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Phenotypic Detection of *Escherichia coli* O157:H7 from Faeces of Slaughtered Cattle in Dutse Abattoir

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Abstract
Escherichia coli O157:H7 is recognized as an important pathogen of man and animals, and a very low infectious dose is needed to propagate the infection and clinical disease associated. The present study was conducted from May to July 2018 on slaughtered cattle in Dutse abattoir in order to detect E. coli with special emphasis on E. coli O157:H7, and to determine the frequency of occurrence of the bacterium. A total of 50 rectal swab samples were randomly collected from freshly slaughtered cattle and were analyzed. Presumptive identification on Eosine Methylene Blue (EMB) agar yielded 30 positive samples confirmed to be E. coli by some biochemical test which showed indole positive, methyl red positive, Voges-proskauer negative, and citrate utilization negative. Sorbitol macConkey agar test yielded 4 positive results. The frequency of occurrence of E. coli was 30 (60%) out of which E. coli O157:H7 were 4 (6.7%) and others were 26 (53.3%). The presence of presumptive E. coli O157:H7 in cattle faeces can be considered as potential pathogens to the individuals having the habit of eating unhygienic meat and undercooked beef. Good manufacturing practices in the processing of meat in abattoirs should be implemented.

Keywords: Abattoir, Cattle, *Escherichia coli* O157:H7, Faeces, and Sorbitol macConkey agar

INTRODUCTION
Foodborne pathogens are the leading cause of illnesses and death in developing countries costing billions of dollars in medical care and social costs. Poor hygienic practices serves as major contributing factor with contaminated raw meat as one of the main sources of foodborne illnesses including the risk of transmission of zoonotic infections (Teka *et al*., 2014).
International food management agencies, especially the World Health Organization (WHO), the Food and Agriculture Organization (FAO) and the International Hazard Analysis Critical Control Point (HACCP) Alliance have already provided guidelines to member countries about safe handling procedures such as HACCP and Good Manufacturing Practices (GMPs) (Nafisa *et al*., 2010).

Despite the extensive scientific progress and technological developments achieved in recent years in developed countries, microbial foodborne illness still remains a global concern. Microorganisms of concern to meat processors may originate from the faeces and skin of animals and also include environmental sources like working utensils presented for slaughter and can be transferred to the carcass during skin removal and evisceration (Elder *et al.,* 2000; Shiaka *et al*., 2015a). *Escherichia coli* (*E. coli*) O157:H7 is one of the most important food borne pathogens, causing diarrhea, hemorrhagic colitis and haemolytic uremic syndrome in humans worldwide (Mersha *et al*., 2010).

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Escherichia coli are genetically heterogeneous group of bacteria whose members are typically non pathogens that are a part of the normal microflora of the intestinal tract of humans and animals. However, certain subsets of this bacterial species have acquired genes that enable them to cause intestinal or extra intestinal disease (Bacon *et al*., 2000; Abdella *et al*., 2009). *E coli* that cause enteric disease have been divided into pathotypes, based on their virulence factors and mechanisms by which they cause disease. One of these pathotypes, called Shiga toxin-producing *E. coli* (STEC), refers to those strains of *E. coli* that produce at least one member of a class of potent cytotoxins called Shiga toxin. The STEC are also called verotoxin producing *E. coli*. The name Shiga toxin (Stx), derived from similarity to a cytotoxin produced by *Shigella dysenteriae* serotype 1 and verotoxin (VT), based on cytotoxicity for Vero cells are used interchangeably (Gill *et al*., 1996 ; Gyles, 2007).

Sporadic cases and outbreaks of human diseases caused by STEC have been linked to ground beef, raw milk, meat and dairy products, vegetables, unpasteurized fruit juices and water. Infections can also be acquired by direct contact with animals and by person to person spread. The organism is destroyed in pasteurization process, but insufficient heat treatment of ground meat and raw milk forms a potential infection risk (Rahimi *et al*., 2012).

Detection of *E. coli* O157:H7 in the clinical laboratory is dependent on distinguishing the pathogenic serotypes from normal fecal flora containing commensal strains of *E. coli (*Chapman *et al.,* 2001; Battisti *et al*., 2006). Fortunately, *E. coli* O157:H7 has two unusual biochemical markers; delayed fermentation of D-sorbitol and lack of β-D-glucuronidase activity, which help to phenotypically separate O157:H7 isolates from nonpathogenic *E. coli* strains. One of these markers (delayed sorbitol fermentation) enables to develop several selective media (e.g Sorbitol-MacConkey; SMAC) which aid in the initial recognition of suspicious colonies isolated from bloody stools (Bindu *et al*., 2010). Detection of *E. coli* O157:H7 from food samples requires enrichment and isolation with selective and/or indicator media, but lacks specificity to identify STEC. Thus, more sensitive methods are required to improve the detect ability of STEC O157:H7 from food and environmental samples. Apart from the traditional culture methods relying on biochemical characteristics, various genotypic methods have been proven useful for species identification, epidemiological typing, and determining genetic relatedness among pathogenic and non-pathogenic bacteria (Ji-Yeon *et al*., 2005).

