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# **Detection of** *Escherichia coli O***157: H7 and Non-O157 Strains in Meat, Human Stool, Abattoir Wastewater and Hygiene Practices among Meat Peddlers in Port Harcourt, Nigeria**

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## *Authors' contributions*

*This work was carried out in collaboration among all authors. Authors CKW, EGN and SEA designed and supervised the study, a uthor SEA wrote the protocol, author NAU wrote the first draft of the manuscript, managed the analyses and the literature searches. All authors read and approved the final manuscript.*

## *Article Information*

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# **ABSTRACT**

**Aim:** To determine the prevalent rates of *Escherichia coli O*157: H7 and non-O157 strains in of isolates from meat, human stool and abattoir waste water, collected from selected sources in Port Harcourt, Nigeria.

**Study Design:** Case-controlled study.

**Place and Duration of Study:** Selected places in Port Harcourt, Rivers State, Nigeria, between November, 2020 to November, 2021.

**Methodology:** Three hundred and forty -nine (349) samples were analyzed, 80 meat and 63 waste waters from five abattoirs cited in the city, 46 meat samples from five selected sites sold by roadside butchers, 109 patient stool samples and 30 stool samples from food sellers, in addition to 20 stool samples from healthy subjects and 1 commercial bottled water which served as control samples. Tryptone soya broth (TSB) as an enrichment media, selective agar media namely; Eosin methylene blue agar, cefixime tellurite-sorbitol MacConkey agar (CT-SMAC), Chromagar STEC were used to isolate STEC from the samples and serology done with O157 latex agglutinating kit to confirm E. coli O157:H7 serogroup.

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**Results:** The results showed that Isolation rates obtained for *E. coli* O157:H7 and non O157 respectively were: abattoir meat 11(13.8%);13(16.3%), roadside meat 5(10.9%); 10(21.7%), clinical stool 7(6.4%); 27(24.7%), food sellers' stool 4(13.3%);6(20%), waste abattoir water 2(3%); 14(22.2%). Food sellers and abattoir effluents were found to be potential sources of STEC dissemination in Port Harcourt. The data obtained were statistically analyzed using chi- square which showed that there was no significant difference (p < 0.05) in the rate of isolation of *E. coli* O157: H7 and non- O157 strain from the samples. **Conclusion:** The detection of *E.coli* O157:H7 and non-O157 strains in raw meat (food), environment and clinical samples in Port Harcourt may give rise to a potential widespread public health hazard if strict adherence to proper hygiene management are not place to regulate food processing in other to reduce contamination and foodborne infections.

*Keywords: Prevalence; Escherichia coli O157: H7 and non-O157 strains; Port Harcourt, Nigeria.*

## **1. INTRODUCTION**

The prevalence of *Escherichia. coli* O157:H7 and non-O157 STEC in many geographical regions suggest a high risk of human infection. Presently in the study area, there is acute scarcity of clinical data on the occurrence of STEC infections, especially the non-O157 STEC which is rapidly rivaling the occurrence of *E. coli* O157:H7 infections [1]. Despite the fact that cattle which are the principal reservoir of STEC [2] are massively slaughtered in local abattoirs daily, where proper sanitation is not in place due to unhealthy practices of meat peddlers and poor infrastructures, yet, residence of the study area purchase and consume meat from these abattoirs on a daily basis.

Lack of adequate laboratory facilities interferes with routine definitive diagnosis; hence patients are misdiagnosed and treated blindly. And an attempt to relate human infection to other sources such as food (meat) and environment (wastewater from abattoirs) thereby, pointing out incident specific determinants and direction of transmission [3]. Isolation of STEC as a pathogen from animals, food, clinical samples and environment has been reported from all continents. Their prevalent nature, the severe disease condition associated with them and other biological characteristics such as; low infective dose, ability to express different virulent factors [4], long survival time in the environment [5] and the difficulty in treatment make STEC an enteric pathogen of major concern worldwide. Although their public health importance is large, the diagnosis of STEC is still largely limited to research laboratories.

