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Research Article

VIRULENCE GENES BEARING AND ANTIBIOTIC RESISTANT *ESCHERICHIA COLI* IN ABATTOIR CONTACT SURFACES AND WATER SOURCES IN SELECTED COMMUNITIES IN OBIO/AKPOR, RIVERS STATE, NIGERIA

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ABSTRACT
The study determined the presence of virulent and antibiotic resistance *Escherichia coli* (*E. coli*) in abattoir surfaces and water in five communities in Obio/Akpor, Rivers State, Nigeria. A total of 50 (40 contacts surfaces and 10 water) samples were examined for the presence of *E. coli* using standard microbiological methods employing Eosin methylene blue agar. Antibiotic sensitivity and virulent genes were also carried out using standard methods. Of the 50 samples, 20 were positive for *E. coli*. The results of the mean total heterotrophic bacteria and coliform count ($log_{10}cfu/cm^2$) revealed the following values for knives (3.11± 0.07, 3.49±1.97), cutting board (3.30±0.07, 3.43±0.99), floor (3.37±0.09, 3.56±1.24) and table (3.19±0.25, 3.33±0.96), respectively. The mean total heterotrophic bacteria and coliform count for the water were: 3.20±0.17 and 3.41±1.07log_{10}cfu/ml, respectively. Antibiotic resistance results showed a 100% against augmentin and tetracycline but varying resistance against ceftriaxone and ofloxacin (42.86%), cefuroxime (78.57%), gentamycin (64.29%) and ciprofloxacin (28.57%). Virulence genes: *eae, ast* and *aggR* were detected in 6, 3 and 6, respectively of the *E. coli* isolates producing the expected bands against the primers. The presence of antibiotic resistance and virulence genes bearing *E. coli* portends danger for the consumer of meats from the abattoirs when not properly cooked or in the event of cross- contamination.

Keywords: *Abattoir, Aggregative Adherence Regulator (AGGR), Contact Surfaces, Virulence Genes*

INTRODUCTION
Slaughter facilities are specially designed and licensed for used at different meat processing stages such as holding, stunning, bleeding, hide removal, evisceration, splitting, washing, inspection, weighing, storage and distribution to the public (Alonge *et al.,* 2005; Abdalla *et al.,* 2009a; Canencia *et al.,* 2016). In Nigeria, the abattoir industry is an important component of the livestock industry providing domestic meat supply to over 150 million people and employment opportunities for teaming populations (Nafarnda *et al.,* 2012). Along the process chain, contamination from the skin of the animal to the personnel hands and the equipment and then to the carcass and cross-contamination from the other carcasses may occur. In most developing countries, traditional methods of handling, processing and marketing of meat undermine quality where poor sanitation leads to considerable loss of product as well as the risk of food-borne diseases (Garcia, 2007). The level of hygiene in some of the abattoirs in developing countries is below the standards due to poor hygiene as most of the employees are poorly trained on meat handling practices (Nwanta *et al.,* 2010; Park *et al.,* 2011). Training on meat handling includes but is not limited to Good Manufacturing Practices (GMPs), Good Hygiene Practices (GHPs) and Hazard Analysis of Critical Control Point (HACCP). The training and implementation of HACCP and other quality management procedures in the developing countries is constrained (Jirathana, 1998).

The external contamination of meat poses a major problem in most developing countries' abattoirs and microbial surface contamination of carcasses has been repeatedly reported to have a significant effect on

the meat shelf life (Hood and Zottola, 1995). Fecal matter is a major source of microbial contamination and could reach carcasses through direct deposition as well as by indirect contact through contaminated and unclean equipment, surfaces, workers and so on (Abdalla *et al.,* 2009b). Presently, operations of abattoir and retail houses are hardly inspected by veterinary and public health officers. There is high possibility of detecting pathogens in meat processing establishments designed for production of meat intended for human consumption (Balcha *et al.,* 2014). Since there is an increasing demand for meat and meat products, it is of paramount importance to assess the contamination levels of meat contact surfaces in the abattoir with pathogens of public health significance such as *Staphylococcus aureus, Escherichia coli*, *Listeria monocytogenes*, *Salmonella* spp. and *Campylobacter* spp. (Buchanan and Doyle, 1997; ESR, 2002; Sobsey *et al.,* 2002; Aighewi *et al.,* 2017). The information on meat production area hygiene status will facilitate designing microbial contamination preventive strategies in abattoir and retail houses and provide baseline data for related studies.

