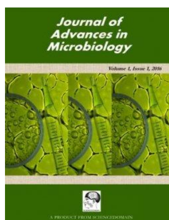

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Incidence of Enterotoxigenic *Escherichia coli* in Slaughter Houses in Sagamu, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author AMDA designed the study and wrote the protocol. Author NOS carried out the practical work. Author JAO managed the analyses of the study. Authors SOM and PAA managed the literature searches. Authors OE and EOO wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Enterotoxigenic *Escherichia coli* (ETEC) is one of the strains of *E. coli* responsible for *E. coli*-associated diarrhea outbreaks world-wide due to the consumption of contaminated foods. Cattle and their environment have been incriminated as the most important sources of pathogenic *E. coli*. The aim of this study was, therefore, to isolate and identify ETEC in abattoirs in Sagamu.

A total of 108 swab samples were collected from different anatomical sites and faeces of selected cattle and floor of slaughter houses in Sagamu, Nigeria. The faeces were collected into a universal bottle with scoop, the tip of sterile swab stick was moistened with sterile water and was used to collect samples from the body coats (Rump and Brisket) before slaughtering, skin (Brisket and Rump) after evisceration and slaughter house floors before and after use. All the samples were homogenized into sterile peptone water and incubated at 37°C for 18-24hrs. Each sample was cultured into MacConkey and Eosin Methylene Blue agar for bacteria isolates. Colonies with typical green metallic sheen after sub culturing into EMB were further identified using BD BBL identification

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system. All the positive isolates were a screen for enterotoxigenic *Escherichia coli* genes (LT and ST) by polymerase chain reaction.
A total of 50 (46.3%) *Escherichia coli* were recovered from the different samples. The percentage of occurrence of *E. coli* in faeces 7 (70%) at Kara abattoir was slightly higher than that of Agbele abattoir 6 (60%) but the difference was not statistically significant ($P > 0.05$). *E. coli* was observed to be higher in brisket area of the body coat 5 (50%) at Agbele than the rump area of the body coat 4 (40%) but *E. coli* in the rump area of body coat 5 (50%) was higher than the region of the brisket of body coat 3 (30%) at Kara. The rump area of the skin had the least isolation rate when compared with the brisket of the skin at the two abattoirs. Furthermore, the molecular identification of enterotoxigenic *Escherichia coli* virulence genes showed that none of the 50 *E. coli* isolated was positive for heat labile and heat stable genes.

Keywords: Abattoir; heat labile gene; heat stable gene; E. coli.

1. INTRODUCTION

Escherichia coli occur as normal flora in the gastrointestinal tract of humans and animals. However, pathogenic *E. coli* strains have been reported to cause life-threatening infections in humans worldwide [1,2]. Among the intestinal pathogens there are six well-described pathotypes: enterohaemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAggEC), enteroinvasive *E. coli* (EIEC) and diffusely adherent *E. coli* (DAEC) [3,4].

Animals carry harmless Escherichia coli in the intestines as part of the normal gut flora. Sometimes, they are carriers of pathogenic E. coli strains that can cause gastrointestinal illness in humans. The importance of these diarrheagenic *E. coli* (DEC) in causing foodborne diseases has been understood in recent years in Africa [5], but very little is known about the reservoirs and routes of the infection on the continent. In general, meat products are considered to be an important source of DEC infections. The meat can be contaminated due to the poor hygiene practices during slaughter. Therefore, adherence to good hygienic practices in slaughter and meat production is essential for prevention of microbial carcass contamination and for ensuring the meat quality and health protection [6]. Healthy asymptomatic animals may carry zoonotic pathogens and represent a reservoir for DEC, which may enter the food chain via the weak points in hygiene practices of the slaughter process [7,8,9].

