



## Occurrence of *E.coli* O157 H7, from meat products sold in obinze abattoir, Rivers State, Nigeria

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### Abstract

This survey was carried out to investigate the occurrence of *Escherichia coli* O157:H7 in meat products from Obinze abattoir, Imo state, South-Eastern Nigeria. A total of 10 beef samples, 10 processing water samples, 10 table swabs and 20 entrails samples were collected. Pour plate technique was used after a tenfold serial dilution to inoculate on Eosin Methylene Blue (EMB) agar and the *E. coli* isolates were cultured on Sorbitol MacConkey agar and incubated for 24 hours at 37°C. The isolates were subjected to biochemical tests for identification before antibiotic sensitivity test was carried out using the disc diffusion (Kirby-Bauer) method. From the survey, the entrails had the highest rate of isolation ( $4.16 \pm 0.56 \log_{10}$  Cfu/ml) followed by the beef samples with ( $3.58 \pm 0.01 \log_{10}$  Cfu/ml). The processing water and the table swabs yielded no growth of *E. coli* O157:H7. The percentage occurrence of *E. coli* O157:H7 was also determined and the entrails samples had the highest with (17, 89.5%) followed by the beef samples with (2, 10.5%). The processing water and table swabs samples did not yield growth of *E. coli* O157:H7 and their percentages were 0. The data obtained were subjected to statistical analysis using the single sample T test which showed that there was no significant difference ( $p > 0.05$ ) in the rate of isolation of *E. coli* O157:H7 from the samples. The *E. coli* O157:H7 isolated showed 100% sensitivity to all the antibiotics used. The presence of *E. coli* O157:H7 implies that these food samples from the Obinze abattoir, if consumed could be a potential public health hazard to the community. Therefore, strict adherence to quality control measures should be implored in order to reduce contamination and food borne illnesses.

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### Keyword

*Escherichia coli* O157:H7, public health, hazard, contamination, occurrence

## Introduction

*Escherichia coli* are Gram-negative rod shaped, facultative anaerobic and non-sporulating bacterium of the family Enterobacteriaceae. The cells are about 2 micrometre long and 0.5 in diameters, with cell volume of 0.6-0.7, commonly found in lower part of the intestine of warm blooded animals (Uhtil *et al.*, 2001; Bavaro, 2012; Coia, 1998). Based on its virulence, the bacterial organism is classified into five groups, namely, enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), attaching and effacing *E. coli* (AEEC), and Shiga toxin-producing *E.coli* (STEC) (Bavaro, 2012; Paton and

Paton, 1998)). Optimal growth of *Escherichia coli* occurs at 37°C, but some laboratory strains can multiply at temperature of up to 45-49°C. Optimal pH requirement range is 5.5 and 7.5 (Palumbo *et al.*, 1995).

It is considered as one ubiquitous pathogens in humans and animals. It is present in the environment and resides in the gastrointestinal tract (GIT) of humans and animals. *Escherichia coli* have been associated with a number of diseases syndromes. Among these are often severe and sometimes fatal infections such as Peheophitis, Septicema, Meningitis, Endocarditis, Urinary tract infections, Epidemic diarrhoea of adults and children. Detection of *E. coli* in foods intended for human consumption shows poor in hygiene during production, processing or preparation. Ultimately, detection of *E. coli* in food is indicative of faecal contamination and presence of other dangerous pathogenic microorganisms which can compromise the health and wellbeing of consumers. In addition to hygienic indicator, some strains of *E. coli* are directly pathogenic to humans. One of such strains of *E. coli* is the *Escherichia coli* O157:H7 (Zhao *et al.*, 1995; Nataro and Kaper, 1998). *E. coli* O157:H7 is a particular serotype of the group referred to as enterohaemorrhagic *E. coli* (EHEC), a sub group of the serotype referred to as verocytotoxin producing *E. coli* (VTEC) or Shiga toxin-producing *E. coli* (STEC) owing to its ability to produce shiga-like toxins that are closely related to the toxin produced by *Shigella dysenteriae*.