Escherichia coli is a species of Gram negative, facultative anaerobic, rod shaped, coliform, non-sporulating bacterium of the family Enterobacteriaceae and genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms and its isolation from abattoir effluents has been reported in South East, Nigeria (Uhitil *et al.,* 2001; Von Baum and Marre, 2005; Gyles, 2007; Bavaro, 2012; Iroha *et al.,* 2016; Menge, 2020). Akinyeye *et al*. (2013) reported that *E. coli* had the highest frequency of occurrence in abattoir effluents contaminated soil in Benin, Edo State. Majority of *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination (Vogt and Dippold, 2005). It is considered that initial *E. coli* contamination in meat is from the faeces of animals shedding the bacterium or from a contaminated skin of cattle which is either shedding or not shedding the bacterium, usually at the stage of slaughter (Elder *et al.,* 2000). *Escherichia coli* from livestock feces is known to survive on grass pasture for at least 5 months, affording opportunity for *E. coli* to be recycled by animals (Avery *et al.,* 2004). The slaughter process is therefore a key step in the control of meat contamination with *E. coli.* Poor meat hygiene and slaughter practices therefore contribute largely to the prevalence and concentration of *E. coli* on the surfaces and surface of meat. Meat hygiene would encompass personal hygiene, slaughter and meat processing hygiene and hygiene of slaughter and meat processing premises and equipment.

In terms of virulence, *E. coli* strains that cause diarrhoea in humans have been classified into groups, namely, enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC) or Shiga toxin-producing *E. coli* (STEC), enteroaggregative E. coli (EAEC), enteroinvasive E. coli (EIEC), and the attaching and effacing *E. coli* (AEEC), (Paton and Paton, 1998; Bugarel *et al.,* 2011; Bavaro, 2012). The groups EPEC and EHEC has as target gene the eae while the EAEC has as it target gene the aggR and ast genes (Bisi-Johnson *et al.,* 2011; Parussolo *et al.,* 2019).

The *aggR*, *eae* and *ast* virulence determinants of *E. coli* are responsible for the control of expression of an Enteroaggregative *Escherichia coli* (EAEC) fimbriae, strain adherence and a heat stable toxin, respectively (Otokunefor *et al.,* 2022). The most injurious *E coli* strains are those able to produce ostensible virulence factors such genes encoding intimin (eae) and the plasmid encoded enterohemolysin, encoded by enterohemorrhagic *E. coli* (EHEC) hlyA (Schmidt *et al.,* 1995; Schmidt and Karch, 1996). The EPEC strains are defined as *eae*-haboring diarrheagenic *E. coli* that possess the ability to form A/E lesions on intestinal cells but do not possess Shiga toxin genes (Kaper, 1996). The aggR regulate most of the EAEC virulence genes, including aggregative adherence fimbriae (AAFs) with four known variants, namely: aggA (AAF/I), aafA (AAF/II), agg3A(AAF/III), and agg4A (AAF/IV) (Nataro *et al.,* 1992; Bernier *et al.,* 2002; Boison *et al.,* 2012).

The study is aimed at determining the occurrence of virulence bearing and antibiotic resistant *E. coli* in contact surfaces and water at abattoirs in selected communities in Obio/Akpor. Rivers State.

MATERIALS AND METHODS

Study Sites

The study covered the following communities: Alakahia, Choba, Ozuoba, Rumuokoro, Rumuosi at close proximity to the University of Port Harcourt, Obio/Akpor Local Government Area, Rivers State.

Collection of samples and preparation

A total of fifty (five on each visit) pooled environmental samples from cutting boards, floor, tables, knives and water sources were examined for the presence of *Escherichia coli* using the methods described in the Compendium of Methods for the Microbiological Examination of Foods (Doores *et al.,* 2013). Sterile swabs previously immersed in 9 ml sterile peptone were used to sample 1 cm^3 area of meat contact surfaces. After sampling, the swabs were placed again aseptically back into peptone water and transported in ice packs to the Microbiology Laboratory for analysis within 2 h of sampling.

Enumeration for total viable bacteria and E. coli Counts

The tubes containing the swab sticks was vortexed for 10s followed by a 10-fold serial dilution in 0.1% sterile buffered peptone water (BPW) (Oxoid, CM0509). Aliquots (0.1 ml) of appropriate dilutions were aseptically spread plated onto freshly prepared Nutrient and eosin methylene blue (EMB) agar plates. The plates were inverted and incubated at ambient temperature (29±2ºC) for 24 h. The resulting discrete colonies were counted. Discrete colonies on Eosin Methylene Blue (EMB) agar with characteristic metallic green sheen were purified by streaking on freshly prepared Nutrient agar plates and pure colonies preserved in Nutrient agar slants kept in refrigerator at 4ºC pending confirmation.

Confirmation of E. coli isolates

The isolated E. coli were confirmed on the basis of the cultural, physiological and biochemical characteristics involving Gram's reaction, indole test, Methyl-red test, Vogues Proskauer test and citrate utilization test (Cheesbrough, 2005; MacFaddin, 2000).

