

Full Length Research Paper

Isolation of *Escherichia coli* O157:H7 from selected food samples sold in local markets in Nigeria

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Food borne illnesses have major social and economic impacts. *Escherichia coli* O157:H7 is associated with food borne illness in human beings. It has been an important food borne pathogen that causes food borne diseases such as diarrhea, hemolytic uremic syndrome and hemorrhagic colitis. This study was conducted to detect the presence of *E. coli* O157: H7 in different food samples sold in Nigerian local markets. A total of 60 different food samples (3 each of meat, fufu, waterleaf, pumpkin, carrot, tomatoes, meat pie, yoghurt, watermelon, cucumber, groundnut, cabbage, garden egg, bread, okra, apple, chicken, unpasteurized milk, salad and pawpaw) were collected randomly from different markets in Calabar, Nigeria. The samples were analyzed using standard microbiological techniques. Isolation was carried out using pour plate technique on sorbitol MacConkey agar. The isolates were identified by morphological and biochemical tests. Out of the 60 samples investigated, 36 (60%) were found to be contaminated with *E. coli* O157:H7 while 24 (40%) were negative by conventional methods. All the isolates obtained from the samples were subjected to various biochemical tests and were all confirmed to be *E. coli* O157:H7. The occurrence of *E. coli* O157:H7 serotype in these food products indicates that there may be a potential risk for public health from consuming these foods. This study clearly indicated the need for proper handling and processing of food products especially ready to eat food products. It is also important that at household level proper hygienic measures should be taken to avoid cross contamination.

Key words: *Escherichia coli* O157:H7, diarrhea, food borne illnesses, food samples, hemorrhagic colitis.

INTRODUCTION

Escherichia coli are large and diverse group of bacteria. It is the type of the genus *Escherichia* that contained mostly motile Gram negative bacilli that fall within the family Enterobacteriaceae. It is the predominant facultative anaerobe of the human colonic flora. The organism

typically colonizes the infant gastrointestinal tract within hours of life, and thereafter *E. coli* and the host derive mutual benefit for decades (Kaper et al., 2004). *E. coli* is a bacterium that normally lives in the intestines of human and animals, the growth and survival of *E. coli* depends

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on the number of environmental factors such as temperature, pH, water activity, composition of the food, carriage by cattle and contamination of surface water (Center for Disease Control, 2001). The temperature range of growth of *E. coli* is 7 to 8 to 46°C, with an optimum temperature of 35 to 40°C. Although most serotypes of *E. coli* are harmless, several produce toxins that cause illness. Some strains of *E. coli* including *E. coli* O157:H7, produce toxins known as shiga toxins and are called shiga toxin producing *E. coli* (STEC). Their virulence characteristics suggest that they may have significant impact on public health (Perera et al., 2015).

During the past two decades, disease caused by *E. coli* O157:H7 has been increasing (Mean et al., 2015). Currently, the Centers for Disease Control and Prevention (CDC) estimated that *E. coli* O157:H7 caused an average of 500 outbreaks that affect >73,000 persons and result in >61 deaths each year in the United States (Charatan, 2014). The epidemiology of *E. coli* O157:H7 has become an important research topic as manure harboring *E. coli* O157:H7 is dispersed, and soil, food, and water are cross-contaminated with feces containing *E. coli* O157:H7 (CDC, 2001; Nakazawa and Akiba, 2001; Mean et al., 2015).

E. coli O157:H7 is an important emerging human pathogen causing haemorrhagic colitis (HC), haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) (Amani et al., 2015; Fan et al., 2019). *E. coli* O157:H7 serotypes are identified as enterohaemorrhagic *E. coli* (Oksuz et al., 2004). The infections by *E. coli* O157:H7 have been reported of increasing frequency from all parts of the world in the form of food poisoning outbreaks (Jo et al., 2004; CDC, 2018). Because of the severity of these illnesses and the apparent low infective dose (<10 cells) (Bach et al., 2002), *E. coli* O157:H7 is considered one of the most serious of known foodborne pathogens (Blanco et al., 2003).

