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Escherichia coli O157:H7 Contamination of Cattle Carcass at Slaughter from Abattoirs in Lagos, Nigeria

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ABSTRACT: Meat obtained from cattle serve as a major source of protein in Nigeria, ensuring its safety will therefore be of great importance. This study was carried out to investigate the contamination of cattle carcass with *E. coli* O157:H7 and determine the antimicrobial susceptibility of the organism. Twenty meat samples from bowel, aitch bone, hide, rib and hunch back were collected from abattoirs in Lagos, Nigeria. MacConkey agar and Eosine methylene blue agar were used for isolation of *E. coli.* Isolates were sub-cultured on sorbitol-MacConkey agar and *E. coli* O157:H7 were differentiated from other strains due to its inability to ferment sorbitol. Identification was based on cultural, morphological and biochemical characteristics. Susceptibility of the isolates to amoxicillin, ceftazidime and cefuroxime was also investigated. All *E. coli* isolates were catalase and indole positive, oxidase, citrate and urease negative. The mean total coliform count varied from $4.45x10^7$ cfu/g (hunch back) to $9.20x10^7$ cfu/g (bowel). *E. coli* O157:H7 occurred in 37% of the meat samples while other *E. coli* strains occurred in 63%. They both occurred in meat samples from the different parts of the carcass investigated. Among the fourteen *E. coli* O157:H7 isolates 57.14% were resistant to ceftazidime, 42.86% to cefuroxime and 78.57% to amoxicillin. *E. coli*, although an enteric bacterium, was found to contaminate different parts of cattle carcass as a result of slaughtering. Of greater concern was the antibiotic resistance and isolation of the pathogenic strain, *E. coli* O157:H7.
Keywords: *Escherichia coli* O157:H7, cattle, carcass, antibiotic resistance

INTRODUCTION

Foodborne pathogens have been known all over the world as common causes of bacterial infections in humans, and food animals are the greatest reservoirs (Akoachere *et al*., 2009). WHO (2011) reported foodborne illnesses in up to 30% of the population in industrialized countries. Many of the morbidity and mortality due to foodborne infections are caused by bacteria (Ateba *et al*., 2008). Diarrheal diseases are the commonest manifestation of food poisoning which are usually as a result of toxin production by the pathogens or by the reaction of the host to the infection (CDC, 2011).

Pathogenic bacteria that have been found to be associated with food animals include amongst others *Salmonella* spp., *Campylobacter* spp., *Staphylococcus aureus, Listeria monocytogenes, Clostridium perfringens, Clostridium botulinum* and *Escherichia coli* O157:H7 (Nyenje *et al*., 2012). During slaughtering, there is every likelihood for pathogens carried by animals in their gut to contaminate other parts of the carcass, slaughter equipment and even the environments and hands of workers. Skinning and gutting are the major cause of contamination of carcass. Galland *et al.* (2001) and Ransom *et al*. (2002) reported that the bovine gut is the primary reservoir of enteropathogenic infection in humans consuming beef and beef products, and *Salmonella* can be isolated in large numbers from the rumen, caecum, colon and rectum. Li *et al* (2004) in their study, reported that out of 335 beef carcass samples they screened for prevalence and spread of *Listeria*, *E. coli*, and *Salmonella*, *E. coli* accounted for about 90% of the pre-dehiding point. There was also high faecal contamination of the beef carcass. *E. coli* in individual cattle were reported to range from 5 to 20% with enumeration rate in faeces at <100 to >104 cfu/g (Zhao *et al*., 2015). Mastitis in cattle has been associated with a number of microorganisms including *E. coli* and *S. aureus* (Khan *et al*., 2003). In the gastrointestinal tract of animals including humans *E. coli* is a normal flora with the exception of the pathogenic strains which cause infections (Ateba and Mbewe, 2011). EHEC has been associated with many outbreaks and O157:H7 has been an important food pathogen. Cattles which are primary reservoirs of O157:H7 and other non O157:H7 strains do not show any pathological signs (Madden *et al*, 2001).

Illness due to *E. coli* O157:H7 infection is usually life threatening, and individuals that are susceptible show different types of symptoms among which are hemolytic colitis, hemolytic-uremic syndrome and thrombocytic purpura (Meng *et al.,* 2001).