The currently accepted methods for the isolation of O157strains consist of assays for the detection of Shiga-like toxins(SLTs), either directly or at the genomic level, coupled with direct plating on sorbitol MacConhkey (SMAC) agar, cefixime-SMAC agar or SMAC agar supplemented with cefixime and tellurite (CT-SMAC) with subsequent stereotyping. Accurate diagnosis of EHEC O157 infections requires the isolation of the pathogen to clarify the etiology of disease and the infectiousness of patients as well as to allow sub-typing of strains for epidemiological purposes. The probability of isolating *E. coli* O157 strains from stool cultures of patients is inversely related to the interval between the onset of diarrhea and the microbiological culture (Helge *et al*.,1995).

Human infection with shigatoxin producing *E. coli* O157:H7 is relatively rare but the consequences could be serious, especially in the immune compromised such as the young and the elderly (Beyi, 2017). *E. coli* O157:H7 has been identified as a pathogen that causes severe and

life-threatening diarrhoea (Momba *et al.,* 2008). This makes the consumption of *E. coli* O157:H7 contaminated raw milk, meat and vegetables a potential high health-risk aspect which can lead to morbidity and mortality (Lye *et al*., 2013).

Shiga toxin-producing *Escherichia coli* O157:H7 (STEC O157) can cause severe enteric infections. Symptoms may include abdominal pain, bloody diarrhea, hemorrhagic colitis and haemolytic uremic syndrome (HUS) (Nataro and Kaper, 1998; Zhao *et al*., 1994). Numerous sporadic infections and outbreaks caused by STEC O157 have been reported in the United States and elsewhere worldwide (Rahimi *et al*., 2012). The majority of STEC O157 infections are food borne; many are associated with bovine sources. STEC O157 was first linked to outbreaks of severe bloody diarrhea in 1982, and is often referred to as a "recently emerged" human pathogen (Wei *et al*., 2006). Outbreaks have been associated with poor hygienic measures during slaughter, evisceration and processing of beef. The detection of *E. coli* O157:H7 is an indicator of fecal contamination and implies presence of other dangerous pathogens which can compromise the wellbeing of consumers (Biruhtesfa *et al*., 2017).

The risk of transmission of *E. coli* O157:H7 to man and animals has increased overtime. The fact that low infectious dose of the organism as low as ten could trigger serious infection is a signal for more researches to be conducted on this disease. This, coupled with the short incubation period of the bacterium could further exacerbate in the disease especially in the elderly and immune-compromised young individuals below five years of age (Weir and Hay, 2006).

The risk of *E. coli* O157:H7 from food animals has not been paid much attention in developing countries (Honise *et al*., 2017). There is also paucity of information regarding the epidemiology of *E. coli* O157:H7 in developing countries. Animals are commercially slaughtered and dressed in unhygienic conditions which compromise microbiological quality and safety of meat obtained from the animals; this consequently risks the health of the consumers.
This research therefore serves as a catalyst for the need to promote surveillance programs in order to identify sources of pathogenic *E. coli* from non-human origin and also to detect *Escherichia coli* O157:H7 from slaughtered cattle in Dutse abattoir.

MATERIALS AND METHODS

Study Area

The study was conducted in Dutse abattoir Jigawa state. Dutse is a city located in North-Western Nigeria and the capital city of Jigawa State with an estimated population of 153,000 based on census conducted in 2006. It is located on the geographical coordinates 11°42′04″N and 9°20′31″E/11.70111°N and 9.34194°E. (https://en.m.wikipedia.org./wiki/Dutse)

Study Design

A cross sectional study was conducted on apparently slaughtered cattle in Dutse Abattoir from May to July 2018.

Collection of Samples

Samples were collected from the rectum of each slaughtered cattle according to the method described by Elder *et al.*(2000) using a sterile swab stick. About 5ml of sterilized peptone water was placed in the swab stick container to moisten the swab stick before use. It was then used to swab the rectum of the slaughtered cattle and was placed back in to the tube. This procedure

was repeated for all other cattle in question. The swab sticks were then placed on icepark and then conveyed to Microbiology laboratory, Federal University Dutse for further analyses. An average of ten cattle were selected at random in each visit, making a total of 50 samples in five visits made.

Laboratory Procedures

On reaching the laboratory, the samples were placed on work bench and were allowed for a period of time to enable them acclimatized to room temperature.

Isolation of E. coli

The sample was inoculated on the surface of already prepared Eosin Methylene Blue (EMB) agar using a sterilized wire loop after which the plate was incubated at 37^{o}C for 24 hours. This was repeated for other samples. The culture plates were observed for greenish metallic sheen. A discrete colony was preserved on nutrient agar slant for further identification. The presumptive *E. coli* isolates were further identified and confirmed by determining theirGram's reaction and subjecting them to selected biochemical tests respectively, following the procedures of Cheesebrough, (2000). The biochemical tests included Indole, Methyl red, Voges-proskauer and Citrate test (IMViC) as stated by Quinn *et al.*(1994).