Most *E. coli* strains harmlessly colonize the gastrointestinal tract of humans and animals as normal commensals, forming part of gut microbiota and are used as indicator bacteria for fecal contamination. However, there are some strains that have evolved into pathogenic *E. coli* by acquiring some virulence factors. These pathogenic *E. coli* strains can be categorized based on serogroups, pathogenicity mechanisms, clinical symptoms, virulence factors or site of infection, [6]. Pathogenic *E. coli* strains are categorized into six pathotypes. The six pathotypes are associated with diarrhea and collectively are referred to as diarrheagenic *E. coli*. They include; Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAEC), Enteroinvasive *E. coli* (EIEC), diffusively adherent *E. coli* (DAEC) and Shiga toxinproducing *E. coli* (STEC) which may also be referred to as Vero cytotoxin-producing *E. coli* (VTEC) or enterohemorrhagic *E. coli* (EHEC) [7].

Epidemiological investigation has identified cattle as the main reservoir for *E. coli* O157:H7 after tracing outbreaks of Shiga toxin enterohemorrhagic diarrhea to domesticated animals, particularly feedlot cattle [8]. Zoonotic transmission of *E. coli* 0157:H7 occurs after consumption of under-cooked meat or deficiently pasteurized dairy products or contact with contaminated fomites laden with Shiga toxin enterohemorrhagic *E.coli* [9]. Other causal etiologies of Shiga toxin enterohemorrhagic *E. coli* include exposure to contaminated water from potable drinking sources, swimming pools and lakes, contaminated food such as inadequately washed leafy greens and fruits, unpasteurized drinks including apple juice, and direct contact with contaminated animals in petting farms [9]. Contamination of fresh fruits and vegetables occurs secondary to fecal contamination in agricultural irrigation water or runoff [10]. *E. coli* O157 has hardy survival characteristics exceeding those found in commensal *E. coli* strains, which enable this food-borne pathogen to survive a wide range of harsh conditions frequently encountered within the human food chain. This pathogen can persist for extended pe riods in the food matrix [10].

Globally STEC causes 2 801 000 acute illnesses annually, with an incidence rate of 43.1 cases per 100 000 person-years. This burden leads to 3 890 cases of HUS and 230 deaths. Among those, a total of 10 200 cases of STEC infections occur in Africa with an incidence rate of 1.4 cases per 100 000 person-years. STEC O157:H7 contributes 10% to this [11]. STEC outbreaks are attributed to its low infectious dose (<100 organisms) and high transmissibility, which can either remain isolated or develop into widespread international outbreaks. Examples of STEC outbreaks in North America include a multistate outbreak resulting in approximately 200 cases of infection following ingestion of fecally contaminated spinach from a single commercial vendor in the fall of 2006 [12]. In 2000, the largest STEC outbreak in Canada occurred in<br>Walkerton, Ontario, due to inadequately Walkerton, Ontario, due to inadequately chlorinated drinking water, which resulted in approximately 2300 cases of infection and seven deaths [13]. Another human outbreak occurred in Canada in 2012 due to STEC O157:H7 infection arising from the fecal contamination of huge volumes of meat in a single processing plant situated in southern Alberta [13]. This study was geared towards detecting the occurrence of *E. coli* 0157:H7 and non-O157 STEC strains within the study area.

# **2. MATERIALS AND METHODS**

## **2.1 Study Area**

Port Harcourt which is the capital of Rivers State, is the oil hub of Nigeria. It is highly congested due to industrialization. Port Harcourt lies along the Bonny River, 66 kilometers upstream from the Gulf of Guinea and is located in the Niger Delta area. Geographically it lies on the coordinates, latitude  $4.75^{\circ}$ N and longitude  $7^{\circ}$ E [14]. Its population was estimated at 2million, making it one of the largest metropolitan areas in Nigeria [14]. Port Harcourt features a tropical wet climate with lengthy and heavy rainy season and very short dry season. Its topography ranges from flat plains with a network of rivers to tributaries [14]. The people of the state depend on different sources of water supply for their drinking water and domestic water needs.