Determination of the presence of virulence genes

Deoxyribonucleic acid (DNA) extraction: The *E. coli* DNA was extracted by the boiling method with modification (Ahmed and Dablool, 2017) Cells were harvested by centrifugation of overnight brain heart infusion broth culture of *E. coli* in 2 ml Eppendorf tube at 10,000 rpm for 2 min and the supernatants discarded. The pellets were re-suspended in 1ml sterile distilled water and re-centrifuged after vortexing at 10,000 rpm for 5 min. The supernatants were again discarded and the pellets re-suspended in 200 µL sterile water and vortexed. The suspensions were heated for 5 min in a boiling bath (100ºC). After cooling and vortexing, the mixtures were centrifuged at 10,000rpm for 5 min. The supernatants were then transferred to a pre-labelled 1.5ml Eppendorf tube while the sediments were discarded. The DNA extracted were stored in deep freezer (-20ºC) until further analysis.

Presence of virulence genes: Oligonucleotide primers for AggRks1, EAS and EAST virulence genes synthesized by Bimers.net, Germany were employed. PCR was conducted in thermocycler in a volume of 25µL, containing 2.5 µL 10×PCR buffer, 1.0 µL $MgCl_2$, 2.0 µL dNTP (deoxy nucleoside triphosphate), 1.0 µL each of appropriate primer, 0.1µL AmphiTaq DNA polymerase, 3.0 µL of appropriate DNA preparation and 13.4 µL sterile distilled water. Amplification following an initial denaturation at 94ºC for 5 min was performed in 35 cycles at 94ºC for 15s, 55ºC for 20s and 72ºC for 30s. A final extension was done for 7 min at 72ºC. An 8µl aliquot of PCR product mixed with a loading dye (10mM, EDTA, 10% glycerol, 0.015% bromo phenol blue and 0.017% sodium dodecyl sulphate (SDS), made up to 100mL) were checked in an ethidium bromide stained 1.5% agarose and the gel read in a UV transilluminator Reaction mixture with no DNA template was incorporated as a negative control in each reaction. The quality of DNA the product of PCR was assessed using gel electrophoresis using a Portable Gel hood built in Blue LED (470nm) (Royal Biotech/Biolympics) 1.5% agarose gel at a constant voltage and 1X TBE for approximately 1 h. They were visualized by Ethidium bromide staining and photographed under ultraviolet light. The ladder used is 1kb base pair ladder from thermo scientific.

Table 1. Primers sequences described by Bisi-Johnson et al. (2011)

Target gene	Primer Nucleotide Sequence (5'- 3')	Amplicon size (bp)
aggR	F 5' GTATACACAAAAGAAGGAAGC 3'	256bp
	R 5' ACAGAATCGTCAGCATCAGC 3'	
eae	F 5' ATGCTTAGTGCTGGTTTAGG 3'	248bp
	R 5' GCCTTCATCATTTCGCTTTC 3'	
ast	F 5' GCCATCAACACAGTATATCC 3'	106bp
	R 5' GAGTGACGGCTTTGTAGTCC 3'	

Antibiotic Sensitivity Pattern of E. coli

Antibiotic sensitivity patterns of all the confirmed isolates were performed by standard disk diffusion method according to Kirby-Bauer on Mueller-Hinton agar (Titan, Biotech Ltd, Indian) following the procedures recommended by Clinical and Laboratory Standard Institute (CLSI) as described in Bauer et al. (1966). Eight commonly used antibiotics (µg/disc) viz. amoxicillin-clavulanate or augmentin (AUG), ceftriaxone (CEF), gentamycin (GEN), nitrofurantoin (NIT), cefuroxime (CRX), ofloxacin (OFX), cefixime (CXM), ciprofloxacin (CPX), tetracycline (TET), Abtek, (UK) were tested. From an overnight culture *E. coli,* 0.5 MacFarland turbidity standard bacterial culture was prepared in sterile saline, from which 0.1 ml was inoculated onto Mueller Hinton agar. Thereafter, antibiotic discs were carefully and aseptically placed on the surface of the agar. The plates were incubated at 37°C for 24 h. Zone of inhibition was measured in millimeter.

RESULTS AND DISCUSSION

Percentage occurrence of E. coli

The results of the percentage occurrence of *E. coli* in the surfaces and water samples examined from the different locations are presented in Table 2. The knives, table and cutting boards were mostly contaminated with positive samples ranging from 50 to 100%. Only the floor samples did not yield the growth of *E. coli* while only the water samples at the Choba abattoir was positive for *E. coli.* The findings of this study were higher than the 0 to 40% occurrence of *E. coli* in 10 each of cutting instruments, floor and tables samples Zailani et al. (2016) at a red meat aabattoir of Bauchi State, North-Eastern Nigeria. On their part, Izevbuwa and Okhuebor (2020) reported the inability to isolate E. coli in processing water and table swabs samples from Obinze abattoir, Rivers State, Nigeria. *Escherichia coli,* which are normal flora of the human and animal intestine, have been identified as a leading cause of food borne illness all over the world (Enabulele and Uraih, 2021), hence its presence of *E. coli* could be of public health importance because of the potential to harbor resistance genes and the risk of contaminating the food chain (Ariel *et al.,* 2020). It is an important indicator which can be used as a measure for hygienic practices, and its presence on abattoir contact surfaces could be related to hygiene practices in abattoirs, and might lead to contamination with enteric bacteria during evisceration, causing occupational diseases which can be transmissible to humans (Wheatley *et al.,* 2010; Shamsul *et al.,* 2016; Ariel *et al.,* 2020).

