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Search for *Escherichia coli* O157:H7 and Other Enterohaemorrhagic *Escherichia coli* in Diarrhoeal Cases in Abuja, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author CCAO designed the study, carried out the sample collection, sample analysis, result analysis and write up. Author OON co-analysed the results and manuscript write up. Author CIN co-designed and carried out the verocytotoxicity assay. Author AAC co-designed and carried out the cytotoxicity study. Author EII co-designed and supervised the entire study, reporting and write up of manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To detect the sporadic involvement of shiga toxin producing *Escherichia coli* (STEC) O157:H7 or any other enterohaemorrhagic *E. coli* in gastro-intestinal (diarrhoeal) infections.
Study Design: The study is a cross sectional survey for bacterial causes of diarrhoea in the study area.
Place and Duration of Study: The study was conducted over a six months period (January to July) within Abuja, Nigeria.
Methodology: One hundred and six faecal samples were collected and analyzed. Direct inoculation of diarrhoeal stool samples on Sorbitol MacConkey agar and Cefixime-Tellurite Sorbitol

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MacConkey agar for the isolation of *E. coli* O157: H7 alongside Enrichment culture in modified peptone water followed by immunomagnetic separation (IMS) with magnetic beads coated with an antibody against *Escherichia coli* O157 was used. For the identification of non-O157 STEC among the Non-sorbitol fermenting *E. coli* isolated, PCR for virulence markers was carried out. Verocytotoxicity Assay was used to detect free faecal toxin in the stool samples for the identification of other Enterohaemorrhagic *E. coli* (EHEC) infections. Routine methods were used for the isolation and identification of bacterial isolates in the specimens.

Results: Enteric bacterial pathogens detected in the study include *V. cholerae* (1.9% of subjects), *S. typhi* (1.9%), *S. paratyphi* B (0.95%), *S. paratyphi* C (0.95%), unidentified *Shigella* species (0.95%), *Shigella dysenteriae* A1 (1.9%), *Shigella flexneri* (4.7%). No *E. coli* O157:H7 was isolated. Eleven NSF *E. coli* isolated and screened for shiga toxin/verocytotoxin (*st*/*vt*) genes via PCR and latex agglutination tests were negative. However, free faecal toxin was demonstrated in 16 (15.09%) of the 106 faecal samples analyzed indicating the possible involvement of non-O157 VTEC in diarrhoeal diseases. None of the non-sorbitol fermenting *E. coli* was found to be STEC.

Conclusion: Findings mean that none of the sorbitol-negative *E. coli* isolated from this study is VTEC/STEC. Results point to the presence of non-O157 VTEC in the study area. This pathogen should be considered as a routinely sought after agent in diarrhoeal illnesses especially among children.

Keywords: Escherichia coli O157:H7; enteric infections; enterohaemorrhagic; diarrhoeal.

1. INTRODUCTION

Diarrhoeal diseases of the bowel make up a veritable aegean stable of entities. Microbiologic agents cause many; others arise in the setting of malabsorptic disorders and idiopathic inflammatory bowel disease [1]. Diarrhoeal disease is a common cause of morbidity and mortality, with worldwide distribution and is of significant public health concern [2,3]. It is one of the commonest illnesses of children and a major cause of infant and childhood mortality in developing countries of which Nigeria is inclusive. The magnitude of the problem cannot be overemphasized with an estimated 1,000 million episodes and 3.3 million deaths (range 1.5 – 5.1 million) occurring each year among under 5 year olds [4]. In the general population, there are an estimated 4 billion episodes of diarrhoeal diseases largely foodborne and waterborne with zoonotic epidemiology resulting in over 2.2 million deaths [5,6].

Susceptibility to diarrhoeal bacterial etiologic agents vary with age, nutrition and immune status of the host as well as the environment (living conditions, public health measures); and special predispositions, such as hospitalization, wartime dislocation, or foreign travels. In 40% to 50% of cases, the specific agent cannot be isolated [1]. Zoonotic pathogens of widespread diarrhoeal illnesses abound of which shiga toxin producing *Escherichia coli* (STEC) O157:H7 and some other enteric bacteria have been incriminated especially among children. Most are results of food borne infections. These outbreaks lead to morbidity, mortality and heavy economic losses. An outbreak of *Escherichia coli* foodborne illness closed a matador restaurant in the Ballard neighbourhood of Seattle, USA [7]. STEC's role in diarrhoeal illnesses is on the increase making it a notifiable disease in most countries. The role and importance of STEC in hemolytic uremic syndrome as an etiology of microangiopathic hemolytic anemia in older people and a common cause of acute renal failure in children cannot be over emphasized [8].