The enterotoxigenic *E. coli* (ETEC) are pathogenic to man and animals such as pig and cattle and may produce types I and II heat-stable (ST) and types I and II heat-labile (LT) toxins. ETEC strains are a major cause of infantile diarrhea in developing countries, are frequently associated with traveler's diarrhea and diarrhea in the very young animals as piglets, lambs, and calves [10]. ETECs are nowadays held responsible for the vast majority of *E. coli*-associated diarrhea outbreaks world-wide [11], which are mostly due to the consumption of contaminated foods and water [12].

Food-borne pathogens have been extensively incriminated worldwide as common causes of bacterial infections in humans with food animals serving as important reservoirs [13,14,15]. Cattle and their environment are among the most important sources of pathogenic *E. coli*, and they may be the origin of contamination of meat and meat products [16].

There has been considerable research on pathogenic *E. coli* in slaughter houses, most studies have focused on VTEC O157: H7 and few studies concerning ETEC have focused on weaned or newborn calves and children. In Nigeria, there is a paucity of information about enterotoxigenic *E. coli* strains in slaughtered cattle in Ogun State and Nigeria. Hence, this study will aim to investigate the incidence of ETEC strains in cattle slaughtered in abattoirs located in Sagamu, Ogun State.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Faeces sample was collected from the rectum of the cattle using a sterile universal bottle with a scoop. The tip of sterile swab stick was moistened with sterile water and was used to collect samples from the body coats (Rump and Brisket) before slaughtering, skin (Brisket and Rump) after evisceration and slaughter house floor before and after use. The

swab samples were put into sterile peptone water. All samples were transported on an ice pack to the laboratory and were processed within 2 hours.

2.2 Isolation and Identification

All peptone water containing the swabs and feces were incubated at 37°C for 24 hours and were inoculated onto MacConkey agar (MAC, Oxoid), Eosin Methylene Blue agar plates and incubated at 37°C for 24 h. Colonies with a green metallic sheen on EMB were inoculated on nutrient agar slants and incubated at 37°C for 24hours and stored for further biochemical identification using BD BBL Crystal identification Systems.

2.3 Gram Staining Technique

A smear of the suspected colony from the culture plate was made on clean, grease-free glass slide. The smear was heat-fixed on the slide by passing the slide over Bunsen burner flame briefly. The slide was then covered with Crystal violet stain and allowed to stain for one minute. The stain was decanted, rinsed with tap water and stained with Lugol's iodine for one minute. The stain was decanted and the film (smear) decolorized with acetone for few seconds. The slide was quickly washed with distilled water and counter stained with Safranin for one minute. The slide was finally washed with water, dried and examined under the microscope using the oil-immersion objective lens. Suspected *Escherichia coli* isolates were Gram-negative rods.

2.4 Molecular Analysis

For optimal performance, beta-mercaptoethanol (user supplied) was added to the Genomic Lysis Buffer to a final dilution of 0.5% (v/v) i.e., 500 µl per 100 ml. The bacterial cells were suspended in 200 µl of isotonic buffer (PBS) and 50-100 mg (wet weight) bacterial cells were taken and transferred to a ZR BashingBead Lysis Tube (0.1 mm and 0.5 mm) and 750 µl Lysis Solution was added to the tube.

The mixture was vortexed at maximum speed for 5 minutes in a bead beater fitted with a 2 ml tube holder. After vortexing, it was centrifuge at 10,000 x g for 1minute. Up to 400 µl supernatant was transferred to a Zymo-Spin IV Spin Filter (Orange Top) in a Collection Tube and centrifuge at 7,000 x g for 1minute. 1,200µl of Genomic Lysis Buffer was added to the filtrate in the collection tube. 800 µl of the mixture was transferred to a Zymo-Spin IIC Column in a collection tube and was centrifuge at 10,000 x g for 1 minute. After centrifugation, the content in the collection tube was poured away. 200 µl DNA Pre-Wash Buffer was added to the Zymo-Spin IIC Column in a new Collection Tube and centrifuge at 10,000 x g for 1minute. 500 µl g-DNA Wash Buffer was added to the Zymo-Spin IIC Column and centrifuge at 10,000 x g for 1minute. Zymo-Spin IIC Column was transferred to a clean 1.5 ml microcentrifuge tube and 100 µl DNA Elution Buffer was added directly to the column matrix and was centrifuge at 10,000 x g for 30 seconds to elute the DNA.