Most EHEC isolates are acid tolerant, capable of surviving in acid foods and during passage through the stomach (Arnold and Kaspar, 1995) and it can cause severe enteric infections with symptoms such as abdominal pain, bloody diarrhea, hemorrhagic colitis, haemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). (Coia, 1998; Zhao *et al.*, 1995; Nataro and Kaper, 1998; Farougou *et al.*, 2012). Since it was first identified in 1982 as a cause of hemorrhagic colitis during outbreaks of bloody diarrhea in Oregon and Michigan, USA, following the consumption of undercooked ground beef, several outbreaks of hemorrhagic colitis and hemolytic uremic syndrome caused by this organism have been epidemiologically linked to consumption of undercooked beef (Riley *et al.*, 1983; Schlundt, 2001) and it has since been a steadily increasing cause of foodborne illness worldwide. Cattle appear to be the main reservoir of *E. coli* O157:H7.

Contamination of carcasses during slaughter is the primary route that ultimately leads to contamination of ground beef. Other foods (e.g., lettuce, sprouts, fruit juices, vegetables, raw milk) and water also have been implicated as vehicles of transmission. Person to person is an important mode of transmission, particularly in day care centres. Direct contact with animals carrying the organism is also a recognized source of infection (WHO, 1997). Mead *et al.*, (1999) has estimated the incidence of *E. coli* O157:H7 to 50% among EHEC serotype in relation to public health problems. This survey was carried out to investigate the occurrence of *Escherichia coli* O157:H7 in meat products from Obinze abattoir, Imo state, South-Eastern Nigeria.

## Materials and Methods

### Collection and Transport of Samples

A total of 50 samples which comprised of 20 entrails samples, 10 beef samples, 10 table swabs and 10 samples of processing water were collected from the Obinze abattoir. The beef and entrails samples were received in cellophane papers and kept in coolers containing ice packs. Sterile swab sticks were used to swab the slaughter tables and kept in their containers immediately. The processing water samples were collected in sterile

universal bottles to prevent allochthonous microbial contaminations. The samples were then transported immediately to the microbiology laboratory for the analysis without delay.

### Preparations for Dilutions

About 9ml of normal saline was dispensed into each of 10 series capped tubes using sterile 10ml pipette. The filled tubes were sterilized alongside with about 500ml normal saline in a glass conical flask at 121°C for 15 minutes in an autoclave.

### Preparation of beef samples for bacterial enumeration

A piece of beef or entrails from each sample was removed from the cellophane paper and mashed with a sterile crushable and pestle. 10g of the mashed beef was weighed and aseptically introduced into a sterile 250ml glass beaker and then topped up to 100ml using sterile normal saline which was properly mixed and labelled as  $10^{-1}$ .

### Preparation of processing water samples for bacterial enumeration

10ml of the processing water sample was transferred from the universal bottle into a sterile beaker using a sterile 10ml pipette and 90ml of sterile normal saline was added to the 10ml of sample to form a homogenous mixture of 100ml.

### Preparation of table swab samples for bacterial enumeration

Eosin methylene blue agar was prepared and sterilized based on the manufacturer's prescriptions. 18 to 20ml of the agar was poured into sterile petri dishes and allowed to solidify followed by the drying of the plates using the hot air oven. The labelled swab sticks were cultured using the streak plate technique. The plates were labelled and incubated at 37°C for 24 hours.

### Ten- fold serial dilution technique

1ml of the sample was transferred from the beaker labelled  $10^{-1}$  containing 10g of sample in 100ml of normal saline into the first test tube labelled  $10^{-2}$  containing 9ml of sterile normal saline using a sterile pipette. The tube was properly mixed by shaking the tube with the cap closed after which, 1ml was transferred to the next test tube labelled  $10^{-3}$ . This dilution continued until the last test tube labelled  $10^{-10}$  where after mixing, 1ml was drawn and discarded, similar to the description of FAO, (1979).