Usual gastrointestinal pathogens routinely sought for in Abuja, Nigeria and in the country at large are *Salmonella* and *Shigella* species. Inclusion of a less frequently encountered pathogen in any given environment in the diagnosis of gastrointestinal disease should be considered when epidemiological and possibly, clinical factors suggest an increased likelihood. The routine use of selective media for these bacteria that have very low prevalence rate or incidence is not justified. Periodic surveys of one's community to establish which pathogens are most common is recommended [9].

Thus, this study is basically a survey to see the involvement of *E. coli* 0157: H7 and other enterohaemorrhagic *E. coli* in diarrhoeal diseases in Abuja, Nigeria while excluding the possible involvement of *Salmonella/Shigella* species in such cases. It is actually a study of bacterial agents associated with diarrhoeal cases in Abuja with special reference to *Escherichia*

coli O157:H7 and other enterohaemorrhagic *Escherichia coli*. Any other bacterial pathogen sought was included when clinical factors suggest an increased likelihood.

2. METHODOLOGY

2.1 Study Area and Location

Abuja is the capital city of the Federal Republic of Nigeria with an estimated population figure of over 1.4 million people [10]. Samples were collected from: National Hospital, Abuja; Julius Berger Clinic, Gwarimpa; Zankli Medical Centre; Nyanya General Hospital; Asokoro District Hospital. Laboratory investigations were conducted in National Hospital, Abuja, Nigeria and National Veterinary Research Institute (NVRI) Vom, Nigeria. Confirmatory analysis of isolates was done in Epidemic Investigations and Surveillance Laboratory, Food borne and Diarrhoeal Diseases Laboratory Section, Centres for Disease Control and Prevention (CDC), Atlanta, Georgia, USA and National Reference Laboratory for *Escherichia coli* and *Shigella*, Food borne and Diarrhoeal Diseases Branch, Centres for Disease Control and Prevention (CDC), Atlanta, Georgia, USA.

2.2 Sample Type and Inclusion Criteria

Diarrhoeal stools with or without blood. Diarrhoea was defined for the purpose of this research as passage of three or more loose or watery stools in a 24-hour period. Bloody diarrhoea, was defined as the presence of one or more loose or watery stools that were red streaked or pink [11].

2.3 Sample Collection, Processing and Analysis

Non probability sampling technique was used and 106 fresh faecal samples were collected from 106 subjects with acute diarrhoea from January to July. The faecal samples were all collected into a clean, dry, leak proof sample container in accordance to standard routine procedure. Faecal specimens were stored at 4°C initially at the hospitals, transported by cold box, and stored at the same temperature in the Microbiology laboratory of National Hospital, Abuja. All samples were analyzed on the same day of collection.

2.4 Isolation of *Escherichia coli* O157:H7

Direct culture (and enrichment cultures followed by immunomagnetic separation (EC - IMS) were used for the isolation of *E. coli* O157:H7 [12]. Direct Culture for *E. coli* O157:H7 was done using Sorbitol MacConkey agar (SMAC) (Oxoid CM 813) and Cefixime–Tellurite sorbitol MacConkey agar (CT-SMAC) (sensitivity of 100% and a specificity of 85%, [13]). *E. coli* O157:H7 latex Agglutination Test kit (Oxoid DR 620 m) (sensitivity and specificity of 100 and 96 % respectively [14]) were used to screen for *E. coli* O157:H7. Approximately 10 µl volumes of faecal samples were inoculated directly on to SMAC [15] and CT-SMAC [16]. The plates were incubated at 37°C for 18 - 24 hours. Non-sorbitol fermenting, (NSF), colonies that appear as colourless colonies on the two plates were tested for *E. coli* O157:H7 using the *E. coli* O157: H7 latex agglutination test kit. After this, the NSF colonies were identified using conventional biochemical tests. All colonies identified as *E. coli* were stored on nutrient agar slants for polymerase chain reaction (PCR) used for detection of virulence genes of Enterohaemorhagic *Escherichia coli*.