2.4.1 PCR for detection of LT and ST toxins of Enterotoxigenic *Escherichia coli*

After the extraction of the DNA, master mix preparation was carried out in a biosafety cabinet in the following composition; PCR Master Mix Reagent (Biolab) in a total volume of 25µl comprising of 0.4 µm each of the primer, 1xPCR Buffer, and 5 µl of extracted DNA, all were prepared according to manufacturer's instruction. Table 1 shows the PCR markers for the detection of virulence genes of *E. coli*.

The PCR cycling conditions were carried out as followed: 94°C for 30 seconds for Denaturation followed by 30 cycles amplification of 20 seconds at 95°C for denaturation and 30 seconds at 58°C for annealing with an extension of 1 minute at 68°C and a final extension of 5 minutes at 68°C.

2.4.2 Agarose gel electrophoresis

Agarose gel electrophoresis was carried out under UV light to view DNA bands shortly after amplification in the thermocycler.

Table 1. PCR markers for detection of virulence gene of *E. coli*

Target gene	Nucleotide sequence (5'→3')	Size of amplified product (bp)	References
St	F: ATTTTTCTTTCTGTATTGTCTT R: CACCCGGTACAAGCAGGATT	190	[17]
Lt	F: GGC GAC AGA TTA TAC CGT GC R: CGG TCT CTA TAT TCC CTG TT	450	[17]

2.4.3 Preparation

Exactly 1.5 g of agarose powder was weighed into a 250 ml Erlenmeyer flask and 100 ml of TAE buffer was added. The mixtures were boiled for 5 minutes with the aid of microwave oven and allowed to cool to a temperature of about 45-50°C. The casting tray with the appropriate combs was assembled. 1.5 µl of Sybr safe was added to Agarose-TAE. The gel was poured and allowed to polymerize for 15 minutes thereafter the combs were removed. Shortly after solidification, the casting block was placed in an electrophoresis tank (filled with TAE Buffer), 1µl of loading buffer and 5 µl of the PCR amplicon were loaded into each well of the gel. 2.5 µl DNA ladder was loaded. The gel was run in an electrophoresis stand at 120 volts for 30 minutes. After 3-4 runs, it was removed and viewed under the imager (UV/Blue light)/trans-illuminator with the picture of the result taken.

2.5 Statistical Analysis

The analysis was done using SPSS version 23, a computer-based statistical software package. The incidence of enterotoxigenic *E. coli* was assessed at different anatomical sites on cattle and feces (feces, body coat, and meat) using Chi-square test.

3. RESULTS

The Table 2a and 2b show the result of 30 different substrates used for biochemical identification of the *E. coli*. *E. coli* utilized over eight different substrates and showed the negative result to the rest using BD BBL Identification System. The positive test result includes Arabinose, Mannose, Rhamnose while the negative test results include Sucrose, Adonitol, Inositol, etc.

The prevalence of *E. coli* in cattle abattoir in Sagamu is 46.3%. The percentages of *E. coli* obtained in this study at Kara cattle abattoir was higher in faeces (70%), hair from the rump (50%) and skin from the rump (50%) when compared with Agbele cattle abattoir. On the other hand, the percentage of *E. coli* at Agbele was higher in hair from the brisket (50%), skin from the brisket (70%) when compared with Kara cattle abattoir. Surprisingly, there was no *E. coli* isolated from slaughter floor samples at both cattle abattoir (Table 3).

Escherichia coli at each anatomical site and faeces at Kara and Agbele were compared with no significant association using Chi-square test at P > 0.05 (Table 4).