### Determination of *E. coli* Counts and Total Aerobic Plate Counts (TAPC)

After the ten-fold serial dilution, 1ml aliquots from  $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$  dilutions were aseptically transferred on petri dishes and about 18 to 20ml of eosin methylene blue agar (EMB) was poured on the petri dishes and swirled in several directions (clockwise, anticlockwise, up and down) for even distribution and then was allowed to solidify. For the total aerobic plate count (TAPC), nutrient agar was used which was prepared and sterilized according to the manufacturers prescription. The plate were inverted and their labels transferred to the other side of the plate containing the agar before the incubation at 37°C for 24 hours then the colonies were counted (Karch *et al.*, 1996).

### Purification and Preservation of Isolates

Using a sterile wire loop, discrete colonies which grew on eosin methylene blue (EMB) agar with a characteristic metallic green sheen were picked and sub cultured on nutrient agar by streaking followed by the incubation at 37°C for 24 hours to obtain pure

isolates. Single colonies were transferred from the sub culture plates into nutrient agar slants aseptically and incubated for 24 hours at 37°C. After incubation, the slants were preserved by wrapping them up within aluminium foil and kept in a refrigerator at 4°C.

### **Cultural Characteristics of Colonies**

Macroscopic characteristics of the microbial growths on the medium were observed and recorded based on their elevations, edges, colours, opacity, and shape. The isolates were microscopically investigated by Gram staining.

### **Biochemical Tests**

Some biochemical tests such as motility, indole production and sugar fermentation with gas production were done for the confirmation of *E. coli*.

### **Identification of *E. coli* O157:H7**

Some tests were carried out in the identification of *E. coli* O157:H7 which included the use of sorbitol MacConkey agar to detect sorbitol fermentation and the serological identification of *E. coli* O157:H7 with the use of the O157:H7 latex kit to check for agglutination.

### **Identification of *E. coli* O157:H7 using sorbitol MacConkey agar (SMAC)**

The sorbitol MacConkey agar was prepared and sterilized based on the manufacturer's prescription and 18 to 20ml was poured into sterile petri dishes which were allowed to solidify and dried using the hot air oven. Single colonies of *E. coli* were picked from the nutrient agar sub culture plate and was cultured on the SMAC using the streak plate technique and incubated at 37°C for 24 hours. *E. coli* O157:H7 does not ferment sorbitol and remained colourless on the sorbitol MacConkey agar (wells *et al.*, 2005).

### **Serological identification of *E. coli* O157:H7 using the O157:H7 latex kit (OXOID England, DR0620)**

*E. coli* O157:H7 was confirmed by using Latex agglutination test Kit (anti- o157:H7 antibody for *E. coli* O157:H7) test kit DR0620M (Oxoid LTD Hampshire, England) as described by Nataro and Kaper (1998).

### **Antibiotic Sensitivity Pattern of *E. coli* O157:H7**

The antibiotic susceptibility test was performed to determine the levels of sensitivity and resistivity of some Gram negative drugs on *E. coli* O157H7 using the Mac Farland standard.

### **Statistical Analysis**

The data obtained from the investigations were edited, coded and subjected to statistical investigation. For the bacteriological analysis, the mean occurrences for the various samples were determined and in the analysis of the variance, single sample T test was used to determine the significance at 95% confidence interval which was not significantly different ( $p > 0.05$ ) using SPSS package.

## **Results and Discussion**

### **Results**

Out of a total of 50 samples which comprised of 20 entrails samples, 10 beef samples, 10 table swabs and 10 samples of processing water which were collected from the Obinze abattoir, 17 out of the 20 entrails samples yielded growth of *E. coli* and they were all *E. coli* O157:H7. 2 out of the 10 beef samples yielded growth of *E. coli* and they were both O157:H7. None of the processing water and table swabs samples yielded growth of *E. coli*.