2.5 Enrichment Culture Followed by Immunomagnetic Separation Technique

In addition to direct culture, stool samples were enriched in buffered peptone water, supplemented with vancomycin, cefsulodine and cefixime. After enrichment, immunomagnetic separation was done followed by culture on sorbitol macconkey agar and CT - SMAC.

Method: About 0.5 g of faeces was inoculated into 5 ml of BPW - VCC. The tube was vortex mixed and broth incubated at 37°C for 6 hours. Thereafter, 1 ml of broth was added to 20 µl of magnetic beads in a 1.5 ml micro centrifuge tube. The suspension was vortex mixed to suspend beads in broth culture. The tubes were then placed in a rotating mixer for 30 minutes at room temperature. After that, tubes were placed in a magnetic separator rack and the magnets put in place for 5 minutes. The culture supernatant was removed by aspiration with a Pasteur pipette. The magnetic plate was then removed from the rack. The beads were washed by re-suspension in 1 ml of PBS, PH 7.2 with Tween – 20, 0.05% v/v (PBST) and the magnetic slide were replaced for 2 minutes. The immediate step above was subsequently repeated. Then, the supernatant was removed and re-suspended in about 50µl of PBS. Beads were then inoculated onto SMAC and CT-SMAC, incubated at 37°C overnight, and examined for non-sorbitol fermenting

colonies as above. Identification of NSF colonies was by standard series of biochemical tests. All isolates confirmed as *E. coli* by biochemical tests were stored for polymerase chain reaction (PCR).

2.6 Polymerase Chain Reaction (PCR) for the Diagnosis of Diarrhoeagenic *Escherichia coli*

An alternative approach for the detection of EHEC serotypes is the use of PCR to detect virulence factors of EHEC such as shiga toxins (*stx*), *E. coli* attaching and effacing (*eae*) and enterohaemolysin (*Ehly*) genes [17].

All primary isolates of *E. coli* from faecal samples by both direct culture and IMS were stored for Polymerase Chain Reaction (PCR). PCR was done using lightcycler™ [18,19,20].

2.7 Detection of Other Serotypes of Enterohaemorrhagic *E. coli* (EHEC)

Other serotypes of Enterohaemorrhagic *E. coli* (EHEC) which do not have a single distinguishing marker such as non-fermentation of sorbitol in 24 hours like *E. coli* OI57:H7 were detected using vero cells in a tissue culture cytotoxicity assay. Faecal extracts were prepared from all samples analysed and stored for vero-cytotoxicity assay using the African Green Monkey Kidney Cells (Vero cells). Vero-cytotoxicity assay was done according to the method of [21,22].

2.8 Identification of Common Bacterial Isolates

Routine methods were used for the isolation and preliminary identification of common bacterial isolates [23].

2.9 Statistical Analysis

Statistical analysis was done using SPSS Version 10 (Computer software).

3. RESULTS

3.1 *Escherichia coli* O157:H7

Samples collected from children (between the ages of one month to fifteen years) were 58.5% while 41.5% were from adults, 46.2% from males and 53.8% from females. The highest incidence of diarrhoeal diseases occurred between the ages of zero to five years i.e. 54.7% (Table 1).

Table 1. Age distribution of diarrhoeal patients in selected hospitals in Abuja, Nigeria

Age group (years)	Number	Percent (%)
0 – 5	58	54.7
6 – 10	3	2.8
11 – 15	1	0.9
16 – 20	3	2.8
21 – 25	2	1.9
26 – 30	9	8.5
31 – 35	7	6.6
36 – 40	8	7.5
41 – 45	6	5.7
46 – 50	2	1.9
51 – 55	4	3.8
≥56	3	2.8
Total	106	100.0

X^2= 306.755, P value = 0.000, df = 11

E. coli O157 was not isolated (either via culture, PCR or Latex agglutination tests) from any of the 106 faecal samples analysed. However, 49 non-sorbitol fermenting organisms were isolated (Table 2).

