

## Seroprevalence Analysis of O157 and Non O157 VTEC Serotypes in Diarrhoeic and Non-Diarrhoeic Sick Hospital Attendees in Abuja, Federal Capital Territory (FCT)

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### Abstract

In Nigeria as in many countries of the world, Verocytotoxigenic *Escherichia coli* (VTEC) have been frequently isolated from cattle and are known as important foodborne pathogen causing substantial proportion of human illnesses. The objective of the study was to analyze sick human faecal samples for the presence of both VTEC O157 and non O157 VTEC. Standard microbiological and biochemical methods were used to isolate various serotypes of VTEC from human faecal samples from both diarrhoeic and non-diarrhoeic hospital attendees from selected hospitals in Abuja, Nigeria. *E. coli* isolates ex - EMB were sub-cultured into cefixime-tellurite sorbitol MacConkey (CT-SMAC) agar and further characterized using commercially procured latex agglutination test kits. Out of the 372 samples collected, 193 were from diarrhoeic patients and 3 samples tested positive for VTEC O157 while 2 were positive for non O157 VTEC. The remaining 179 samples were from non diarrhoeic patients and one tested positive for VTEC O157 while 2 were for non O157 VTEC. The isolation of various VTEC serotypes illustrates the significance of studying the broader group of VTEC organisms from a public health perspective. One hundred and twelve (112) well-structured questionnaires distributed to respondents at risk were returned. Thirty five (35) agreed strongly to have had associations with food animals and animal products at the time; 15 agreed; 20 were undecided; 12 disagreed and 30 strongly disagreed. There was a strong indication that humans get infected by consuming contaminated beef and beef products. Proper personal and environmental hygiene should be observed in order to curb and control the prevalence of VTEC.

**Keywords:** Seroprevalence; Verocytotoxigenic *Escherichia coli*; Serotypes; Diarrhoeic; Apparently Healthy; Humans

### Introduction

Verocytotoxigenic *Escherichia coli* (VTEC) also known as Shiga toxin-producing *Escherichia coli* (STEC) is a group of food and water-borne pathogens that are known to cause human gastrointestinal illnesses with diverse clinical spectra, ranging from watery and bloody diarrhea to hemorrhagic colitis and haemorrhagic uraemic syndrome (HUS) [1,2]. VTEC are particularly hazardous due to their low infective dose (may be as low as 10 colony forming units) and their ability to survive in the infective environment [3].

Although a single serotype, O157:H7 (referred to as O157 STEC), is most associated with outbreaks, epidemiological surveillance has reported that non-O157 STEC serotypes are responsible for approximately 30% of diseases in humans [4]. The consumption of raw or undercooked meats as well as water and other food products from fecal-contaminated environments with infected animals are common routes of transmission of STEC [5].

The system of cattle production in Nigeria is somewhat free range. Cattle roam unrestricted in both urban and rural areas dropping their dung indiscriminately along as they move. Humans get exposed by matching or touching the dung thereby being at risk of VTEC infection. Ruminants (cattle, sheep and goat) that harbour the pathogen in their hindguts and shed them in feces [6] have been shown to be important reservoirs for non-O157 as well as O157 STEC. There is lack of awareness on the zoonotic implications of VTEC in Nigeria where food animals (cattle, sheep and goat) interact closely with their owners and handlers and pose a risk in the epidemiology of the infection, cattle remaining the major ruminant reservoir of VTEC [7].

Although there have been cases of VTEC infections in Nigeria, not enough research work has been conducted. In this study, an analysis of O157 and non O157 VTEC was carried out in both diarrhoeic and apparently healthy hospital attendees in Abuja, FCT to establish the seroprevalence of the infection.

## **Materials and Methods**

### **Study area**

The study was carried out in Abuja, the Federal Capital Territory of Nigeria which has a landmass of approximately 7,315 km<sup>2</sup> (about 724,473.9 hectares), and is situated within the Savannah region with moderate climatic conditions. Lying between latitude 8.25 and 9.20 North of the equator and longitude 6.45 and 7.39 East of Greenwich Meridian [8], Abuja is geographically located in the center of the country.

### **Study design**

The cross sectional epidemiological study method was used in this work. The sampling technique adopted was simple random sampling, used to select three (Abuja Municipal, Gwagwalada and Kuje) out of the six Area Councils. Simple random sampling was also used to collect samples from diarrhoeic and non-diarrhoeic individuals visiting the hospitals. The samples were analyzed microbiologically and biochemically at the University of Abuja Teaching Hospital (UATH) Gwagwalada between May, 2011 and April 2012. Ethical permit was sought and obtained from the Ethical Committee of the Teaching Hospital.