Antibiotic resistance is a critical problem in animal and human health. Foodborne pathogens have been found to be resistant to many antibiotics (Shekh *et al*., 2013). Food contaminated with antibiotic-resistant bacteria is of danger to public health, because the antibiotic resistance determinants can be transferred to other pathogenic bacteria (Van *et al*., 2007). The public health implication of the transfer of antibiotic resistant bacteria to humans through food is that it will lead to an increase in foodborne illness as well as treatment failure (Adesiji *et al*., 2011). From the farm, food of animal origin could be contaminated and if the food is not properly handled during slaughtering and processing it may lead to multiplication of pathogens (Ghosh *et al*., 2007). The conditions under which these foods are handled raise questions regarding their microbiological quality. Studies conducted in different countries to investigate the microbiological quality of food of animal origin reported the presence of potential human pathogens (Akoachere *et al*., 2009). In Nigeria, a large proportion of the population relies on beef as their source of protein which could expose them to infection if contaminated (Ateba and Mbewe, 2011).

The aim of the present study therefore, is to investigate the contamination of cattle carcass at slaughter in some abattoirs in Nigeria with *E. coli* O157:H7 and determine the antimicrobial susceptibility of the organism.

MATERIALS AND METHODS

Collection of samples

Samples of meat cuts or trimmings obtained with sterilized knives and forceps were collected in sterile ziploc polythene bags from 5 different parts of the slaughtered cattle carcass which included the aitch bone, bowel, hide, hunch back and rib with 4 samples each. A total of 20 samples were therefore collected from abattoirs in Lagos. The samples were transported in ice packs without delay to the Microbiology laboratory, University of Lagos for analysis.

Microbial analysis

Isolation and identification of *Escherichia coli*

Ten grams of meat sample was weighed, ground and homogenized in 90ml of sterile distilled water. The stock solution was diluted serially. Using a micropipette, 0.1 ml from 10^{-5} and 10^{6} dilutions were pipetted and each plated in duplicates on McConkey agar (Oxoid) using the spread plate method. Incubation was for 24-48 hrs. at 37°C. The McConkey agar plates were observed for growth and the pink colonies which represented lactose fermenters were enumerated as coliform count (Mhone *et al*., 2011). The average number of colonies were multiplied by the reciprocal of 0.1ml inoculum and the reciprocal of the dilution factor to obtain the colony forming unit/g. The pink colonies were sub-cultured on Eosine Methylene Blue (EMB) agar (Oxoid) plates and incubated for 24-48 hrs. at 37°C. Colonies that produced green metallic sheen which is characteristic of *E. coli* were sub cultured on sorbitol-McConkey agar (Oxoid) and incubated at 37°C for 24 hrs. in order to distinguish between *E. coli* O157:H7 which are non-sorbitol fermenters that appeared pale cream in colour and other non O157:H7 strains of *E. coli* which fermented sorbitol to produce pink colonies. Pure cultures of both were obtained by repeated sub culturing and stored on slants at 4°C.

Identification of isolates was based on cultural, morphological and biochemical characteristics among which were catalase, oxidase, citrate, urease and triple sugar ion (TSI) test according to Buchanan and Gibbons (1974).

***In vitro* Antibiotic susceptibility tests**

All the *E. coli* isolates obtained from cattle carcass were subjected to in-vitro antibiotics susceptibility test using disk diffusion method on Muller Hinton agar plates (Oxoid). Antibiotics tested include; amoxicillin (5µg), ceftazidime (30µg) and cefuroxime (1µg). The diameter of zones of inhibition was measured and compared with the reference data provided by the Clinical and Laboratory Standards Institute (CLSI, 2014) as reflected in the breakpoints in Table 1.

Table 1: CLSI Table for the Antibiotics Used

Bacterial Family	Antibiotic	Potency	Susceptibility	Intermediate	Resistance
Enterobacteriaceae	Amoxicillin	30µg	≥18	14-17	≤13
Enterobacteriaceae	Cefuroxime	30 µg	≥18	15-17	≤14
Enterobacteriaceae	Ceftazidime	30 µg	≥21	18-20	≤17

RESULTS

The total coliform counts obtained from MacConkey agar varied for the different meat samples. For bowel it was 7.90×10^7 to 9.60×10^7cfu/g, aitch bone (7.15×10^7 to 9.00×10^7cfu/g), hides (7.80×10^7 to 9.05×10^7cfu/g), hunch back (7.05×10^7 to 8.65×10^7cfu/g) and rib (7.90×10^7 to 8.35×10^7 cfu/g). The Bowel samples had the highest mean coliform count followed by aitch bone, hide, rib and hunch back (Figure 1).

E. coli O157:H7 and other *E. coli* strains were isolated from meat samples from the different parts of the cattle carcass. The frequency of occurrence of *E. coli* O157:H7 from the total samples was 37% while that of other *E. coli* strains was 63% (Figure 2).