(i.e. NSF *E. coli* other than *E. coli* O157:H7) constituted 22.4% of all the NSF's isolated and 10.4% of the total number of samples analysed. Other non-sorbitol fermenters include *Vibrio cholerae* (1.9% of the total number of samples analysed), *Klebsiella pneumoniae* (0.9%), *Pseudomonas* spp (8.5%), *Shigella flexneri* (4.7%), *Shigella dysenteriae A1* (1.9%), and unidentified *Shigella* species (0.9%) (The isolate was not typable but gave all the biochemical reactions of *Shigella* species but did not react with the three anti-*Shigella* antiserum available i.e. anti-*Shigella dysenteriae* A1, A2 and anti-*Shigella flexneri*). The rest of the non-sorbitol fermenters isolated included *Morganella morganii* (1.9%), *Proteus mirabilis* (1.9%), *Proteus vulgaris* (2.8%), *Providencia retgerii* (0.9%) and *Enterobacter aerogenes* (0.9%). There were nine non-sorbitol fermenters (8%) isolated that were not identified due to oversight at the early stages of the study. However, isolation of non-sorbitol fermenters in sorbitol macconkey agar was more significantly associated with *E. coli* and *Pseudomonas* specie, statistically, than other bacterial species (P<0.05).

3.2 Non-sorbitol Fermenting *E. coli* Other than *E. coli* O157:H7

Ten samples yielded eleven NSF *E. coli* isolates (Table 3). One yielded two clearly different serotypes of NSF *E. coli*. The residence of subjects from whom these organisms were

isolated seems to be fairly spread out within Abuja i.e. there is no geographical clustering of these cases. One sample was from a retroviral disease (RVD) patient resident in Makurdi on referral to National Hospital, Abuja.

Three (068, 084, 087) of the 11 NSF *E. coli* were non-motile while eight were motile. Two of the three non-motiles (084, 087) were equally non-lactose fermenting. Thus, they are non-sorbitol fermenting (NSF), non-lactose fermenting (NLF) and non-motile (NM) *E. coli* (NSF, NLF, NM *E. coli*). The only non-motile *E. coli* was lactose fermenting (NSF, LF, NM, *E. coli*). However, all the eight motile *E. coli* were lactose fermenting. None of the ten samples yielded any other bacterial pathogen. Generally, sorbitol macconkey agar (SMAC) supported the growth of these NSF *E. coli* better than cefixime-tellurite macconkey agar. Inclusion of the Immunomagnetic separation procedure did not improve the rate of isolation.

Analysis of the eleven NSF *E. coli's* for virulence markers by PCR for shiga-toxin or vero-cytotoxin production showed that none was positive for the shiga toxins, *Stx*1 and *Stx*2 and enterohaemolysin (*Ehly*) genes (Table 4). Two were positive for the *E. coli* attaching and effacing (*eae*) gene. One of the two that were positive for eae gene serotyped as *Escherichia coli* O Rough: Non-Motile. The other serotyped as *Escherichia albertii* (formerly called *Shigella boydii* 13).

3.3 Other Serotypes of Entero-haemorrhagic *E. coli* (EHEC)

Cytotoxic activities of the stool filtrates were examined for all 106 faecal samples. Free faecal toxin was demonstrated in 15.09% (16/106) (Table 5). Cytopathic effect (CPE) on the cells showed cells rounding up and detachmentof cell sheet from matrix within 24 hrs. The undiluted stool filtrates of all the samples except one induced the above effect in about 25% of monolayer within 24 hrs. The exception, sample no 058, from the five year old induced the CPE in 50% of the monolayer within 24 hrs. Of the 16 samples that showed CPE, one yielded a common enteric pathogen – *Shigella dysenteriae* A1 from stool culture (Table 5). Three of these 16 samples yielded non-sorbitol fermenting *E. coli*.

75% (12/16) were children between the ages of 0 to 3 years. This shows age predominance is statistically significant ($P< 0.05$). No sex predominance was observed. The male: female ratio is 9:7. There was no clustering of cases geographically or in time within the city. No specific food item could be implicated as the source of infection. Food history could not be obtained from majority of subjects. There was no history of recent travel abroad for all subjects. Two of the children had a member of their family also down with diarrhoea at the same time. Subject's clinical presentations of diarrhoea are as shown on Table 7.