Enterohemorrhagic *E. coli* O157:H7 is an important food borne pathogen a causative agent of HC and HUS. Globally, STEC caused 2,801,000 acute illnesses annually, with an incidence rate of 43.1 cases per 100,000 persons per year. This burden led to 3890 cases of HUS and 230 deaths (Lupindu, 2017). Large outbreaks of EHEC infection were reported throughout the world. *E. coli* O157:H7 is the most commonly recognized STEC in the United States, however, many other STEC serogroups including O26, O103, O111, and O145, have been associated with outbreaks and sporadic cases of HC and HUS worldwide (Essendoubi et al., 2019). One of the largest *E. coli* O157:H7 (one of the serotypes of EHEC) outbreaks associated with food consumption occurred in Sakai City, Japan in 1996. About a quarter of African countries have reported isolation of STEC O157:H7 either from humans, animals, food or the environment (Lupindu, 2017; Yusuf et al., 2018).

The overall aim of this study was to investigate the

presence of *E. coli* O157:H7 in different food samples sold in Calabar Metropolis, Nigeria and to determine its incidence rate.

MATERIALS AND METHODS

Sample collection

A total of 60 different food samples (3 each of meat, fufu, waterleaf, pumpkin, carrot, tomatoes, meat pie, yoghurt, watermelon, cucumber, groundnut, cabbage, garden egg, bread, okra, apple, chicken, unpasteurized milk, salad and pawpaw) were collected randomly from different markets in Calabar, Nigeria. All the samples were collected aseptically in sterile universal containers and polyethylene bags and immediately placed in pre-cooled containers containing ice packs and then transported to the laboratory for analyses.

Preparation of samples

About 25 g of food samples was taken and homogenized with 225 ml of buffered peptone water 0.1% in a stomacher for 15 min, after which the homogenate was used for the isolation.

Isolation of *E. coli* O157:H7

Isolation was carried out after pre-enrichment of the samples by selective plating as described by Kim et al. (2005). One milliliter of the homogenate was used for ten-fold serial dilution after which 0.1 ml of 10³ dilution factor was inoculated on Sorbitol MacConkey agar (SMAC) supplemented with cefixime (0.05 mg/l) and potassium tellurite (2.5 mg/l) in triplicate. The plates were then incubated at 28°C for 18 to 24 h. After the incubation period, the plates were observed for the growth of *E. coli* O157:H7 colonies.

Purification and maintenance of isolates

Each discrete colony on a Petri dish was transferred using a sterile inoculating loop into plates containing freshly prepared Nutrient agar (NA) and were incubated at 37°C for 24 to 48 h, respectively. The isolates were then preserved on NA slants stored in the refrigerator at 4°C.

Biochemical confirmation of isolates

The suspected colonies of *E. coli* O157:H7 were subjected to various tests and confirmed based on the biochemical characteristics. The individual colonies of EHEC from CT-SMAC agar were transferred to tryptic soy broth (TSB) and incubated at 37°C for 24 h. Primary identification tests like Gram's staining, catalase test and oxidase test were performed. Secondary identification tests like indole production, methyl red (MR) reaction, voges proskauer (VP) reaction, citrate utilization test, urease activity, and carbohydrate utilization test were carried out as per the standard procedures.

RESULTS AND DISCUSSION

The percentage of *E. coli* O157:H7 in the food samples analyzed is shown in Figure 1 where the highest rate of