The diameter of zone of inhibition of the growth of all *E. coli* isolates ranged from 6-26mm, 9-34mm and 10-17mm when amoxicillin, ceftazidime and cefuroxime were used respectively. However, among the 14 isolates of the pathogenic *E. coli* O157:H7, results of individual zones of inhibition (Table 2) translated according to CLSI (2014) (Table 1) are as follows: 11 (78.57%) were resistant to amoxicillin, 6 (42.86%) to cefuroxime and 8 (57.14%) to ceftazidime. Only 2 (14.29%) *E. coli* O157:H7 isolates were susceptible each to ceftazidime and amoxicillin. None was susceptible to cefuroxime. Also, 1 (7.14%) of them was intermediate to amoxicillin, 8 (57.14%) to cefuroxime, and 4 (28.57%) to ceftazidime (Table 3).

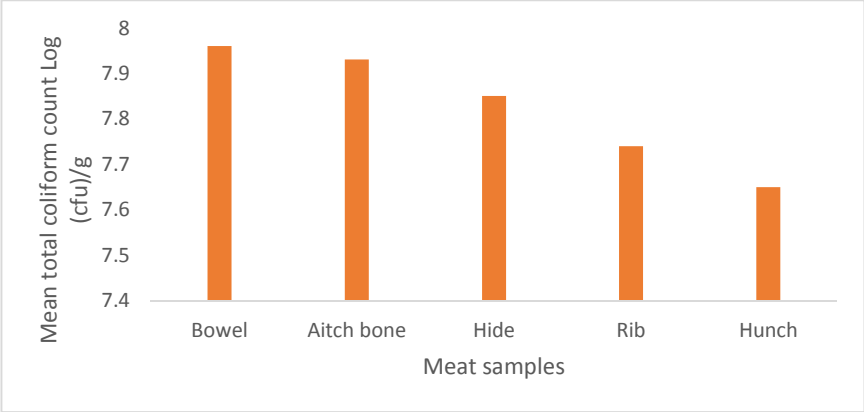

Figure 1: Mean total coliform count of meat samples from cattle carcass at slaughter in abattoirs in Nigeria

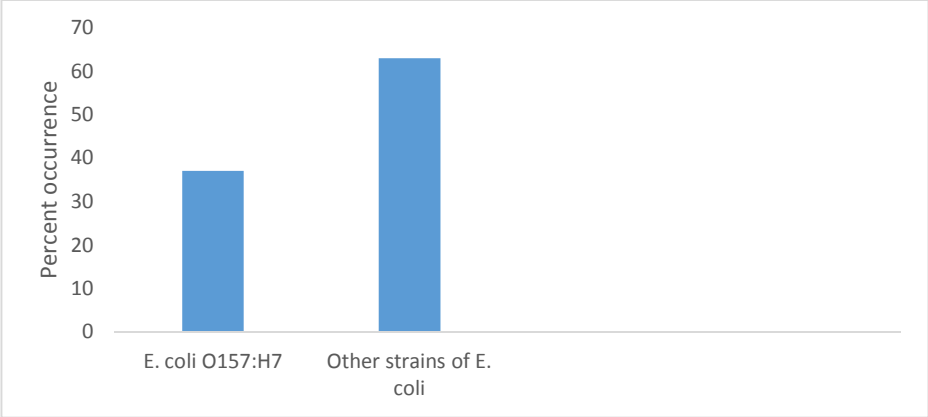

Figure 2: Incidence rates of *E. coli* O157:H7 and other *E. coli* strains in meat samples from cattle carcass at slaughter in abattoirs in Nigeria

Table 2: Antibiotic susceptibility pattern of *E. coli* O157:H7 and other *E. coli* strains isolated from cattle carcass.

Isolate code	Amx (5µ)	Caz (30µ)	Crx (1µ)
Hi (p)	R (9)	R (9)	R (9)
Ab(w)	R (9)	R (13)	R (9)
Bo(p)	R (9)	R (17)	R (11)
Ab(p)	R (9)	R (9)	R (9)
Hi(p)	I (14)	S (34)	I (15)
Ri(p)	R (6)	I (20)	I (15)
Bo(p)	R (9)	I (18)	R (12)
Ab(w)	R (9)	R (16)	I (15)
Ri(w)	R (13)	R (16)	I (15)
Bo(p)	R (11)	R (15)	R (13)
Bo(p)	S (18)	S (23)	R (14)
Ab(w)	R (12)	S (29)	R (12)
Hu(p)	I (15)	S (22)	R (14)
Ri(p)	R (9)	I (20)	I (15)
Hu(w)	R (13)	I (19)	R (14)
Ab(p)	R (9)	R (17)	R (13)
Ri(w)	I (14)	R (16)	I (15)
Ab(w)	R (9)	R (17)	I (16)
Hu(w)	R (12)	I (20)	I (16)
Hu(w)	S (24)	I (18)	R (13)
Hi(w)	S (26)	I (20)	I (17)