### **Methodology**

A total of 372 faecal samples were collected and analyzed. One hundred and ninety three (193) were from diarrhoeic patients while 179 were from apparently healthy persons. The samples were collected using gloves and sterile plastic universal bottles and transported to the laboratory for analysis under aseptic conditions. Buffered peptone water (BPW) was prepared and supplemented with 8 mg/litre vancomycin, 10 mg/litre cefsulodin and 0.05 mg/litre cefixime (BPW-VCC). The preparation served as enrichment media to suppress the growth of gram positive organisms. Then 0.5g of faecal sample was inoculated into 5 ml of the BPW-VCC and incubated at 37°C for 6 - 8 hours [9]. Samples were then cultured on eosin methylene blue (EMB) agar (Oxoid) and incubated at 37°C for 20 hours to observe the typical greenish sheen colouration characteristic of *E. coli* on EMB agar. Biochemical tests (indole, methyl red, vogesproskauer, citrate utilization, urease production and hydrogen sulphide production) were then carried out to confirm the isolates as *E. coli* [10,11].

Sub-culturing of *E. coli* isolates ex-EMB into plates of CT-SMAC was the next step. They were then incubated at 37°C for 24 hours [12]. Non-sorbitol fermenting isolates that appear as colourless or neutral gray with smokey centre (1 - 2 mm in diameter) were presumptive of *Escherichia coli* O157 while sorbitol fermenting that remained pinkish were presumptive of non O157 VTEC [13]. Both sorbitol fermenting and non-sorbitol fermenting isolates were stored at 4 - 8°C in nutrient agar slants in bijoux bottles for further characterization. The isolates were further characterized serologically using commercially procured latex agglutination test kits from Oxoid Ltd, Hampshire, England according to the manufacturer's instructions. The non O157 were first tested with polyvalent serocheck test kits before using the specific seroscreen test kits.

### **Result**

The isolates exhibited similar IMViC pattern of + + - - and showed negative to both urease and hydrogen sulphide production (Table 1).

Biochemical test	Reaction observed
Indole test	+
Methyl Red (M/R)	+
Voges Proskauer	-
Citrate utilization	-
Urease Production	-
H <sub>2</sub> S production	-

**Table 1:** Biochemical tests and reactions observed.

The total number of both diarrhoeic and non diarrhoeic patients tested was 372 out of which 4 were positive for O157 and 5 positive for non O157 VTEC. Of the 372 samples, 193 were diarrhoeic patients and 3 tested positive for O157 and also 3 was for non O157. The remaining 179 were for non diarrhoeic and 1 tested positive for O157 while 2 tested positive for non O157 (Tables 1 and 2).

Patient	No tested	No positive	% positive
Diarrhoeic Patients	193	3	1.55
Non diarrhoeic Patients	179	1	0.56
Total	372	4	1.08

**Table 2:** Seroprevalence of O157 VTEC in diarrhoeic and non-diarrhoeic patients.

The non O157 VTEC detected were 5 in both diarrhoeic and non diarrhoeic patients. The specific serotypes and the number detected (prevalence) were recorded (Table 3 and 4).

Patient	No tested	No positive	% positive
Diarrhoeic Patients	193	3	1.55
Non diarrhoeic Patients	179	2	1.12
Total	372	5	1.34

**Table 3:** Seroprevalence of non-O157 VTEC in diarrhoeic and non-diarrhoeic patients.

Serotype n = 179	No positive	% positive
O26	2	1.12
O103	1	0.56
O145	1	0.56
O91	-	-
O111	-	-
Untyped	1	0.56

**Table 4:** Specific non-O157 VTEC serotypes isolated.

Out of the total of 220 structured questionnaires distributed to human respondents, 112 were returned. Thirty five (35) agreed strongly to have had associations with food animals and animal products in recent time; 15 agreed; 20 were undecided; 12 disagreed and 30 strongly disagreed (Figure 1).