Total aerobic plate count

The results in Table 3 shows the total aerobic bacteria count (TABC) on Nutrient agar. The TABC ranges for water samples (Log_{10}cfu/ml), knives, cutting board, floor and table (Log_{10}cfu/cm^2) were 3.06±0.08 to 3.56±0.63, 3.07±0.10 to 3.15±0.11, 3.15±0.04 to 3.45±0.03, 3.24±0.02 to 3.48±0.08 and 2.67±0.58 to 3.39±0.06, respectively. The findings of this study are way below the mean counts of 9.51 (3.27×10^9), 7.57 (3.70×10^7) and 10.59 (3.85×10^{10}) log_{10}cfu/cm,^{2} respectively for abattoir floor, knives and tables reported by Uzoigwe et al. (2021) in an aassessment of bacterial contamination of beef in slaughterhouses in Owerri zone, Imo State, Nigeria. The TABC range obtained in this study for cutting board (3.15±0.04 to 3.45±0.03log_{10}cfu/cm^2), floor (3.24±0.02 to 3.48±0.08log1$_0$cfu/cm^2) and table (2.67±0.58 to 3.39±0.06 log_{10} cfu/cm^2) were also lower than the range of 6.4±0.6 to 7.6±0.2; 6.9±0.8 to 7.6±0.2 and 6.9±0.8 to

7.8±0.3 $log_{10}cfu/cm^2$, respectively reported by Zailani et al. (2016) in meat contact ssurfaces in red meat abattoirs of Bauchi State, North-Eastern Nigeria

Table 2. Percentage occurrence of *E. coli*

Location	Contact surface	No of samples	No of positive samples	% Occurrence
Choba	Knife	2	2	100
	Cutting board	2	1	50
	Floor	2	-	0
	Table	2	2	100
	Water source	2	1	50
Rumuosi	Knife	2	1	50
	Cutting board	2	1	50
	Floor	2	1	0
	Table	2	2	100
	Water source	2	-	0
Alakahia	Knife	2	1	50
	Cutting board	2	1	50
	Floor	2	-	0
	Table	2	1	50
	Water source	2	-	0
Ozuoba	Knife	2	2	100
	Cutting board	2	1	50
	Floor	2	-	0
	Table	2	1	50
	Water source	2	-	0
Rumuokoro	Knife	2	1	50
	Cutting board	2	-	0
	Floor	2	-	0
	Table	2	1	50
	Water source	2	-	0

The average of 2.4$log_{10}cfu/ml$ reported by Bersisa *et al.,* (2019) in water obtained from abattoir and butcher shops in Bishoftu, Central Ethiopia is less than the average of 3.20±0.17 $log_{10}cfu/ml$ obtained in this study (Table 4). The counts of 6.58 and 6.10 $log_{10}cfu/cm^2$ reported by Bersisa *et al.,* (2019) for cutting tables and knives were also higher the average of 3.30±0.07 and 3.11±0.07 $log_{10}cfu/cm^2$ obtained in this study. Bhandare et al. (2009) report of 7.19±0.18$log_{10}cfu/cm^2$ and 3.90±0.07 $log_{10}cfu/ml$ in abattoir floor and water were higher than the 3.37±0.09 $log_{10}cfu/cm^2$ and 3.20±0.17 $log_{10}cfu/ml$ obtained in this present study. The mean count of 3.12±0.18$log_{10}cfu/cm^2$ TABC from knives reported by Pradhan et al. (2018) agrees with the 3.11±0.07 $log_{10}cfu/cm^2$ mean value of this study; whereas, the mean 6.27±0.48$log_{10}cfu/cm^2$ and 6.18±0.71$log_{10}cfu/ml$ reported for abattoir floor and water from contact surfaces and water samples in retail Chevon meat shops and abattoirs of Kolkata, West Bengal, India were higher than the values obtained in this present study.

