ORIGINAL ARTICLE

Occurrence of *Escherichia coli* O157:H7 infection and its effects on haematological parameters among patients in Central Hospital, Benin City, Nigeria

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ABSTRACT

Objectives: *Escherichia coli* O157:H7 has emerged as an important food-borne pathogen of considerable public health concern. This study was conducted against the background of the paucity of data on its prevalence in secondary healthcare facilities in Benin City, Nigeria.

Methods: Stool and blood specimens were collected from 420 patients with various gastrointestinal complaints, accessing care at Central Hospital, Benin City, Nigeria. The stool specimens were cultured on MacConkey agar to recover *Escherichia coli*. The recovered *Escherichia coli* isolates were further subjected to sorbitol fermentation and the sorbitol non-fermenting isolates were further subjected to *Escherichia coli* O157:H7 latex agglutination serology. The blood specimens were used to determine the full blood count of the patients.

Results: A total of 107 (25.48%) *Escherichia coli* were recovered from the 420 stool specimens. The gender of the patients did not significantly affect the distribution of the *Escherichia coli* isolates (p=0.9114). Patients within the age group of 11 – 20 years had significantly (p<0.0001) higher rates of *Escherichia coli* recovery and recovery of *Escherichia coli* was significantly associated with diarrheagenic stool specimens (OR= 2.376; 95%CI= 1.079, 5.232; p= 0.0489). A total of 7 (6.54%) of the 107 *Escherichia coli* isolates were sorbitol non-fermenting and 5 (4.67%) of these were confirmed to be *Escherichia coli* O157:H7 serologically. The presence of *Escherichia coli* O157:H7 did not significantly alter the haematological parameters.

Conclusion: The prevalence of *Escherichia coli* O157:H7 in this study was 1.19%, and its presence did not cause any changes in haematological parameters. Measures to prevent the spread of this food-borne pathogen are advocated.

Keywords: *Escherichia coli* O157:H7, infection, haematological parameters, Hospital patients, Nigeria.

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INTRODUCTION

Escherichia coli (*E. coli*) is a Gram-negative bacteria and strains that cause diarrhoea in humans are enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), diffusely adherent *E. coli* (DAEC) or verocytotoxigenic *E. coli* (VTEC) (1). One of the VTEC stains associated with diarrhoea, bloody diarrhoea, haemorrhagic colitis and haemolytic uremic syndrome (HUS) is the Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 (2, 3). Shiga toxin-producing *E. coli* (STEC) are important causes of diarrhoea, haemorrhagic colitis, bloody diarrhoea, and haemolytic uremic syndrome (HUS) (4). STEC O157: H7 is transmitted via the faecal-oral route by ingestion of contaminated food and water as well as from person to person (5). *Escherichia coli* O157:H7 has emerged as an important food-borne pathogen of considerable public health concern, because of the severity of infection which it causes (6). This is due to characteristics such as low infective dose, ability to express different virulence factors, long survival time in the environment, unusual acid tolerance, difficulty in treatment, and their apparent special but inexplicable association with ruminants that are used for food (7-13). Studies in Nigeria have demonstrated the presence of *E. coli* O157:H7 in humans, animals and the environment (14-16). No studies among patients accessing care at a secondary health facility in Benin City, Edo State, Nigeria or studies on the effect of *E. coli* O157:H7 on haematological parameters in Nigeria were found in the literature. This is important as *E. coli* O157:H7 is reported to be associated with haemorrhagic colitis, bloody diarrhoea, and HUS (4).

Against this background, this study aimed to determine the prevalence of *E. coli* O157:H7 among patients with gastrointestinal complaints seeking care at a public secondary healthcare facility in Benin City, Nigeria. The effect of *E. coli* O157:H7 on some haematological parameters was also determined.

MATERIALS AND METHODS

Study area

The study was carried out in Central Hospital, Benin City (Oredo Local Government Area of Edo State, Nigeria), located on latitude 6.3298°N and longitude 5.6225°E. Oredo LGA has a population of 374,515 (17). This population figure was from 17 years ago, as no official census has been done since then. Central Hospital is a government-owned secondary-level hospital that serves the health needs of people in Benin City as well as other LGAs in Edo State as it attends to referral cases from primary health care centres. The study was conducted between June 2017 and May 2018.

Study population

A total of 420 patients with gastrointestinal complaints (diarrhoea, stomach discomfort and abdominal pain) attending Central Hospital, Benin City, Nigeria, were recruited for this study. Information on patients' age, gender and whether or not they had diarrhoea was obtained. Informed consent was obtained from all subjects or their parents/guardians in case of children prior to specimen collection. Approval for the study was given by the Ethical Committee of the Edo State Ministry of Health, Benin City.

Specimen collection and processing

Blood and stool specimens from each patient were collected into ethylene diamine tetra-acetic acid (EDTA) containers and sterile universal containers respectively and analysed in the laboratory within one hour of collection. The full blood count of all patients was determined using a haematology autoanalyzer – Sysmex K21N (Sysmex Corporation, Kobe, Japan) following the manufacturer's instructions.