Table 2a. Biochemical pattern of *E. coli* using BD BBL identification system

Substrate	Results
Arabinose (ARA)	+
Mannose (MNS)	+
Sucrose (SUC)	_
Melibiose (MEL)	+
Rhamnose (RHA)	+
Sorbitol (SOR)	+/_
Mannitol (MNT)	+
Adonitol (ADO)	_
Galactose (GAL)	+
Inositol (INO)	_
p-n-p-phosphate (PHO)	_
p-n-p-α-β-glucoside (BGL)	_
p-n-p-β-galactoside (NPG)	+
Proline p-nitroanilide (PRO)	_
p-n-p bis phosphate (BPH)	_
p-n-p xyloside (BXY)	_

Table 2b. Biochemical pattern of *E. coli* using BD BBL identification system

Substrate	Results
p-n-p-α-arabinoside (AAR)	_
p-n-p-phosphorylcholine (PHC)	_
p-n-p-β-glucuronide (GLR)	+
p-n-p-N-acetyl glucosaminide (NAG)	_
γ-L-glutamyl p-nitroanilide (GGL)	_
Esculin (ESC)	_
p-nitro-DL-phenylalanine (PHE)	_
Urea (URE)	_
Glycine (GLY)	_
Citrate (CIT)	_
Malonate (MLO)	_
Tetrazolium (TTC)	+
Arginine (ARG)	_
Lysine (LYS)	+
Indole (IND)	+
Oxidase (OXI)	_

The result of molecular identification of ETEC virulence genes is presented in Table 5. Out of 50 *E. coli* isolated, none was positive for heat labile and heat stable toxin.

4. DISCUSSION

The present study was conducted to establish the occurrence of enterotoxigenic *E. coli* in samples collected from slaughter houses in

Table 3. Occurrence of *Escherichia coli* at Kara and Agbele cattle abattoir

Sample sites	Samples collected at each abattoir (n)	% of *E. coli* in Kara	% of *E. coli* in Agbele	Total N
Faeces	10	7 (70)	6 (60)	13 (65)
Hair from Brisket	10	3 (30)	5 (50)	8 (40)
Hair from Rump	10	5 (50)	4 (40)	9 (45)
Skin from Brisket	10	6 (60)	7 (70)	13 (65)
Skin from Rump	10	5 (50)	2 (20)	7 (35)
Slaughter floor	4	0 (0)	0 (0)	0 (0)
Total	54	26 (48.1)	24 (44.4)	50 (46.3)

Table 4. Relationship between site of isolation of *E. coli* and Abattoir

Sites		Frequency (%) by location		Chi –square	P-value
		Kara	Agbele		
HB	Positive	3 (30)	5 (50)	0.83	0.65
	Negative	7 (70)	5 (50)		
HR	Positive	5 (50)	4 (40)	0.20	1.00
	Negative	5 (50)	6 (60)		
SB	Positive	6 (60)	7 (70)	0.22	1.00
	Negative	4 (40)	3 (30)		
SR	Positive	5 (50)	2 (20)	1.98	0.35
	Negative	5 (50)	8 (80)		
Faeces	Positive	7 (70)	6 (60)	0.22	1.00
	Negative	3 (30)	4 (40)		

Key: H. B (Hair from the Brisket); H. R (Hair from the Rump); S. B (Skin from the Brisket); S. R (Skin from the Rump); P > 0.05

Table 5. Prevalence of enterotoxigenic *E. coli* (ETEC) in Sagamu slaughter houses

Sample sites	*E. coli*	ST	LT
Feces	13	0	0
Hair from Brisket	8	0	0
Hair from Rump	9	0	0
Skin from Brisket	13	0	0
Skin from Rump	7	0	0
Slaughter house floor	0	0	0
Total	**50**	**0**	**0**

Key: ST (Heat Stable toxin); LT (Heat-Labile toxin)

Sagamu. In this study, fifty *Escherichia coli* were isolated from one hundred and eight samples and the observed prevalence of 46.3% agree with the work of Anuradha et al. [18], Adesiji et al. [19] that reported the prevalence rate of 48%. However, this work disagrees with the findings of Nicoline et al. [20], that reported a high prevalence rate of 67.5% from cattle and pig slaughtered at Vhembe abattoir, South Africa. The high prevalence of *E. coli* could be due to external or internal contamination of meat.