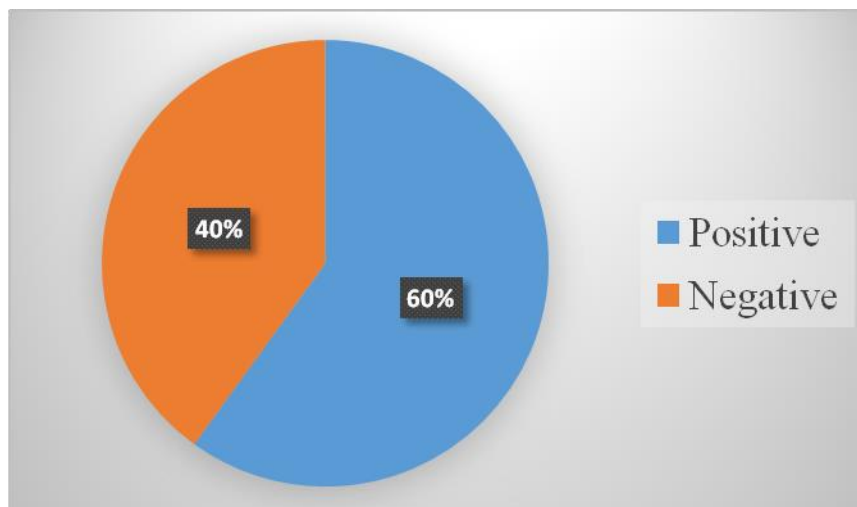


Figure 1. Incidence rate of *E. coli* O157:H7 in selected food samples.

Table 1. Microbial loads of *E. coli* O157:H7 in selected food samples.

S/N	Food sample	Mean count (CFU/g or ml)
1	Meat	3.8×10^2
2	Fufu	1.13×10^2
3	Pumpkin	2.04×10^2
4	Meat pie	2.9×10^2
5	Yoghurt	2.2×10^2
6	Cucumber	1.27×10^2
7	Cabbage	1.43×10^2
8	Garden egg	1.16×10^2
9	Okra	1.52×10^2
10	Salad	2.42×10^2
11	Unpasteurized milk	1.86×10^2
12	Chicken	2.7×10^2

occurrence of *E. coli* O157:H7 was observed to be 60% with 40% representing negative occurrence. The results of the microbial load of *E. coli* O157:H7 in food samples analyzed are shown in Table 1. The highest *E. coli* O157:H7 load was recorded of meat sample with a mean load of 3.89×10^2 CFU/g while the least load was observed in fufu (1.13×10^2 CFU/g) sample.

All these isolates were positive for catalase test, motility, indole production, MR, triple sugar iron agar reaction and Lysine decarboxylase. These isolates were negative for oxidase, VP, citrate utilization, urease and sodium chloride tolerance test. The results of these tests clearly indicated that they belonged to the category of enterohaemorrhagic *E. coli*. These isolates were then subjected to carbohydrate utilization tests for identification of species. The results suggested that all the 16 isolates were showing reaction similar to that of *E.*

coli O157:H7 shown in Table 2.

The results from the study revealed an overall incidence of *E. coli* O157:H7 as 60% (12/20) in all the collected food samples from different locations in Calabar, Nigeria. Kumar et al. (2004) found that 100% of different beef samples positive for *E. coli* O157:H7 in a study conducted in Mangalore, India. The United States Department of Agriculture (USDA, 2007) reported that the Food Safety and Inspection Service (FSIS) identified more than 75% of the ground beef and vegetable samples were positive for the presence of *E. coli* O157:H7. Grant et al. (2011) reported that the prevalence of non O157 EHEC in raw beef as 2.4 to 49.6% in Canada and United States it ranged from 5.7 to 26.2%. These suggested that foods, particularly beef are an important source of *E. coli* O157:H7 infections. In the present study also, the highest mean load was observed

Table 2. Biochemical characteristics and identification of *E. coli* O157:H7.

S/N	Biochemical test and sugar fermentation	Meat			Fufu		Meatpie			Yoghurt			Cabbage		Cucumber		Okra	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	Gram's reaction	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
2	Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	Oxidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	Motility	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	Indole production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	Methyl red	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	D-Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	Citrate utilization	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

in meat sample. This may be due to the fact that the gastro intestinal tract of the cattle is the most important predilection site of the organism (Vijayan et al., 2017). The range of food samples positive in this study for *E. coli* O157:H7 is of concern to consumers and food processors. More of concern is the fresh vegetables that are eaten raw especially in salads and the already prepared foods.