## **2.2 Sampling Locations**

Samples were collected from Rumuokoro, Egbelu, Igwuruta, Mile 3 and Mbuogba Abattoirs; some roadside butchers; some roadside casual restaurants (Buka)/street stalls that sell food prepared in advance; and some hospital, clinics and laboratories all in Port Harcourt. Majority of the stool samples were collected at Obio Cottage Hospital in Port Harcourt. Stool samples were also collected from Ebony Clinic, one of the most popular and well attended clinic located at Rumuokwuta, Port Harcourt. Few additional stool samples were collected from two private laboratories, Antel Medical Laboratory and Healthwise Medical Laboratory located at Rumuigbo and Rumuokwuta in Port Harcourt, which conducted medical test for private patients.

# **2.3 Study Population**

The study was conducted between November 2020 and November 2021. The stool samples were collected from patients with gastrointestinal complaints who attended clinics and food sellers in the city. Both sexes from these two groups of subjects were sampled, including age groups between the ages of less than 20 and above 40 years. It was a mixed socio-economic population including the poor and rich, those living in wellorganized settlements and those living in areas with poor sanitary conditions. Also, individuals without gastrointestinal complaints, who do not eat outside their homes and are not regularly in contact with raw meat were included as control population.

# **2.4 Sample Collection**

One hundred and twenty -six (126) meat samples examined in this study were collected from two sources: the abattoir butchers (80) and the roadside butchers (46). Five abattoir locations in the city were sampled: Rumuokoro, Egbelu, Igwuruta, Mile 3 and Mbuogba abattoirs. Raw meat of different parts was purchased in the mornings, immediately after the cattle are slaughtered and are ready for sale, from the abattoir butchers tables, in two replicates (two different days), minced and aseptically packed into sterile universal bottles. Meat from five roadside butchers located in different areas of the city was sampled: Rumuola, Rumuigbo, Mgbuoba, Mile3 and Mile4. The sampling took place in the afternoons, it was collected in two replicates, the meat was minced on the butchers table and packed into sterile universal bottles. The one hundred and thirty- nine (139) stool samples investigated in this study was sourced from two premises namely; patients (89) and food sellers (30). Patients' samples were collected from four locations: Obio cottage hospital, Ebony clinic, Healthwise medical laboratory and Antel medical laboratory. Stool samples from food sellers operating local restaurants (Buka) that sell food prepared in advance was also sampled in four different locations in the city: Rumuigbo, Iwofe, Airport and Ozuoba. Samples were collected aseptically with sterile universal bottles (Smart diagnostics 2019, China). The stool macroscopy was observed and noted immediately after collection. Sixty-three (63) abattoir wastewater was collected from five abattoir located at Mgbuoba, Rumuokoro, Egbelu, Igwuruta, Mile 3. Wastewater used in washing different parts of the cattle carcass was aseptically collected from the wash bowls using sterile universal bottles (Smart diagnostics 2019, China).

# **2.5 Sample Analysis**

## **2.5.1 Media preparation**

All the agar-based media and broth used in this study were reconstituted and sterilized according to the manufacture's instruction. The molten agar was allowed to cool to about  $45^{\circ}$ C, supplemented (where applicable) and poured into sterile plastic petri dishes (about 20ml per Petri dish). They were allowed to solidify, packed and stored in the refrigerator for subsequent uses.

## **2.5.2 Enrichment and recovery**

#### *2.5.2.1 Meat sample*

Each of the meat sample was macerated on the butchers table and 2.5g of the macerated meat sample was suspended in 22.5ml of tryptone soya broth (TSB) (Oxoid CM0129) using sterile universal bottle mixed by shaking and incubated at  $37^{\circ}$ C for 6-18 hours.

#### *2.5.2.2 Abattoir waste water sample*

One (1) millilitre of abattoir wastewater sample was added to 9.0ml of tryptone soya broth (TSB) (Oxoid,) contained in Bijou bottles, mixed by shaking and incubated at  $37^{\circ}$ C for 6-18 hours.