Table 2. Non-sorbitol fermenting organisms isolated from diarrhoeal cases in selected hospitals in Abuja, Nigeria

Species	Number of isolates	Percentages (%)	
		Per total no. of NSF's isolated	Per total no. of samples analysed (n)
NSF *E. coli* (Not O157)	11	22.4	10.4
Vibrio cholerae	2	4.1	1.9
Klebsiella pneumoniae	1	2.0	0.9
Pseudomonas specie	9	18.4	8.5
Shigella flexneri	5	10.2	4.7
Shigella dysenteriae A1	2	4.1	1.9
Shigella specie	1	2.0	0.9
Morganella morgani	2	4.1	1.9
Proteus mirabilis	2	4.1	1.9
Proteus vulgaris	3	6.1	2.8
Providencia retgerri	1	2.0	0.9
Enterobacter aerogenes	1	2.0	0.9
NSF's (not identified)	9	18.4	8.5
Total	49		

X^2 = 40.408, P value = 0.000, X^2 = 40.408, P = 0.000, df = 12

Table 3. Analysis of non-sorbitol fermenting *E. coli* from diarrhoeal cases in selected hospitals in Abuja, Nigeria

Sample No	Age (years)	Sex	Residence	Appearance of samples	Isolates obtained from				Remarks
					SMAC	CT-SMAC	IMS-SMAC	IMS-CT-SMAC	
50	28	F	Nyanya	Brownish watery, No Blood Nomucus		-	+	Not cultured on this	Isolate showed poor growth and no growth at all on CT-SMAC. It was this isolate that showed the need to inoculate SMAC after IMS.
54	47	F	Wuse	Brownish, mucoid, Bloody.	+	-	+	-	This isolate seemed to require heavy inoculums to obtain a positive MR Result.
66	45	M	Gwagwa	Brownish, loose, mucoid, No Blood.	+	+	+	-	Isolates from SMAC, CT-SMAC & IMS-CT-SMAC were exactly the same. IMS-SMAC yielded mixed growth of NSF of Motility (RVD Patient)
68	$1^{2}/_{12}$	M	Old Karu	Brownish, watery, No blood, No mucus	+	-	-	-	Sample yielded just one colony on SMAC. On subculture showed poor growth on CT-SMAC. Isolate is non-motile (NM, NSF, LF *E. coli*).
75	$^{4.5}/_{12}$	F	Area 11, Garki.	Brownish, loose, mucoid with blood stains	-	-	-	+	Identified as *Proteus vulgaris*. Only IMS-CT-SMAC yielded NSF *E. coli*. Since the plate showed mixed growth of NSF's, it is possible that growth of NSF *E. coli* may have been masked in the other plates.
84	$^{4}/_{12}$	M	Mararaba	Watery, No blood, No mucus	+	+	+	+	All four plates yielded NSF *E. coli* that gave the same biochemical result. NSF, NM, NLF *E. coli.*
87	44	M	Kubwa	Dark brownish watery, No blood, No mucus	+	-	+	-	Case of chronic renal failure (CRF) biochemically similar to the immediate one above, NSF, NM, NLF *E. coli*. R. I. P.
98	40	M	Markurdi	Yellowish, watery, No blood, No Mucus.	+	+	+	+RVD Patient	Yielded 2 serotypes of NSF *E. coli*: (a) showed autoagglutination ie positive reaction on both test and control latex of *E. coli* O157:H7 serological kit. This isolate also showed gas production +. (b) The second isolate was completely negative on both test and control latex. It showed gas production ++. Both isolates were observed on SMAC plate. Only (b) was seen on CT-SMAC. Growth was generally poorer on CT-SMAC than SMA C. The 2 isolates showed larger colonies on SMAC – 2-3mm smooth slightly raised moist NSF colonies with entire edges.
101	62	M	Maitama	Brownish, Semi-formed, No blood, Nomucus	+	-	+	-	NSF's isolated on all 4 plates. Isolates on SMAC yielded NSF *E. coli*. Isolates on CT-SMAC & IMS-SMAC yielded *Morganella morganii.* Bio on NSF from IMS-CT-SMAC was not found. CONCLUSION – isolate have poor growth on CT-SMAC.
102	$1^{6}/_{12}$	M	AdisaEstate, Gudu District	Brownish, watery mucoid, No blood seen	-	+	-	-	Isolated only on CT-SMAC. Showed no motility.