In table 1, the mean *E. coli* counts was determined in which the entrails samples had the highest mean of (4.16 ±0.56 log<sub>10</sub> Cfu/ml) followed by the beef samples which yielded (3.58 ± 0.01 log<sub>10</sub> Cfu/ml). The processing water and table swabs samples did not yield growths of *E. coli* and their means were 0.

In table 2, the percentage occurrence of *E. coli*, *E. coli* O157:H7 and non *E. coli* O157:H7 isolated from entrails, beef, table swabs and processing water samples was determined where the entrails samples had the highest percentage of (89.5%) followed by that of beef which gave (10.5%). The processing water and table swabs samples did not yield growths of *E. coli* and their percentages were 0.

In table 3, the antibiotic sensitivity pattern of *E. coli* O157: H7 isolated from entrails and beef samples was determined where all the Gram negative antibiotics which included Septrin, Chloramphenicol, Sparfloxacin, Ciprofloxacin, Amoxicillin, Augumentin, Gentamycin, Peflacin, Tarivid and Streptomycin were all 100% sensitive.

**Table 1: The mean occurrence of *E. coli* counts isolated from entrails, beef, table swabs and processing water samples from the Obinze abattoir, Imo state.**

Samples	Log <sub>10</sub> CFU/ml
	<i>E. coli</i>
Entrails	4.16 ±0.56
Beef	3.58 ± 0.01
Processing water	0.0 ± 0
Table swab	0.0 ±0

**Table 2: The percentage occurrence of *E. coli*, *E. coli* O157:H7 and non *E. coli* O157:H7 counts isolated from entrails, beef, table swabs and processing water samples from the Obinze abattoir, Imo state.**

Samples	<i>E. coli</i> counts (%)	O157:H7 (%)	Non O157:H7 (%)
Entrails	17 (89.5)	17 (89.5)	0 (0)
Beef	2 (10.5)	2 (10.5)	0 (0)
Processing water	0 (0)	0 (0)	0 (0)
Table swab	0 (0)	0 (0)	0 (0)
Total			

**Table 3: The antibiotic sensitivity pattern of *E. coli* 1057 H7 isolated from entrails and beef samples from the Obinze abattoir, Imo state.**

Antibiotics	(Concentration)	Susceptible (%)	Resistant (%)
Septrin	(30µg)	19 (100)	0 (0)

Chloramphenicol	(30µg)	19 (100)	0 (0)
Sparfloxacin	(10µg)	19 (100)	0 (0)
Ciprofloxacin	(10µg)	19 (100)	0 (0)
Amoxicillin	(30µg)	19 (100)	0 (0)
Peflacin	(30µg)	19 (100)	0 (0)
Gentamycin	(10µg)	19 (100)	0 (0)
Augmentin	(30µg)	19 (100)	0 (0)
Tarivid	(10µg)	19 (100)	0 (0)
Streptomycin	(30µg)	19 (100)	0 (0)

### Discussion

The data obtained revealed that *Escherichia coli* O157: H7 were isolated from meat products sold in Obinze abattoir in Imo State, Nigeria. Previous studies also indicated the presence of *E. coli* O157: H7 in meat and meat products (Enabulele and Uraih, 2009; Itelima and Agina, 2011; Salome *et al.*, 2014).