#### *2.5.2.3 Stool sample*

Pea size stool or 1ml stool sample was inoculated into sterilized Bijou bottle containing 10ml of TSB mixed or emulsified by shaking, incubated at  $37^{\circ}$ C for 6-18 hours.

#### **2.5.3 Microbiological analysis**

A loopful of each sample from the enrichment broth was plated on Eosin methylene blue agar (TM, India) and incubated at  $37<sup>0c</sup>$  for 24 hours. A colony was picked from colonies exhibiting characteristic deep red *E. coli* colonies with metallic greenish sheen appearance and sub cultured into nutrient agar plates, for biochemical test. Thereafter, the *E. coli* isolates were sub cultured into supplemented Chromagar STEC (France). About two Mauve colonies typical of Shiga toxin producing *E. coli* were picked and again sub cultured into sorbitol MacConkey agar (SMAC) (Bio mark, India) supplemented with  $2.5$ mg<sup>1-1</sup> of potassium tellurite (Bio mark, India). All colourless colonies from SMAC were further tested using *E. coli* O157 latex agglutination (Oxoid DR0620) test kit for O157.H7 identification. In addition, antibiogram (Kirby-Bauer method) of the STEC strains was performed and noted.

## **2.5.4 Identification of isolates**

The identification of isolated bacteria was done by examining the cultural morphology, colour of the bacterial colonies cultured on indicator agar plates, serology testing, and conventional biochemical tests such as citrate utilization, indole reactions, and methyl red tests.

## **2.6 Statistical Analysis**

GraphPad Prism 2.01 was used to perform the statistical analysis. Prevalent rates were presented in percentages. Pearson Chi-Square was used for the analysis of categorical data and p-values < 0.05 were considered statistically significant.

## **3. RESULTS AND DISCUSSION**

The present study was carried out to assess the occurrence of *E. coli* O157 and non-O157 in abattoir meat, abattoir waste water and meat sold by roadside butchers, clinical stool samples and food sellers stool samples in Port Harcourt, Rivers State. Results from this study revealed that Shiga toxin producing *E. coli* (STEC) were isolated from the study materials analyzed. The number of STEC isolates obtained in this study varied in some locations and was equal in others. The results however showed that there was no significant difference (p>0.05) in the occurrence of STEC with respect to location (Table 1). Rumuokoro abattoir gave the highest isolation of *E. coli* O157 and non-O157 serogroups from meat 6(7.5%), 4(5%) and abattoir waste water 2(3.17%), 11(17.4) respectively (Table 1). Rumuokoro abattoir was the busiest abattoir visited in terms of human traffic as a result of a big market situated within the abattoir. Large number of cattle were slaughtered daily, and many dumpsites littered the environment. The area had cattle dungs which contaminated the

environment. Animal hides are the main source of beef contamination at the slaughter because of its easy contact with contaminated soil and animal dung. STEC pathogens on the hide can be transferred to the carcass during the skinning process. Again, cattle were slaughtered on the same concrete slab one after the other and same cutting equipment used. These conditions could aid contamination of meat with STEC in this location and consequently the waste wash water would be implicated also.





*Key AB Meat- Abattoir Meat\* AB WW- Abattoir waste water\* RS Meat- Roadside Butchers' meat. NS- Not significant*





*Key CS – Patients Stool sample\* FS - Food sellers' Stool NS- Not significant*

Egbelu, Igwuruta, and Mgbuoba abattoirs were private abattoirs located in Port Harcourt. They are smaller, cleaner and less crowded, fewer number of cattle are slaughtered compared to the bigger government owned abattoirs, this factor could have influenced the rate of STEC isolation in these locations. Also, the use of antibiotics on food animals to prevent diseases and to enhance growth may render the animals momentarily sterile giving rise to no bacterial isolation. Previous studies had detected STEC pathogens in abattoir meat and abattoir waste water [15-18]. The roadside butchers operating at the five different locations sampled, purchased meat for sale from the abattoirs sampled in this study. The isolation rate of STEC from the roadside butchers was equal in some locations but varied in others (Table 1), the reason behind this variation could be as a result of the sanitary condition of the environments where these meats are sold. The meat parts are displayed in the open without any shield from droplets, dust and flies. Some roadside butchers' site their stands near open drainage filled with dirty water, the chances of losing the specific pathogen (STEC) from a diversity of many other contaminating bacteria (total coliform) could arise. Previous studies detected STEC pathogens in abattoir meat, abattoir waste water and meat retailed in the open market [15-18].