Table 3: Total aerobic plate bacteria count (TABC)

Location	Contact surface	TABC (cfu/cm^2 or cfu/ml)	TABC log_{10} cfu/cm^2 or cfu/ml (Mean ± SD)
Choba	Knife	$1.1 \times 10^3 – 1.35 \times 10^3$	3.09±0.06
	Cutting board	1.5×10^3 -1.93×10^3	3.32±0.08
	Floor	2.3×10^3 -2.8×10^3	3.40±0.06
	Table	2.2×10^3 - 2.7×10^3	3.39±0.06
	Water source	1.5×10^3 - 1.6×10^3	3.19±0.02
Rumuosi	Knife	1.2×10^3 - 1.7×10^3	3.15±0.11
	Cutting board	2.1×10^3 -2.5×10^3	3.36±0.05
	Floor	2.8×10^3 - 3.3×10^2	3.48±0.08
	Table	1.0×10^3 - 3.0×10^3	3.24±0.34
	Water source	1.2×10^3 - 1.3×10^3	3.10±0.02
Alakahia	Knife	1.1×10^3 - 1.6×10^3	3.12±0.02
	Cutting board	2.5×10^3 -3.2×10^3	3.45±0.08
	Floor	2.9×10^3 - 3.1×10^3	3.48±0.02
	Table	2.0×10^3 - 2.7×10^3	3.37±0.09
	Water source	1.1×10^3 - 1.5×10^4	3.11±0.10
Ozuoba	Knife	1.16×10^3 -1.5×10^3	3.12±0.08
	Cutting board	1.4×10^3 - 1.9×10^3	3.21±0.08
	Floor	1.2×10^3 - 2.7×10^2	3.26±0.25
	Table	1.8×10^2 - 1.2×10^3	2.67±0.58
	Water source	1.3×10^3 - 1.0×10^4	3.56±0.63
Rumuokoro	Knife	1.0×10^3 -1.4×10^3	3.07±0.10
	Cutting board	1.3×10^3 - 1.5×10^3	3.15±0.04
	Floor	1.7×10^3 - 1.8×10^3	3.24±0.02
	Table	1.4×10^3 - 2.4×10^3	3.26±0.17
	Water source	1.0×10^3 -1.3×10^3	3.06±0.08

Table 4. The mean of total aerobic bacteria count (TABC)

Contact surface sampled	Number of samples	TABC Log_{10}cfu/cm^2 of cfu/ml
Knife	10	3.11±0.07
Cutting board	10	3.30±0.07
Floor	10	3.37±0.09
Table	10	3.19±0.25
Water source	10	3.20±0.17

Total coliform count

Adeyemi and Adeyemo (2007) have asserted that the presence of faecal coliforms is attributed to poor sanitary hygiene standards and lack of efficient waste management control systems in rivers and streams, which is common in developing countries, including Nigeria.

Table 5. shows the total coliform count for the sampled surfaces and water. Generally, the counts ranged from 2.11±1.51 to 4.70±2.20 log_{10}cfu/cm^2 and 2.54±2.07 to 4.29±1.72 log_{10}cfu/ml for contact surfaces and water, respectively. The result from the count showed that the table was more contaminated in general while, the water source was least contaminated. The results of the average count of coliform for knives, cutting board, floor and table were: 3.49±1.97, 3.43±0.99, 3.56±1.24 and 3.33±0.96 log_{10}cfu/cm^2, respectively while the water had average count of 3.41±1.07log_{10}cfu/ml (Table 6). The mean count for abattoir floor (3.56±1.24log_{10}cfu.cm^2) in this study is comparable to the mean counts of 5.58 log_{10}cfu/cm^2 reported by Uzoigwe et al. (2021). The means for knives and tables of 3.84 and 6.43log_{10}cfu/cm^2 reported by Uzoigwe et al. (2021) in an aassessment of bacterial contamination of beef in slaughterhouses in Owerri zone, Imo State, Nigeria did not agree with the findings of this study. The mean count of 2.49±1.11og$_{10}$cfu/cm^2 reported by Çetin et al. (2006) in food contact surfaces at red meat processing plants in Istanbul, Turkey is within the range of 2.11±1.51 to 4.70±2.20log_{10}cfu/cm^2 obtained in this present study.

Table 5. Total coliform count (TCC)

Location	Contact surface	TCC (cfu/cm^2 or cfu/ml)	TCC log_{10} cfu/cm^2 or cfu/ml (Mean ± SD)
Choba	Knife	6.2×10^1 - 1.2×10^4	2.94 ±1.62
	Cutting board	1.1×10^1 -1.5×10^3	2.11 ±1.51
	Floor	3.4×10^2 - 1.8×10^4	3.39 ±1.22
	Table	2.9×10^1 - 1.4×10^3	2.30 ±1.19
	Water source	1.2×10^2 - 1.3×10^4	3.10 ±1.44
Rumuosi	Knife	1.0×10^1 -1.1×10^5	3.02 ±2.86
	Cutting board	1.0×10^2 - 1.7×10^5	3.62 ±2.28
	Floor	1.2×10^1 - 1.5×10^3	2.13 ±1.48
	Table	1.1×10^3 - 1.0×10^4	3.52 ±0.68
	Water source	1.2×10^1 - 1.0×10^4	2.54 ±2.07
Alakahia	Knife	4.1×10^1 - 1.6×10^5	3.12 ±0.12
	Cutting board	1.2×10^1 - 1.7×10^6	3.45 ±0.08
	Floor	1.1×10^4 -1.4×10^4	3.48 ±0.02
	Table	1.3×10^3 - 1.4×10^4	3.37 ±0.09
	Water source	1.8×10^2 - 1.2×10^4	3.11 ±0.10
Ozuoba	Knife	2.1×10^4 - 3.0×10^4	4.48 ±4.40
	Cutting board	1.3×10^3 - 2.8×10^4	3.78 ±0.94
	Floor	1.6×10^3 - 1.1×10^5	4.12 ±1.30
	Table	1.5×10^3 - 2.4×10^3	3.38 ±3.28
	Water source	1.2×10^3 - 3.2×10^5	4.29 ±1.72
Rumuokoro	Knife	1.9×10^3 - 2.9×10^4	3.87 ±0.84
	Cutting board	1.2×10^4 - 2.0×10^4	4.19 ±0.16
	Floor	1.4×10^3 - 1.8×10^6	4.70 ±2.20
	Table	1.0×10^4 - 1.3×10^4	4.06 ±0.08
	Water source	1.0×10^4 - 1.5×10^4	4.00 ±0.00