The occurrence and isolation of *Escherichia coli* O157:H7 from entrails, beef, processing water and table swabs from the Obinze abattoir in Imo state indicates contamination which is potentially hazardous. The *E. coli* O157:H7 counts observed from the beef samples may be attributed to poor handling procedure in the abattoir. It is therefore apparent that proper cooking must be done before the consumption of beef purchased from the abattoir. This is in agreement the report of Enabulele and Uraih (2009), who stated that the high rate *E. coli* O157:H7 occurrence is indicative of the poor sanitary environment under which the animals are slaughtered and sold. These animals were reported to be slaughtered on the abattoir floor that is not properly disinfected after every kill, with butchers and retailers walking between carcasses as they transact their business, while those in the market are displayed on tables in the open for sale. Also, Nkanga and Uraih (1981) reported that meat is frequently found to be contaminated due to poor sanitary environment during slaughter, transportation and usage and through handling. Cattle are the natural reservoir of *E. coli* O157:H7 with a prevalent rate of more than 35%. Various food items contaminated with cattle faeces are implicated as the source of human infections, beef and beef derived products being the most important. Also, the abattoir environment is an ideal reservoir harbouring this pathogen. The presence of *E. coli* O157:H7 in the ground beef obtained from the abattoir indicates that the consumer of beef and beef products from this abattoir if not properly cooked are at risk of acquiring food poisoning. This agrees with Elder *et al.*, (2000) report, stating evidence of contamination as the most potential source of *E. coli* O157:H7 in beef was hide and or faeces during slaughter process. Evidently, *E. coli* O157:H7 has been isolated from faeces or gastrointestinal tract of cattle, sheep, horses, pigs, turkeys, dogs and a variety of wild animals (Hancock *et al.*, 1998).

17 out of the 20 entrails samples yielded growth of *E. coli* and they were all *E. coli* O157:H7 and then 2 out of the 10 beef samples yielded growth of *E. coli* and they were both O157:H7 and these accounted for 89.5% and 10.5% respectively. The 89.5% prevalence rate in the entrails in this study is a clear indication of heavy contamination and this agrees with a similar report of Dahiru *et al.*, (2008) who reported 53%. One possible reason for this high prevalence of *E. coli* O157:H7 found in the entrails is contamination from the faeces of the

animal. The 10.5% prevalence rate in beef samples agrees with a similar report of Hancock *et al.*, (1994) who reported a prevalence rate of *E. coli* O157:H7 in herds of dairy cattle to be 8.3% and 16% from beef. However some reports highlighted lower prevalence rate (Enabulele and Uraih, 2009; Itelima and Agina, 2011; Salome *et al.*, 2014).

In this study, *E. coli* O157:H7 from the samples were susceptible to all ten (10) gram negative antibiotics used as seen in table 3 above. Total sensitivity of some Gram negative antibiotics to *E. coli* O157:H7 is a great advantage which implies that the early detection and the use of antibiotics will be helpful in the treatment of *E. coli* O157:H7 infections. There are very rare studies that reports susceptibility of *E. coli* O157:H7 to all same antibiotic agents used in this study. However, some reports have stated the susceptibility of *E. coli* O157:H7 to gentamycin and ciprofloxacin which were also used in this study (Salome *et al.*, 2014; Walsh *et al.*, 2006; Okolocha *et al.*, 2006). A possible reason for the susceptibility of the isolate to these drugs may be due to the fact that these antibiotics are very expensive compared to others and therefore, not indiscriminately used (Okeke *et al.*, 2000). Contrary to the findings in this study, Salome *et al.*, (2014) reported resistance to chloramphenicol, streptomycin and amoxicillin. Also Chigor *et al.*, (2010) reported *E. coli* O157:H7 resistance to ciprofloxacin. This could be attributed to difference in host habitat.

## Conclusions

The total occurrence of *E. coli* O157:H7 from all the *E. coli* isolates indicates the prevalence of *E. coli* O157:H7 amongst other strains of *E. coli* associated with meat. Therefore, the need to improve the sanitary condition of the abattoir should be implored in order to prevent the spread of the pathogen. Educating the workers in abattoirs on good sanitary practices during the processes of cow slaughtering and the possible dangers of consuming contaminated products should be ensured. The National Agency for Food and Drugs Administration and Control (NAFDAC) should see to the compliance of sanitary practices in abattoirs. Furthermore preserving the products in safe condition is necessary. Federal Government should set standard committee to ensure the standard of processed food and the compliance to WHO specification. Also, organizational seminars and conference should be done to enlighten and educate the masses on the dangers of consuming undercooked beef.

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