Similarly, the results obtained in this study showed that STEC was prevalent among patients attending clinics with gastrointestinal complaints as well as food sellers in Port Harcourt (Table 2). Obio Cottage hospital, a location where most of the stool samples were collected gave the highest isolation of STEC strains compared with other sampling locations,

the reason being that the hospital with its reputation of high standard health care provision attracts a wide range of patronage from every part of Port Harcourt and Rivers state at large. Patients both children and adults with long standing health challenges sort the hospital for medical help. Futher, the present study revealed that STEC pathogens were prevalent among food sellers located in different parts of Port Harcourt. The occurrence of STEC among food sellers may have varied at the different locations as a result the level of hygiene practice observed by the food handlers.

The data obtained in this study showed that there was no significant difference based on the rate of isolation of STEC from the sample types (Table 3). This can be attributed to the nature of *E. coli* that enables it to live on a wide variety of substrates and uses mixed acid fermentation in anaerobic condition [19] however, the incidence of STEC in the various sample types varied slightly (Table 3). The highest prevalence of *E. coli* O157:H7 was obtained from abattoir meat 13.8%. Previous studies had confirmed raw meat and meat products as primary sources of STEC pathogens [2]. The prevalence of 13.8% *E. coli* O157:H7 and 16.3% non-O157 serogroup detected in this study was in agreement with the findings of Tadese et al. [20] who reported a prevalence of 9.1% for *E. coli* O157:H7 from abattoir meat in a study at Ambo town, Ethopia and 46.3% prevalence of non-O157 STEC serogroup was obtained from meat by Ayoade et al. [16] in research conducted at Osun state Nigeria. The difference in the detection rates may have been influenced by factors such as the season of the year, geographical location, age and diet of cattle sampled, and sampling method.







#### **Table 4. Sociodemographic Characteristics of Abattoir butchers, Roadside butchers and food sellers**



<u>Age</u>	<b>Total Sampled</b>	<b>Positive</b>	<b>Negative</b>	
$20$	55	15 (27.3%)	40 (72.7%)	
$21 - 40$	71	25 (35.2%)	46 (64.8%)	
>41	13	4 (30.8%)	9(62.3%)	
P value	0.6351			
Pearson Chi sq.	0.9080			
Remark	ΝS			
$K_{\Omega V}$ : $N\Omega$ - $N\Omega t$ Simplicant				

*Key: NS - Not Significant. Chi sq- Chi square*





Furthermore, the prevalence of *E. coli* O157:H7 isolated from abattoir waste water 3.2% (Table 3) agreed with the work done by Bello et al. [15, they obtained a prevalence of 4.2% on *E. coli* O157:H7 isolation from water used in washing up carcasses in a study conducted at abattoirs in North-West, Nigeria. Slaughter houses are known globally to contaminate the environment either directly or indirectly from their several procedures. The closer to the abattoir, the less portable water is for consumption [21]. Prevalence of 10.9% obtained for *E. coli* O157:H7 from the roadside meat samples (Table 3) was consistent with the 3.43% occurrence recorded by [18] obtained from retail markets in Plateau State. The variation in detection rate could be attributed to the difference in geographic regions, the sampling method and level of hygiene practice by the butchers.