The occurrence of *E. coli* O157:H7 of the present study was comparatively lower than a study done by Zahraa et al. (2016). They got a prevalence of 82% (135/164) from different food samples. Almost similar result was found in other studies conducted by Vinothkumar et al. (2014) in Puducherry, India who observed 84% prevalence in food samples. Momtaz and Jamshidi (2013) in Iran found 71.2% of the food samples were positive for *E. coli* O157:H7. These findings suggested that cross-contamination of these food samples may occur in retail food shops and markets with higher prevalence in animals. Cattle act as a reservoir host for EHEC O157:H7 resulting

in higher food contamination (Bindu and Krishnaiah, 2010). This is a serious health issue that the public health department of any government to take off. It is important that the regulatory agencies put up an enlightenment campaign to educate consumers on how to control or eliminate cross contamination by cooking meat properly, drinking pasteurize milk and juice, wash produce thoroughly, wash utensils very well, keep raw foods separate and to wash hands thoroughly after handling raw meats.

Similar study carried out in Iraq, from 100 samples of meat only two isolates and from 98 dairy product samples were detected as *E. coli* O157:H7 (Dhaher et al., 2010). In Iran, another study proved that, from 130 bulk tanks of milk just one isolate was *E. coli* O157:H7 (Brenjchi et al., 2011). While, in another study, from 125 samples of soft cheese prepared from raw milk, found 5 isolates of *E. coli* O157:H7 (Najand and Khalilli, 2007). Out of 50 ground beef samples, 7 strains of *E. coli* O157:H7 were detected, while none was isolated from chicken drumsticks in Turkey (Fatma

and Murat, 2000).

In Turkey, studies conducted to detect *E. coli* O157 and/or *E. coli* O157:H7 revealed that *E. coli* O157 have been isolated from different food products with an occurrence varying from 0 to 55% (Elmali et al., 2005). Few studies, however on the isolation of *E. coli* O157:H7 from ground beef and vegetable products in Turkey have revealed negative results (Siriken and Pamuk, 2004; Siriken et al., 2004). Likewise, there is also no report of any outbreaks due to *E. coli* O157:H7 in Turkey (Agaoglu et al., 2000). It is likely that these kinds of cases have not been reported or the causative agents of food poisonings have not been identified. Lack of direct link between isolates from humans and other sources makes it difficult to point out incident specific determinants and direction of transmission (Lupindu, 2017). But, it seems that incidences found in this study seem to be higher than those previous studies for other food products. Vegetables, milk and water have also been implicated in *E. coli* O157:H7 poisoning outbreaks (Kayisoglu et al., 2003).

In Africa, a study carried out in Gwagwalada, Federal Capital Territory, Nigeria, among children between the ages of 0 to 24 months found 31.1% positive for STEC (Onanuga et al., 2014), which could be as a result of the mothers hygienic status.

Conclusion

The findings from this study suggested that most raw foods are contaminated with *E. coli* 0157:H7. This is a serious health issue. Cross-contamination of foods may be occurring in retail meat shops because studies have indicated higher prevalence of *E. coli* 0157:H7 in animals. Cattle act as a reservoir host for EHEC 0157:H7 resulting in higher food contamination. Mainly in the beef retail shops, beef carcasses were not hoisted but kept on the tables or the floor for dressing. More precautions are therefore needed processing and handling of meat to avoid cross contamination of foods. This study has also highlighted the contaminated levels of the various food products. The hygienic environments and handling could have contributed immensely to the cross contamination of other food products not prone to contamination by *E. coli* 0157:H7. Undocumented frequent food borne disease outbreaks in this part of the country could be attributed to *E. coli* 0157:H7.

Efforts should therefore be made to control this bacterium in Nigerian food products in order to avert any sickness or death resulting from eating food contaminated with *E. coli* 0157:H7.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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