Key: + = Growth - = No Growth

Table 4. Screening of non-sorbitol fermenting *E. coli* isolates from diarrhoeal cases in Abuja, Nigeria for virulence markers using PCR

Sample no	*Stx* 1 gene	*Stx* 2 gene	*Eae* gene	*Ehly* gene	Serotype
Is 50	-	-	-	-	-
Is 54	-	-	-	-	-
Is 66	-	-	-	-	-
Is 68	-	-	-	-	-
Is 75	-	-	-	-	-
Is 84	-	-	+	-	*Escherichia albertii* (formerly *Shigella boydii* 13)
Is 87	-	-	-	-	-
Is 98a	-	-	-	-	-
Is 98b	-	-	-	-	-
Is 101	-	-	-	-	-
Is 102	-	-	+	-	*Escherichia coli* O ROUGH: NON-MOTILE.

Key: + - Positive, - - Negative, PCR – Polymerase Chain Reaction, CDC – Center for Disease Control, Atlanta Georgia USA, eae - E. coli attaching and effacing, Stx 1 - Shiga toxin 1, Stx 2 – Shiga toxin 2, Ehly – Enterohemolysin

The demographics of the sixteen subjects are shown on Tables 5, 6, and 7. Age distribution was 0.08 yr – 44 yrs with the mean being ± 6 yrs (Table 6).

4. DISCUSSION

This study showed that the highest frequency of diarrhoeal diseases in Abuja occurs within the age group of 0 to 5 years (54.7%). This is in line with the internationally reported facts that children suffer more from diarrhoeal diseases than adults. A fact attributed partly to lower immunity seen in children than adults, which makes them more susceptible to infections with enteric pathogens.

4.1 *Escherichia coli* O157:H7

E. coli O157:H7 was not isolated in this study. This is in contrast to studies in some parts of the world, mostly the developed countries tailored towards detecting sporadic occurrences of *E. coli* O157:H7 in diarrhoeal diseases [15,24]. Most reported outbreaks of haemorrhagic colitis and haemolytic uraemic syndrome due to *E. coli* O157 has been in the developed countries with only a few reports coming from Africa [25,26]. Most outbreaks are food borne. Numerous studies have incriminated this pathogen in the gastrointestinal tract of varying percentages of cattle [27] from where they could lead to contamination of their meat products. There is a paucity of literature in Nigeria to show the presence or absence of this organism in the bowel flora of healthy cattle. Traditionally, in most parts of Nigeria, meat and meat products are excessively cooked before consumption. It can then be adduced that in the event of contamination, heat from excessive cooking could reduce the bacterial load or denature the organisms in such a way as to render them incapable of causing human infection. This may also be a reason for the inability to detect any sporadic case of diarrhoeal disease due to *E. coli* O157:H7 from this study. In addition, sorbitol macconkey agar does not detect all *E. coli* O157: H7, which are non-sorbitol fermenting [28] so, failure to detect *E. coli* O157: H7 by this technique does not mean that there is no *E. coli* O157: H7.

4.2 Non-sorbitol Fermenting *E. coli* and Other Bacteria Other than *E. coli* O157:H7

Non-sorbitol Fermenting (NSF) *E. coli* other than *E. coli* O157:H7 was observed (Table 3).The ability to ferment sorbitol within a 24-hr period [26] as phenotypic characteristic of most *E. coli* serotypes was observed. *E. coli* O157:H7 is the only *E. coli* among clinical isolates which does not ferment sorbitol within 24hrs and which is glucuronidase negative [29,30]. The isolation of NSF *E. coli* other than O157 is in line with the findings of Chapman and Siddons (12). However, they did not state clearly whether these NSF *E. coli* strains should be regarded as normal gastrointestinal flora or not.

Table 5. Cytotoxic activity of stool filtrates on vero cells and bacterial yield analysis of samples with cytotoxic activity in Abuja, Nigeria

