meet drinking water standards. It must be free of chemical substances or microorganisms in amounts that could cause hazards to health. The bacterial load of the process water of all the slaughterhouses of this study also exceeded the Federal Environmental Protection Agency (FEPA, 1999; Okoyomon *et al.*, 2021) and (WHO 2010) recommended limits hence making the source of water unfit for meat processing. The high microbial load may also be attributed to the source of water for cleaning and meat processing. As reported in a similar study by Adebowale *et al.*, (2012).

This study isolated 115 *E. coli* out of which 13(11.30%) were *E. coli* O157:H7 strains. The 11.30% isolation rate of *E. coli* O157:H7 in this study underscores a significant public health concern, particularly regarding foodborne zoonoses. This prevalence rate is substantially higher than the 0.4% reported by Mailafia *et al.* (2017) in Suleja, Nigeria, and the 2.8% observed in human clinical samples in Ile-Ife by Odetoyi *et al.* (2016). Such disparities in isolation rates are frequently documented in the literature and can be attributed to a nexus of geographical, environmental, and methodological factors. The high occurrence (11.30%) observed in the current study is likely a reflection of suboptimal hygienic standards during the slaughtering process. As *E. coli* is a well-known commensal of the gastrointestinal tract of diverse livestock (Rosa *et al.*, 2017; Buharshak *et al.*, 2019), the risk of carcass contamination during evisceration is high. The stark contrast with the 53% isolation rate reported by Dahiru *et al.* (2008) in Kano suggests that meat processing environments in certain regions may suffer from extreme levels of cross-contamination, likely due to the lack of modern sanitary infrastructure and poor water quality used for washing carcasses.

The variation between these findings and international data—such as the low rates in Poland (0.7%) and Finland (1.2%) reported by Tutenel *et al.* (2002)—may stem from differences in sampling sites and detection sensitivity. For instance, Elder *et al.* (2000) utilized rectal swabs, which often yield higher isolation rates (27.8%) because the terminal rectum serves as the primary colonization site for *E. coli* O157:H7 in cattle (Naylor *et al.*, 2003). Furthermore, the use of advanced detection techniques, such as Immunomagnetic Separation (IMS), significantly enhances the recovery of the pathogen from complex matrices compared to conventional culture methods alone. Geographic location and climate also play a pivotal role in the shedding patterns of this pathogen. Higher temperatures in tropical regions like Nigeria may favor the environmental persistence and proliferation of *E. coli* O157:H7 in water and soil, facilitating a continuous cycle of reinfection among herds (Salo *et al.*, 2011). This environmental factor, combined with varying livestock management systems (extensive vs. intensive), likely contributes to the differences in the isolation rates seen across different states and countries.

The *E. coli* O157:H7 isolates were highly resistant to tetracycline (76.9%), cefixime (69.2%) and ampicillin (61.5%). However, 69.2%, 69.2%, 61.5% and 53.8% of these isolates were respectively susceptibility to colistin sulphate, amoxicillin, imipenem and chloramphenicol. The resistance to tetracycline and ampicillin found in *Escherichia coli* in this work is higher than that

reported by Nyamboya *et al.* (2013). They recorded 70.4% and 66.7% of the bacteria to exhibit resistance to tetracycline and ampicillin. Resistance to these antibiotics may be attributed to several factors such as source of drinking water of the animals which may be polluted by antibiotic agents, and secondly introduction of antibiotics supplemented commercial feed may have initiated resistance (Nyamboya *et al.*, 2013). The high isolation rate found in this study highlights the urgent need for stricter enforcement of Hazard Analysis and Critical Control Points (HACCP) in Jos abattoirs to mitigate the risk of foodborne outbreaks. It is worthy of note that despite the efficacy of colistin sulphate on presumptive *E. coli* O157:H7 obtained in this study, the result should be interpreted with caution as Colistin disk diffusion (DD) testing is not recommended by the CLSI and EUCAST due to high error rates and poor reproducibility

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