A discrete colony of the *E. coli* was streaked onto Sorbitol MacConkey Agar supplemented with Cefixime and Tellurite (CT-SMAC Oxoid, UK) and incubated at 37°C for 24 h (Cheesebrough, 2000). The colourless colonies were considered as presumptive *E. coli* O157: H7
The results obtained from this research were presented in Tables and Charts.

RESULTS

Characteristic green metallic sheen colonies on EMB agar were identified as *E. coli.* These were positive for 30 (60%) out of 50 samples collected as shown in Table1. They were all Gram negative rods and positive for indole and methyl red, negative for voges-proskauer and citrate tests.

Table1: Isolation of *E. coli*

No of visits	No of samples	No of isolate +ve on EMB	C.M	G.R	Biochemical characteristics	Inference
5	50	30	Greenish metallic sheen	-	I.E + M.R + V.P - C.E -	*E. coli*

EMB= Eosine methylene blue, C.M= Colonial morphology, G.R= Gram's reaction, I.E= Indole, M.R= Methyle red, V.P= Voges-proskauer, C.E= Citrate, + = Positive, - = Negative.

Upon sub-culturing, characteristics colourless colonies on SMAC agar were considered as presumptive *E. coli* O157:H7 which were positive for only 4 (6.7%) isolates.

Table 2:The cultural identification of *E. coli* O157:H7.

Isolates	Appearance on SMAC	No of positive samples	Presumptive identification
E. coli	Colorless	4	*E. coli* O157:H7
E. coli	Pink	26	Non -*E. coli* O157:H7

SMAC= Sorbitol macCkonkey

Table 3: Isolation frequency of *E. coli* and presumptive *E. coli* O157:H7 from the samples

N	*Non-E. coli* (%)	*E. coli* positive (%)	Presumptive *E. coli* O157:H7 (%)
50	20(40)	30(53.3)	4(6.7)

N=number of samples collected

DISCUSSION

E. coli is frequently isolated in abattoirs as obtained in previous and similar researches (Ukut *et al*., 2010 and Haileselassie *et al*., 2013 and Shiaka *et al*., 2015b). This might be due to the ability of cattle to adequately harbor this organism in the gastrointestinal tract and more so, poor hygienic practices of the workers during meat processing. Cattle have been identified as major reservoirs and food contaminated with faecal materials of cattle is a frequent source for human infection (Kiranmayi *et al*., 2010). This has been recognized as a major cause of large scale epidemics of gastrointestinal illnesses in animals and man (Deshmukh and Karpe, 2006).

The appearance of colorless colonies by certain *E. coli* strains on SMAC agar is due to their inability to ferment D-sorbitol due to lack of betaglucuronidase. This serves as a typical characteristic of *E. coli* O157:H7 (Cheesebrough, 2000).

Shiga-toxin producing *E. coli* infections may result in life threatening sequelae such as heamolytic-uremic syndrome, an important cause of acute renal failure in children and morbidity and mortality in adults and haemorrhagic colitis. Cattle are known to harbour not only strains pathogenic to animals but also strains which cause asymptomatic infections in animals and which can pass through the food chain to cause clinical diseases in man (Arshad *et al*., 2006).

The rate of isolation of *E. coli* O157:H7 was far lower than those of *E. coli* species. This was in agreement with the reports of Balcha *et al.* (2014) in which the isolation rate of *E. coli* O157:H7 and *E. coli* were 18% and 62.5% respectively. More so, they were higher than the 6.7% and 53.3% of the results obtained in this research, probably due to better hygienic practices and GMPs by the workers in Dutse abattoir. This result was also in consistent with the report of Taye *et al*. (2013) in which the overall prevalence of carcass contamination with *E. coli* was 30.97% and that of *E. coli* O157:H7 was only 2.65%. These results have indicated that abattoir meats are more contaminated by *E. coli* species than *E. coli* O157:H7. This could be justified by the fact that highly pathogenic organisms are known to be fastidious.

E. coli O157:H7 infection is particularly a challenge for the Nomads as they live in close proximity of cattle and have little or no knowledge about pathogenicity of bacteria and the transmission of diseases. The isolation rate of *E. coli* O157:H7 obtained from this study was found to be very low but could present a more challenging effect especially in cases involving human exposure, since a very low dose of the pathogen is required to establish infection (Podolak *et al*., 2010).This research is limited to only cultural identification of the bacterium with no emphases on the serological and molecular analysis due to lack of facilities and financial constrain.

CONCLUSION

The present study showed a considerable presence of *E. coli*(53.3%) and less of *E. coli* O157:H7 (6.7%) in faeces of slaughtered cattle in Dutse abattoir. In spite of the less isolation rate of *E. coli* O157:H7, they might proliferate, spread from animal to human and as a result, could be a concern to public health.

RECOMMENDATIONS

Hygienic measures and GMP must be key in abattoirs during any meat processing and also proper cooking by the consumers. Public enlightenment on the risk of food borne diseases is necessary. Further investigations on this research should involve serological and molecular techniques as well as developing strategies for minimizing cross contamination during meat processing and other food items of animal origin.

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