The stool specimens were inoculated onto MacConkey agar plates. The plates were incubated at 37°C overnight. Lactose fermenting colonies were processed to get pure cultures, and the pure cultures were identified as previously described (18). An isolate was identified as *E. coli* if it was a Gram-negative bacillus, oxidase negative, lactose fermenting, motile, indole positive, citrate negative and urease negative.

Screening for Escherichia coli O157: H7

Isolates identified as *Escherichia coli* were screened using MacConkey sorbitol agar to determine if they were presumptive *E. coli* O157: H7. The *E. coli* isolates were cultured on MacConkey sorbitol agar and the plates were incubated at 37°C overnight. Isolates that did not ferment sorbitol (appear colourless) were taken as presumptive *E. coli* O157: H7.

Confirmation of *Escherichia coli* O157:H7

Confirmation of *Escherichia coli* O157: H7 was done using a latex agglutination kit (Rim *E. coli* O157: H7 latex test, Remel Inc., Santa Fe, CA, USA) following the manufacturer's instructions. For each isolate to be tested, one drop of Test Latex reagent was dispensed into a well of the test slide, labelled test. One drop of *E. coli* Control Latex was dispensed into a separate well of the test slide labelled control. Using a plastic stick (provided with the kit), a small portion of a non-sorbitol fermenting *E. coli* colony from the Sorbitol MacConkey agar plate was emulsified in the *E. coli* O157 Test Latex on the slide. Using a fresh plastic stick, the remaining portion of the non-sorbitol fermenting *E. coli* colony was emulsified in the drop of the *E. coli* Control Latex. The mixtures in the slide were rotated for 1 minute and agglutination watched out for. If there is agglutination in the test *E. coli* O157 area labelled test and no agglutination in the area labelled control, the isolate is positive for *E. coli* O157. The positive isolate was sub-cultured on blood agar, incubated overnight and the emergent colonies used to repeat the above process but using the *E. coli* H7 Latex reagent. However, the control latex is not used in this step. Agglutination confirms that the non-sorbitol fermenting *E. coli* was *E. coli* O157: H7.

Statistical analysis

The parametric data were analysed using unpaired student t–test. The non-parametric data were analysed using Chi square (χ^2) test and odd ratio (OR) analysis. The statistical software IN-STAT® (Graph Pad Software Inc., San Diego, CA, USA) was used for the analysis.

RESULTS

A total of 107 (25.48%) *Escherichia coli* were recovered from the 420 stool specimens processed. Gender was not significantly associated with *E. coli* recovery (OR= 1.053; 95 % CI= 0.673, 1.653; p= 0.9114), while the prevalence of *E. coli* was significantly (p<0.0001) higher among patients within the age group of 11 – 20 years compared to the other age groups (Table 1). The recovery of *E. coli* from stool specimens was significantly associated with diarrhoea (OR= 2.376, 95%CI = 1.079, 5.232, p = 0.0489) (Table 1). Of the 107 *E. coli* isolates, 7 (6.54%) were non-sorbitol fermenting while 5 (4.67%) were confirmed to be *E. coli* O157:H7 by serology. *Escherichia coli* O157:H7 infection did not significantly (p>0.05) affect haematological parameters (Table 2).

Table 1: Distribution of *Escherichia coli* isolates among the studied subjects

Characteristics	Subjects Tested	Subjects with *E. coli* (%)	OR	95%CI	P value
Gender			1.053	0.672, 1.653	0.9114
Male	161	42 (26.09)			
Female	259	65 (25.10)			
Age (Years)					<0.0001
≤1 – 10	30	8 (26.67)			
11 – 20	46	25 (54.35)			
21 – 30	83	12 (14.46)			
31 – 40	93	24 (25.81)			
41 – 50	73	16 (21.92)			
≥51	95	22 (23.16)			
Type of stool			2.376	1.079, 5.232	0.0489
Diarrhoea	29	11 (37.93)			
Non-diarrhoea	391	80 (20.46)			

OR = odd ratio, CI = confidence interval

Table 2: Haematological parameters of patients infected with and without *E. coli* O157: H7

Parameters	Patients infected with *E. coli* O157 H7 (n=5)	Patients infected with non-*E. coli* O157: H7 (n=102)	P value
Haemoglobin (g/L)	116.7 ± 7.3	131.1 ± 25.0	0.5816
Haematocrit (%)	35.43 ± 2.51	38.72 ± 6.11	0.6198
White blood cell count (x103/µL)	3.27 ± 0.90	5.36 ± 2.57	0.4447
Neutrophil count (%)	40.71 ± 8.94	50.78 ± 12.09	0.5085
Lymphocyte count (%)	40.86 ± 6.23	37.90 ± 10.51	0.8097

Figures are in mean ± standard error of the mean (SEM)

DISCUSSION

The prevalence of *E. coli* O157:H7 in this study was 4.67%. Gender of study participants did not significantly affect this prevalence. *E. coli* O157:H7 was recovered mostly from participants within 11 – 20 years of age and those with diarrhoea. The presence of *E. coli* O157:H7 did not alter the studied haematological parameters.