The mean value of 4.16±0.32 and 2.14±0.09log10cfu/cm2 for floor and knives and 3.88±0.28log10cfu/ml for water samples reported by Pradhan *et al.,* (2018) from retail Chevon meat shops and abattoirs of Kolkata, West Bengal, India did not agree with the value obtained in this study.
Total coliform counts for the water samples exceeded the maximum limits of 1.0 × 10^2 cfu/mL recommended by EPA (2002) in drinking water. This is indicative of faecal contamination of the processing water sources. All water samples were found to have coliform counts, which exceeded surface water limits of 200/100 mL.

Table 6. The mean of total coliform count (TCC)

Contact surface sampled	Number of samples	TAC $Log_{10}cfu/cm^2$ of cfu/ml
Knife	10	3.49±1.97
Cutting board	10	3.43±0.99
Floor	10	3.56±1.24
Table	10	3.33±0.96
Water source	10	3.41±1.07

Antibiotic resistance
Bacterial resistance to antibiotics is identified globally to be in high rate and could result in a trend toward emergence of multidrug resistance; posing an emerging challenge to public, animal and environmental health, calling for its strict judicious and stewardship use in food animals (Akpan *et al.,* 2020). Antibiotic resistance in *E. coli* is of particular concern because it is the most common Gram-negative pathogen in humans, multidrug-resistant strains and is easily transferable to other strains (Rasheed et al. 2014). The results of the susceptibility pattern of the confirmed *E. coli* is presented in Table 7. The isolates showed varying resistance against the selected antibiotics, with the highest being tetracycline and augmentin (100%), followed by cefuroxime (78.57%) while the least was cefixime and nitrofurantoin (0%). Others are: Gentamycin (64.29%), ofloxacin and ceftriaxone (42.86%) and ciprofloxacin (28.51%).

Table 7: Percentage antibiotics susceptibility pattern of *E. coli*

Susceptibility/antibiotic	No of *E. coli*	Number of *E. coli* with profile (N) in percentage (%)								
		CEF	CRX	GEN	CXM	OFX	AUG	NIT	CPX	TET
Resistant	28	12 (42.86)	22 (78.57)	18 (64.29)	0 (0)	12 (42.86)	28 (100)	0 (0)	8 (28.57)	28 (100)
Intermediate	28	2 (7.14)	4 (14.29)	0 (0)	0 (0)	2 (7.14)	0 (0)	0 (0)	4 (14.29)	0 (0)
sensitive	28	14 (50.0)	2 (7.14)	10 (35.71)	28 (100)	14 (50.00)	0 (0)	28 (100)	16 (57.14)	0 (0)

CEF. =Ceftriaxone; CRX. = Cefuroxime; GEN. = Gentamycin; CXM. = Cefixime; OFX. = Ofloxacin; AUG. = Augmentin; NIT. =Nitrofurantoin; CPX. = Ciprofloxacin and TET. = Tetracycline

The reports of susceptibility of *E. coli* from abattoirs contact surfaces and water sources from different locations and countries against some of the antibiotics employed in this study revealed varying resistant percentages. The 64.29% resistance against gentamycin in this study however, agrees with 64.2% reported by Edward *et al.,* (2020) from one of three locations while not agreeing with the 54.5 and 60.4% from the other two locations. A number of authors have reported values of 0, 22.2, and 100% resistance of *E. coli*