Sample Nos	Age (years)	Sex	Residence	Food history	Travel history	Diarrhoeal illness in family members	Activity of stool filtrates on vero monolayer.	Organisms isolated from the stool culture.
010	$2\,^{5}/_{12}$	M	Wuse II, Urban	Cerelac, small qty of rice	Nil	Yes	+	_
025	$^{6}/_{12}$	F	Asokoro, Urban	Breast milk	Benin	No	+	_
026	27	M	Suleja, Urban	NA	NA	NA	+	_
028	$^{6}/_{12}$	F	Karmo, Periurban	NA	NA	NA	+	_
030	$^{9}/_{12}$	M	BPE, Abuja, Urban	NA	NA	NA	+	_
043	$1^{8}/_{12}$	F	Area1, Garki, Urban	Frisocrem, Tea	Kano	NA	+	_
053	$^{11}/_{12}$	M	Wuse, Urban	NA	NA	NA	+	_
058	5	F	Maitama, Urban	NA	Gurara falls	No	++	_
075	$4^{5}/_{12}$	F	Area11, Garki, Urban	Infant formular purchased at kaduna	Kaduna	Yes (older Brother)	+	NSF *E. coli*
081	$^{6}/_{12}$	M	NA	NA	Nil	No	+	_
083	3	F	Gausau (Zamfara State) Urban	NA	Nil	No	+	*Shigella dysenteriae* A1
084	$^{4}/_{12}$	M	Maraba, Periurban	NA	Nil	No	+	NSF, NLF, NM *E. coli*
087	44	M	Kubwa, Urban	NA	NA	NA	+	NSF, NLF, NM *E. coli*
094	$^{1}/_{12}$	M	Maitama, Urban	Breast Milk	Nil	No	+	_
097	$2^{6}/_{12}$	M	Wuse II, Urban	NA	NA	NA	+	_
105	$1^{8}/_{12}$	F	NA	NA	NA	NA	+	_

Key: NA – Information not available, + - Rating of morphological effect corresponding to roughly <25% of cells affected, ++ - Rating of Morphological effect corresponding to roughly 50% of cells affected

Table 6. Age groups of diarrhoeal patients with cytotoxic activity from stool filtrates in selected hospitals in Abuja, Nigeria

Age group (years)	Nos of subjects	Percent (%)
0 – 3	12	75.0
4 – 5	2	12.5
≥ 5	2	12.5
Total	16	100.0

$X^2 = 12.500$, *P value = 0.002, df = 2*

Table 7. Clinical features of diarrhoeal patients in Abuja, Nigeria whose stool filtrates gave cytotoxic activity on vero cell

Symptoms	Yes (%)	No	No response
Nausea	4 (25.00)	11	1
Abdominal Cramps	6 (37.50)	8	2
Watery Diarrhoea	13 (81.25)	3	-
Bloody Diarrhoea	3 (18.75)	13	-
Presence of mucus	8 (50.00)	8	-
Vomiting	5 (31.00)	9	2
Fever	10 (62.5)	3	3
Total no. of Patients	16		
Male/Female Ratio	9: 7		
Age Distribution	0.08 year – 45 years		
Mean	6 years		

Apart from NSF *E. coli*, eleven other non-sorbitol fermenters were isolated including known pathogens (Table 3). These isolates are similar to those obtained by Chapman and Siddons [12]. They are *Vibrio cholerae*, *Shigella dysenteriae* A1 and *Shigella flexneri*. The *Vibrio cholerae* were isolated from two sporadic cases of cholera. There was no outbreak during the study period.

This finding shows how limited the usefulness of SMAC as a screening method for *E. coli* O157: H7 may be due to its low specificity of 85% [13]. This in line with the findings of Smith et al. [28] who noted that the sensitivity of sorbitol macconkey agar method when compared with DNA probes was 73% while the specificity was only 39%. Similarly, non-specific adherence of sorbitol non-fermenting microorganism other than *E. coli* O157 to magnetic beads in the IMS technique observed in this study has been reported by other workers. Ten different types of these organisms with the two common groups being *E. coli* strains of other serogroup (32.4%) and *Proteus* spp. (19.6%) were reported by Chapman and Siddons [12]. This study reports 12 different types of these organisms with two common groups being *E. coli* strains of other serogroup (10.4%) and *Pseudomonas specie* (8.5%) (2).

No sex or age predominance was seen among subjects from whom NSF *E. coli* was isolated. Sorbitol MacConkey agar (SMAC) yielded more isolates of NSF *E. coli'* than Cefixime-Tellurite Sorbitol MacConkey agar (CT-SMAC) (Table 3). Inclusion of the immunomagnetic step (IMS) procedure did not improve the rate of isolation. One of the recognized phenotypic characteristics of *E. coli* O157:H7 is its ability to resist cefixime and tellurite while most other normal stool flora are sensitive to them. This property led to the incorporation of these two agents into SMAC to improve its selectivity for *E. coli* O157:H7. Thus, the finding here simply shows that these NSF *E. coli* strains are most probably sensitive to Cefixime and Tellurite. In addition, the IMS procedure is specifically meant to improve the isolation of *E. coli* 0157:H7 because the magnetic beads are coated with an antibody against *E. coli* O157. Therefore, inclusion of the IMS procedure should not really improve the rate of isolation of other NSF *E. coli* strains.