The occurrence of *E. coli* in faeces from the two abattoirs is 7 (70%) from Kara and 6 (60%) from Agbele. There was no statistically significant difference between the numbers of *E. coli* isolated from the two abattoirs. However, the overall percentage of *E. coli* in faeces was 13 (65%) and this finding conforms to the work of Anuradha et al. [18] that reported the prevalence rate of 66% in faeces. *E. coli* is normal flora of the intestinal tract of man and animal, they are passed out in faeces.

The occurrence of *E. coli* in the brisket area of the body coat 5 (50%) is higher than the rump area of the body coat 4 (40%) at Agbele cattle abattoir and this is in line with the work of Reid et

al. [21] in which the brisket area was reported as the most contaminated region. On the other hand, the rump region 5 (50%) is contaminated than the brisket region 3 (30%) in Kara cattle abattoir and this study disagrees with the findings of Reid et al. [21]. The contamination of hides could have emanated from the feedlot, during transportation and lairage environment.

The rump area of the skin was the site with the least isolation rate at the two abattoirs with 2 (20%) at agbele and 5 (50%) at Kara when compared with the brisket of the skin and this also conforms to the work of Nicoline et al. [20]. This could be due to the fact that microorganism requires enough nutrients and oxygen to grow and proliferate, these are probably absent in the rump because they are mostly muscles [20]. Initial contamination of meat most likely arises during slaughtering [22].

Surprisingly, there was no *E. coli* isolated from the floor of the slaughter house. This finding disagrees with the works carried out by Anuradha et al. [18] that reported a prevalence rate of 16% and Gun et al. [23] that isolated *E. coli* O157:H7 in abattoir environment and slaughtering floor. These authors observed that generally, the environmental condition of the local slaughter houses was very poor. The absence of *E. coli* in this study reflects the level of cleanliness of the floor of the slaughter house.

Furthermore, the molecular identification of ETEC virulence genes showed that none of the *E. coli* was positive for heat labile and heat stable toxin. This study contradicts the report of Kagambe`ga et al. [24], that reported 4% prevalence rate of ETEC in faeces of cattle slaughtered at the abattoir, in Burkina Faso. Nicoline et al. [20] also reported 3.8% in South Africa. The non-isolation of ETEC in this study might probably be due to the fact that the cattle are healthy and the feces samples were collected directly from the rectum.

This study shows that food animals like cattle and pigs need ETEC vaccination to eliminate the incidence of ETEC infection. Recently, Nandre et al. [25] did substantial extraordinary research work in the ETEC vaccine development in pigs. Similar ETEC vaccine development is needed to prevent ETEC infection in food animals including cattle. Also, this would lead to reducing the incidence of ETEC infection in humans due to consumption of ETEC contaminated meat and meat products.

5. CONCLUSION

Although *E. coli* was isolated from the feces and body of the cattle, none were observed to carry LT and ST virulence genes. Also, none isolation of *E. coli* on the slaughter house floor is indicative of good hygienic practices which should be encouraged further. In addition, ETEC vaccination in food animals is required to eliminate the prevalence of ETEC infection.

6. RECOMMENDATIONS

Studies like this should be encouraged to create baseline data especially for Epidemiological surveillance and to monitor the occurrence of ETEC in the future. Hence, good hygienic practices should also be encouraged in all abattoirs in order to prevent the outbreak of ETEC. Also, ETEC vaccination in food animals at the farm is encouraged to prevent ETEC infection.

Enterotoxigenic *Escherichia coli* is one of the six pathogenic strains of *E. coli* causing diarrhea, so more researches is suggested to be carried out in the two abattoirs to screen for other pathogenic *E. coli* strains in meat.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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