against gentamycin (Atnafie *et al.,* 2017; Izevbuwa and Okhuebor, 2020; Shiaka *et al.,* 2020; Tadese *et al.,* 2021). The resistance against augmentin (amoxicillin-clavulanate) was 100% in this study and a reflection of high values of 55.5, 76, 80.0, 87.5% previously reported in similar studies (Atnafie *et al.,* 2017; Edward *et al.,* 2020; Shaika *et al.,* 2020; Ajuwon *et al.,* 2021; Tadese *et al.,* 2021). The 28.57% resistance against ciprofloxacin in this study is comparable to the 25.00% reported by Ajuwon *et al.,* (2021) whereas other authors reported 0 and 66.7% resistance against ciprofloxacin (Shiaka *et al.,* 2020; Izevbuwa and Okhuebor, 2020; Tadese *et al.,* 2021). The 100% resistance of E. coil against tetracycline in this study is not comparable to the 27.7 and 73.7% reported by Tadese *et al.,* (2021) and Atnafie *et al.,* (2017), respectively. The resistance of 42.86% of the study against ofloxacin was less than the 87.1, 92.6 and 95.5% reported by Edward et al. (2020) from three locations in Abia State, Nigeria. In this study, 0, 42.86 and 78.57% resistance were obtained against nitrofurantoin, ceftriaxone and cefuroxime, whereas 50.00, 100.00 and 94.4%, respectively were reported by Ajuwon et al. (2021), Atnafie *et al.,* (2017) and Tadese *et al.,* (2021).

Table 8. Multidrug resistant pattern of *E. coli* isolated from abattoir contact surfaces and water

Antibiotics	No. of isolates with resistance profile (N) in %	Resistance category
AUG-GEN-TET	18 (64.29)	Multi-drug resistant
AUG-GEN-TET-CRX	14 (50.00)	Multi-drug resistant
AUG-GEN-TET-CRX-OFX	4 (14.29)	Multi-drug resistant
AUG-GEN-TET-CRX-OFX-CEF	2 (7.14)	Multi-drug resistant

CEF. =Ceftriaxone; CRX. = Cefuroxime; GEN. = Gentamycin; CXM. = Cefixime; OFX. = Ofloxacin; AUG. = Augmentin; NIT. =Nitrofurantoin; CPX. = Ciprofloxacin and TET. = Tetracycline

The present result also shows the potential dissemination of multidrug-resistant *E. coli* strains in the abattoir contact surfaces and water samples examined. The *E. coli* were multi-drug resistant, i.e., resistant to three or more antibiotics employed in this study (Table 8). Shiaka *et al*. (2020) have reported a 92% (46 of 50) of *E. coil* isolates from selected abattoirs in North-western Nigeria found to be resistant to minimum of three classes of antibiotics, among them is augmentin and tetracycline also employed in this study. Similarly, Edward et al. (2020) reported a 99.8% multiple antibiotic resistant (MAR) of *E. coli* from abattoir wastewaters in Abia State, Nigeria in a study employing cefuroxime, gentamycin, ciprofloxacin, ofloxacin, augmentin and nitrofurantoin also employed in this study. The MAR index range of 0.3 to 0.8 agrees with the 0.2 to 1.0 previously reported (Falodun and Ajala, 2018; Edward *et al.,* 2020; Shiaka *et al.,* 2020). Consistent with the findings of this study, Shiaka *et al*. (2020) and Falodun and Ajala (2018) reported that majority of the *E. coil* were resistant to a combination of 3 (64.29%) and 4 (50.00%) antibiotics while few were resistant to 5 (14.29%) and 6 (7.14%) combinations.
The development of drug resistance in *E. coli* in this this study can be linked to indiscriminate use of antibiotics for treatment, growth promotion in humans and food producing animals, poor abattoir hygiene, selective pressure to extensive use of antibiotics in the animal industry and indiscriminate dumping of antibiotics in the environment (Edward *et al.,* 2020; Shiaka *et al.,* 2020).

Presence of virulence genes

The result of the present study has further confirmed that ruminants are an important source of serologically and genetically diverse intimin-harboring *E. coli* strains which have been described in association with human diseases (Blanco *et al.,* 2005). An assessment of the virulence potential associated with the confirmed *E. coli* isolates showed that the *ast* gene was detected in 3 isolates; *aggR* was detected in 6 isolates while *eae* gene was detected in 6 isolates (Figures 1, 2 and 3). The presence of these tested virulence genes differentiates commensal and pathogenic strains of the isolated *E. coli*. Detection of *eae* possibly

indicates the presence of an EPEC or EHEC strain since the afimbrial adhesin intimin, *eae* encodes for is usually typical of these pathotypes while ast and aggR indicates the presence of EAEC which regulates the aggregative adherence fimbriae (AAFs) (Nataro *et al.,* 1992; Meng *et al.,* 1998; Bernier *et al.,* 2002; Boison *et al.,* 2012). It is pertinent to state that there is paucity of information on the detection of these virulence genes in E. coli isolated from abattoir contact surfaces and processing water.