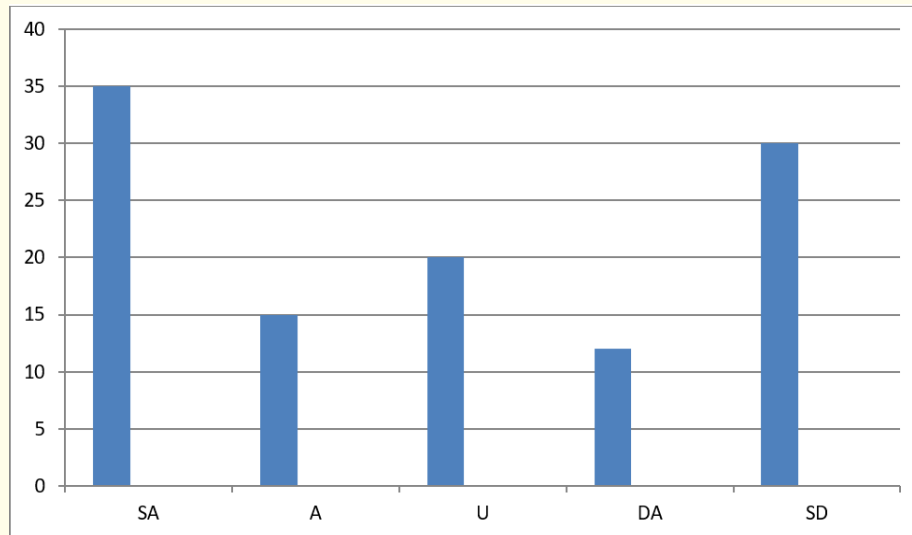


Figure 1: Response to the questionnaire.

Key: SA: Strongly Agreed; A: Agreed; U: Undecided; DA: Disagreed; SD: Strongly Disagreed.

## Discussion

Shiga toxin producing *Escherichia coli* O157 and non-O157 serogroups are known to cause serious diseases in human. Although in general, *E. coli* O157:H7 causes severe illness more frequently than non-O157 STEC, pathogenic non O157 STEC have been shown to cause the same range of symptoms as *E. coli* O157:H7, ranging from mild non-bloody diarrhea to more significant health outcomes, including HUS and death, especially in young, elderly or immune-compromised individuals [14,15].

The prevalence of O157 for both diarrhogenic and non diarrhogenic patients was 4 (1.08%) and that of non O157 was 5 (1.34%). The study established the presence of both VTEC among hospital patients in Abuja, Nigeria. According to [16], VTEC were isolated from stool samples of 126 (2.5%) of the 5054 patients investigated. *E. coli* O157:H7 was detected in 24 patients (0.5%), whereas non-O157 VTEC were detected in 104 (2.1%).

In this study, 3 (1.55%) positive VTEC O157 were isolated from diarrhogenic patients while 1 (0.56%) were from non diarrhogenic patients. In the same vein, 3 (1.55%) positive Non-O157 VTEC were isolated from diarrhogenic patients while 2 (1.12%) were from non-diarrhogenic patients. The result indicated that Non O157 isolates were slightly higher than the O157 isolates [17] in their work in Minnesota stated that Non-O157 STEC isolates were recovered from stool specimens obtained from ill patients slightly more frequently than O157 when results from the sentinel sites were combined. This suggests that non-O157 serotypes account for a substantial proportion of STEC infections in Minnesota and is consistent with other studies in the United States on the relative incidence of O157 versus non-O157 [18,19].

The result of the study indicated that there were more positive cases amongst the diarrhoeic patients than the non diarrhoeic for both the O157 and non O157 VTEC. This signifies a relationship between faecal consistency and infection with both O157 and non O157 VTEC. Diarrhea is associated with VTEC infection. In a 1997 study of 30,000 diarrheal stool samples, *E. coli* O157:H7 was the fourth most prevalent bacterial enteric pathogen [20,21] were the first to address the prevalence (4.2%) of non-O157 STEC in diarrheal samples from the Great Plains region of the northern United States, where cattle and other animal reservoirs of STEC are abundant. In Abuja Nigeria, cattle and sheep roam indiscriminately in both urban and rural settings constituting a serious risk factor for VTEC infection to man. The result of the structured questionnaire showed that more people agreed to have had association with animals and animal products.

The specific non O157 VTEC isolates tested in this study include O26, O103, O145, O111 and O91. These serogroups are among the 6 most common serogroups in the United States [3,14]. According to [22] report, a restricted range of serotypes (O157 followed by O26, O103, O91, O145 and O111) were associated with public health risks. The result of this study showed that 3 non O157 serogroups were isolated (O26 - 2; O103 - 1; O145 - 1). In a similar study, three serogroups (O26, O103, and O111) accounted for 67% of the non-O157 isolates of known serogroup [17]. One untyped serogroup was also isolated. It tested positive with the polyvalent serocheck agglutination test kits. Among patients with non-O157 STEC infections, serogroups O26, O45, O103, O111, O121, and O145 were the most common cause of hospitalizations [23].

## **Conclusion**

The findings in this work suggested that VTEC could be a significant cause of human illnesses in Abuja, Nigeria. The data also confirmed that human infections with non O157 VTEC were as common as those of O157 in Nigeria. Diarrhoeic patients were more associated with VTEC infection than the non diarrhoeic. The isolation of various VTEC serotypes illustrated the significance of studying the broader group of VTEC organisms from a public health perspective. There is a strong indication that humans get infected by consuming contaminated beef and beef products as more people agreed to have had association with animals and animal products.

Proper personal and environmental hygiene should be observed in order to curb and control the prevalence of VTEC.

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