Table 2 cont'd

Isolate code	Amx (5µ)	Caz (30µ)	Crx (1µ)
Hi (p)	R (9)	R (9)	R (9)
Ab(w)	R (9)	R (13)	R (10)
Bo(p)	R (9)	R (17)	R (11)
Ab(p)	R (9)	R (9)	R (9)
Hi(p)	I (14)	S (34)	I (15)
Ri(p)	R (9)	I (20)	I (15)
Bo(p)	R (9)	I (18)	R (12)
Ab(w)	R (9)	R (16)	I (15)
Ri(w)	R (13)	R (16)	I 15)
Bo(p)	R (11)	R (15)	R (13)
Bo(p)	S (18)	S (23)	R (14)
Ab(w)	R (12)	S (29)	R (12)
Hu(p)	I (15)	S (22)	R (14)
Ri(p)	R (9)	I (20)	I (15)

Key: S: Susceptible, I: Intermediate, R: Resistant, Amx: Amoxicillin, Caz: Ceftazidime, Crx: Cefuroxime. Values in bracket= diameter of zones of growth inhibition, isolate code with (w): *E. coli* O157:H7, isolate code with (p): other *E. coli* strains

These measurements of zones of growth inhibition were interpreted according to Clinical and Laboratory Standards Institute (CLSI), 2014 (Table 1).

Table 3: Antibiotic susceptibility of *E. coli* O157:H7 isolated from cattle carcasses

Antibiotics (µg)	Number susceptible (%)	Number intermediate (%)	Number resistant (%)
Amx (5)	2 (14.29)	1 (7.14)	11 (78.57)
Caz (30)	2 (14.29)	4 (28.57)	8 (57.14)
Crz (1)	0	8 (57.14)	6 (42.86)

Key: Amx: Amoxicillin, Caz: Ceftazidime, Crz: Cefuroxime

DISCUSSION

There were variations in the coliform count obtained from the meat samples. The meat samples from the bowel had the highest coliform count followed by the aitch bone, the hide, the rib and the hunch back. This may be because the bowel and aitch bone are of close proximity to the intestinal materials. The occurrence of *E. coli* O157:H7 and other *E. coli* strains in our meat samples was 37% and 63% respectively. The 37% is lower than 53% prevalence of *E. coli* O157:H7 reported by Dahiru *et al*. (2008) in fresh beef meat in Kano City, Nigeria. *E. coli* is a known enteric organism and an indicator of faecal contamination. The presence of *E. coli* on the hide, rib and hunch of the cow could be an indication of contamination from the intestinal materials during de-hiding process, slaughtering floor and contact with workers' hands and tools. Compared to our findings, many other authors however reported lower prevalence of *E. coli* O157:H7 in food animal carcass and meat products. Zelalem *et al* (2019) in their research reported that the pooled prevalence of *E. coli* O157:H7 isolated from meat and meat products in Ethiopia was 5%. McEvoy *et al.* (2003) reported the prevalence of *E. coli* O157:H7 in carcass to be 3.2%. Mekuria *et al*. (2014) showed that 23.7% samples from food of bovine origin harboured *E. coli*.

Bekele *et al*. (2014) in their study reported an overall prevalence of *E. coli* O157:H7 in beef to be 13.3%. Hussein and Bollinger (2005) in their study of the prevalence of STEC O157:H7 in cattle reported the prevalence rate as 26.7% and in New York 1.3% was reported (Schroeder *et al*., 2002). Pooled prevalence of *E. col*i O157:H7 in foods of animal origin was found to be 4% by Assefa (2019). Cattle have been implicated as the principal reservoir of *E. coli* O157:H7 (Chapman *et al*., 2001). Variations in the prevalence of *E. coli* O157:H7 might be due to difference in location, sampling techniques, hygienic conditions of the handlers, slaughtering environment and the carcass.

Resistance to the antibiotics amoxicillin, ceftazidime, and cefuroxime observed in our study were similar to the resistance shown in a study conducted by Doughari *et al.* (2011) on virulence factors and antibiotic susceptibility among verotoxic non O157: H7 *Escherichia coli* isolates. The 14.29% susceptibility of our *E. coli* O157:H7 isolates to ceftazidime is in contradiction with the study of Tanih *et al*. (2015*)* who reported 72.3% susceptibility of their *E. coli* isolates to the antibiotic.

CONCLUSION

Meat samples from the hunch back, hide, bowel, rib and aitch bone were contaminated with both *E. coli* O157:H7 and other strains of *E. coli* and the organisms were multidrug resistant. Strict hygiene practices should therefore be maintained during slaughtering of cattle in abattoirs. Gut content should be prevented from other parts of the carcass.

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