Clinical stool samples had a prevalence of 6.4% *E. coli* O157:H7 in this study which is consistent with the 6% documented in a previous study by Olorunshola et al. [22]. The highest prevalence of non-O157 serogroup in this study 24.7% was obtained from the clinical stool samples, confirming the finding that illnesses linked to STEC serotypes other than O157:H7 appear to be on the increase worldwide [1] indicating that some of these may be new emerging pathogens.Many of which originated from new genetic informations transmitted through mobile genetic elements such as prophages, transposons,plasmids and genomic islands [23]. As more laboratories are testing for these organisms in clinical samples, more cases are uncovered. Some cases of non-O157 STEC illness appear to be as severe as cases associated with O157, although in general cases attributed to non-O157 are less severe [1] and may be self- limiting.

Additionally, prevalent rates as high as 13.3% of *E. col*i O157:H7 and 20% of non-O157 obtained from food sellers stool samples (Table 3) was not surprising as the population belonging to this group handles beef of various parts on a daily basis, purchased from roadside butchers who already have the meats on their tables contaminated, in the process of conveyance from abattoir to their selling points. The personal hygiene of washing hands with soap after handling meat, most often was not observed among this group (food sellers). Moreover, while meat is mostly consumed well cooked in Nigeria, thereby eliminating infections from meat consumption, food sellers are in the habit of cooking meat half cooked to prevent the meat cube from shrinking into smaller sizes, this practice and others, could lead to STEC transmission both to the cooks and the people that patronize them. It can be deduced from this study that some local food vendors in the state are asymptomatic carriers of STEC and could serve as added vehicle through which STEC infection could spread.

The consistency of beef contamination with STEC both at the abattoirs, retail roadside butchers' shops and detection among food sellers could have been influenced by the unhygienic and deplorable condition of the environments where meat are processed and sold. A good percentage of the respondents decried the state of their work place (Table 4). Secondly, the unhealthy practices by some butchers and food sellers enhanced cross contamination of meat and other food. For instance, 62.5% of the abattoir butchers do not wash the slaughtering slab and equipment adequately before the next use, such that carcass can be contaminated with gut contents of previous slaughters [15], 50% of the abattoir butchers wash meat entrails before selling, 75% of the roadside butchers said meat entrails are sold without additional washing, they are actually packed, transported and displayed for sale alongside with other beef parts while only 50% of the food sellers wash it well before cooking it. Furthermore, only 12.5% of the respondents wash their hands regularly while at work, and 25% of the respondents wash their hands before snacking while at work. The shortcomings observed in the implementation of personal hygiene can be attributed to low educational standard of the meat handlers, 25% of the

abattoir butchers, 62.5% of roadside butchers and 87.5% of food sellers had the secondary form of education which was the highest category in education. Formal and/or informal training on hygiene and sanitation of meat handling could guaranty better and safer handling. Those of them that are married but lack meat safety knowledge could serve as transmission vehicle to their family members. In this study, age of subjects did not have any influence on the result obtained (P > 0.05). STEC prevalence was found to be more among the age bracket 21 to 40 years. Anyone can be infected with STEC, but young children, older adults, and those with weakened immune systems are more likely to have severe illness (Table 5).

Statistically, sex did not affect the prevalence of STEC in this study (Table 6). STEC occurred more among females probably as a result of regular exposure to beef and beef products while cooking. This could be likened to the outbreak of O157:H7 infections linked to fresh spinach, that occurred in United States of America in October 2006 among women; 71% women were the most affected, probably reflecting that woman are more likely to consume fresh vegetables [24]. In other words, women could be said to be at risk of contracting STEC infection as a result of their natural inclination towards variety of foods and their duties as cooks.

## **4. CONCLUSION**

This study demonstrated the prevalence of STEC in Port Harcourt, with non-O157 strains occurring higher, which thus demands attention. This also indicates the need for proper hygiene management at the abattoir and among meat handlers to prevent the spread of STEC.

## **CONSENT**

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this research and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

## **ETHICAL APPROVAL**

Ethical approval was obtained from Rivers State Ministry of Health, the state hospital board ethical review committee and the state ministry of environment. Informed consent was obtained from food vendors after the purpose of the study was explained to them.

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# **COMPETING INTERESTS**

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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