The 11 NSF *E. coli* screened for shiga toxin/verocytotoxin (*st*/*vt*) genes were negative. However, two were positive for the *E. coli* attaching and effacing (*eae*) gene (Table 4). The presence of *eae* gene alone has not been shown to be of clinical significance. Findings mean that none of the sorbitol-negative *E. coli* isolated from this study is VTEC/STEC. This is not surprising as most non-O157 STEC strains are known to ferment sorbitol [31]. It further highlights the need for further studies aimed at identifying the specific serotype responsible for the CPE observed during cytotoxicity test.

4.3 Other Serotypes of Entero-haemorrhagic *Escherichia coli*

In the last decade, VTEC have emerged as important pathogens of the gastrointestinal tract of individual of all ages but with an increased incidence and severity in young children and the elderly [32] as also seen in this study. The

incidence found was much higher in children between age one month to three years (75%) as against 12.5% for children between ages 5-10 years and 12.5% for adults. No specific food item could be implicated as being the source of infection (Table 5).

The lack of isolation of *E. coli* O157:H7 strains in less-developed areas of the world does not mean that STEC strains should be exempted completely as part of the burden of diarrhoeal disease in children from these areas [33]. The findings from this study have given more credence to this statement. *E. coli* belonging to serotypes other than O157:H7 have been associated with both outbreaks and sporadic diseases in animals and humans in various parts of the world [34,35].

One of the 16 samples also yielded *Shigella dysenteriae* A1 from stool culture. It is known that certain sub-lines of Hela and Vero cells are highly sensitive to shiga toxins [36]. Therefore, the finding that stool filtrate of a sample that yielded *Shigella dysenteriae* A1 gave cytotoxic activity on vero cells is not completely out of place. This finding is indicative of the possibility of a mixed infection of *Shigella dysenteriae* A1 and non-O157 VTEC.

The incidence of 15.09% makes non-O157 VTEC infection more common than any other enteric pathogen isolated during the study. This is similar to the finding in a study of stool specimens submitted to a children's Hospital in Seattle where non-O157 VTEC were found to be more common than *Yersinia* or *Shigella* species [22]. In a study from Germany carried out in hospitalized Children with diarrhoea, EHEC infections were found to be the second most common bacterial cause of diarrhoeal diseases [37]. In this study, it can be said to have been found the leading cause with its incidence of 15.09% being higher than those of other enteric pathogens isolated.

4.4 Enteric Bacterial Pathogens Isolated from the Study

The high yield of none *E. coli* O157:H7 bacteria pathogens from samples analysed shows that the greater percentage of enteric infections within the territory remains undiagnosed possibly because they may be caused by pathogens other than the ones routinely looked for. This indicates the need for inclusion of non-routine pathogens that may be significantly associated with diarrhoea in the search for causes of diarrhoeal diseases. Findings concur with that of Purwar et al. [38] who reported that due to high rate of faecal-oral transmission in developing countries it is prudent to look beyond O157:H7 serotype of *E. coli* in such epidemiological settings because of very high potential to spread across larger geographical regions and cause life threatening illness.

5. CONCLUSION

Though, this study did not yield any *E. coli* O157:H7, it does not completely rule out its involvement in diarrhoeal diseases in Abuja. There is need for a population-based study in search of this organism before its involvement in diarrhoea diseases within this region could be conclusively ruled out. The study showed involvement of 16 (15.09%) non-O157 STEC and various common enteric bacterial pathogens in diarrhoeal diseases. The new knowledge acquired on the aetiology of diarrhoea in studied subjects can inform further studies into various aspects of diarrhoeal diseases in the country.

CONSENT

Subject's informed consent was sought and received by direct explanation of the study design and purpose to subjects or their parents followed by administration of consent form and questionnaire.

ETHICAL APPROVAL

Ethical clearance was sought and received from the Management and Research Ethics Committee of National Hospital, Abuja.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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