Among the bacterial agents responsible for diarrhoea and other gastrointestinal complaints, *E. coli* has been reported to be the most prevalent (1,19). In this study, a total of 107 (25.48%) of the 420 stool specimens yielded *E. coli*. This is comparable to the 27.3% reported by Ifeanyi et al. (20).

Gender had no effect on the prevalence of *E. coli* in this study (p= 0.9114). This is in agreement with a previous report (20), albeit the study subjects in Ifeanyi et al (20) study were children ≤5 years of age. Although Nweze (19) reported a higher prevalence of *E. coli* generally in females and among EPEC (enteropathogenic *E. coli*), EIEC (enteroinvasive *E. coli*), EAEC (enteroaggregative *E. coli*) and EHEC (enterohaemorrhagic *E. coli*), statistical analysis was not performed on the data.

The effect of age on the prevalence of *E. coli* was significant (p<0.0001) with the age group of 11 – 20 years having the highest prevalence. The reason for this is unclear. However, diarrhoea is a leading cause of morbidity and mortality among children less than five years of age in developing countries and the bacterial pathogen most associated with endemic forms of childhood diarrhoea is *Escherichia coli* (21, 22). The use of antibiotics in Nigeria is unregulated and over-the-counter sales of antibiotics without prescriptions are rife (23). It is possible that children less than five years of age would have been treated at the primary health centre before they arrived at the secondary health centre, and one may surmise that this may be responsible for the lower prevalence in children less than five years of age in this study.

A higher prevalence of *E. coli* was recovered from diarrheagenic stool specimens compared to non-diarrhoea stool specimens. *E. coli* is the most prevalent bacterial agent associated with diarrhoea (21, 22). This agrees with the findings in this study.

The *E. coli* O157 serogroup does not usually ferment sorbitol unlike typical *E. coli* and this characteristic is usually used to screen for *E. coli* O157 (24). Of the 107 *E. coli* isolates, 7 (6.54%) were sorbitol non-fermenting, a finding that is comparable to the 6% reported in Lagos, Nigeria (14). The prevalence reported in this study is higher than the 3.6% reported in Tanzania (25) but lower than the 11.4% reported in Zaria, Nigeria (15). The higher value reported by Chigor et al (15) may be because the isolates were recovered from water and faecal specimens. The lower prevalence reported by Raji et al (25) compared with the findings in this study may be due to the type of patients used and geographical location. Raji et al. (25) study sampled only patients with diarrhoea compared to this study where patients with gastrointestinal complaints (not only diarrhoea patients) were used.

Serological analysis revealed that 5 (71.4%) out of the 7 non-sorbitol fermenting *E. coli* was *E. coli* O157:H7. Other authors have reported that not all non-sorbitol fermenting *E. coli* are *E. coli* O157:H7 (26, 27) and this agrees with the finding in this study. The prevalence of *E. coli* O157:H7 in this study was 4.67%. In relation to total faecal specimens, the prevalence of 0157:H7 in this study was 1.19% (5/420). This is lower than 6% reported in Lagos (14) and 5.4% in Zaria (15). The difference could be due to geographical location and the type of patients used. Both studies (14, 15) used patients with diarrhoea. This study used patients with and without diarrhoea. This study was conducted in Benin City – south-south geopolitical zone; Chigor et al. (15) study was carried out in Zaria – Northwest geopolitical zone while Olorunshola et al. (14) study was in Lagos – southwest geopolitical zone. Also, the prevalence of the infection varies among children who had diarrhoea depending on geographic locations, regions within the same country, and even over time in the same location and population (28).

The presence of *E. coli* O157:H7 did not significantly (p>0.05) alter the studied haematological parameters. Patients infected with *E. coli* O157:H7 may be asymptomatic, may initially experience watery non-bloody diarrhoea or it may lead to haemorrhagic colitis, the haemolytic uremic syndrome (HUS), thrombocytopenia purpura and death (29). Patients with haemorrhagic colitis, HUS, and thrombocytopenia purpura usually have altered haematological picture. None of the patients used in this study presented with bloody diarrhoea. There was no physician to help ascertain if the patients had haemorrhagic colitis, HUS and/or thrombocytopenia purpura at the point of specimen collection. It is safe to assume that the patients recruited for this study came with signs and symptoms that did not significantly affect the haematological parameters. This may help to explain the results of this study.

CONCLUSION

In conclusion, the prevalence of *E. coli* O157:H7 observed in this study had no effect on haematological parameters despite the gastrointestinal complaints it sustained. Further studies in this regard are highly recommended and measures to prevent the spread of this food-borne pathogen are advocated.

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