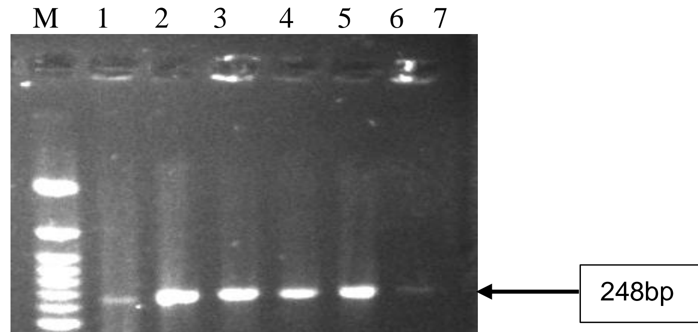

Figure 1: Gel picture of *eae* virulence gene on *E. coli* samples. M is 100bp DNA ladder and wells 1, 2, 3, 4, 5 are positive samples while 6 and 7 are negative isolates

The intimin, a product of the *eaeA* gene is involved in the attaching and effacing adherence phenotype (Wang et al. (2002). The 30% of positive samples for STEC high virulence gene (*eae*) in this study is comparable to 40% reported by Blanco et al (2005) in faecal samples collected from cattle at slaughter in Switzerland. Oloyede et al. (2016) have reported the none detection of the intimin (eaeA) gene in *Escherichia coli* O157:H7 Isolated from raw beef sold in Abeokuta, South-West Nigeria.

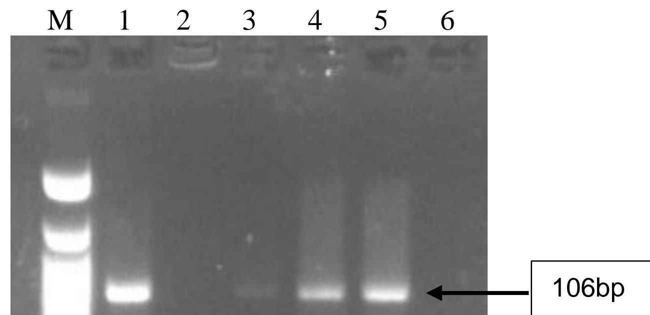

Figure 2: Gel picture of *ast* virulence gene on *E. coli* samples. M is 100bp DNA ladder and wells 1, 4 and 5 are positive samples while 2, 3 and 6 are negative isolates

In this study 3 (30%) of the isolates showed the presence of ast virulence gene. Bisi-Johnso et al. (2011) have reported the predominance in diarrheagenic *E. coli* of EAEC heat-stable enterotoxin *astA* genes in clinical isolates of *E. coli* from a tertiary hospital in the Eastern Cape, South Africa. Silva et al. (2014) concluded that the presence of an intact *astA* gene may represent an additional virulence determinant in both EPEC groups in a study on the Detection and genetic analysis of the enteroaggregative *E. coli* heat-stable enterotoxin (EAST1) gene in clinical isolates of enteropathogenic Escherichia coli (EPEC) strains.

Aggregative adherence regulator (aggR), a transcriptional regulator of enteroaggregative *Escherichia coli* (EAEC) which has been proposed as the defining factor for typical EAEC strains was detected in 6 (30%) isolates. It is shown to regulate most of the EAEC virulence genes, including aggregative adherence fimbriae (AAFs) with four known variants, namely: aggA (AAF/I), aafA (AAF/II), agg3A(AAF/III), and agg4A (AAF/IV) (Nataro *et al.,* 1992; Bernier *et al.,* 2002; Boison *et al.,* 2012). The aggR also controls anti-aggregation protein gene (aap) and activated island (aaiC) (Boison *et al.,* 2012).

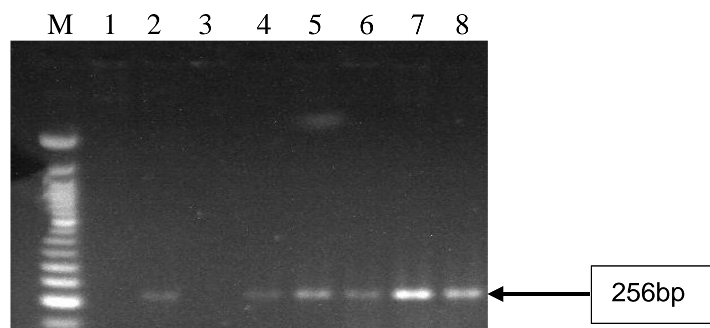

Figure 3: Gel picture of *aggR* virulence gene on *E. coli* samples. M is 100bp DNA ladder and wells 2, 4, 5, 6, 7 and 8 are positive samples while 1 and 3 are negative isolates

CONCLUSION

The study therefore, revealed high heterotrophic and coliform counts in both the water and contact surfaces examined, though lower in most cases than values reported in previous reports. The presence of antibiotic resistant *E. coli* carrying eae, ast and aggR genes that are target sites for EPEC, EHEC and EAEC are a serious risk to public health. There is therefore, the need for a strict surveillance of Escherichia coli in abattoir contact surfaces and water since shiga toxin-producing *Escherichia coli* can survive in foods meat for long periods with the possibility of transmitting infections to the consumer.

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