A study on the prevalence of *Escherichia coli* O157:H7 among patients attending some public hospitals in Adamawa State, Nigeria

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Abstract: A total of three hundred stool specimens were collected from patients with complains of stomach pain (some of them with accompanied diarrhea) attending Federal Medical Centre, Yola; General Hospital, Mubi and General Hospital, Numan in Adamawa State, Nigeria. The specimens were cultured on Sorbitol MacConkey agar medium (SMAC) and 280 isolates were obtained (47 colonies were colorless whereas 233 colonies were pink in color). Biochemical tests (IMViC and sugar utilization tests) revealed that all the isolates were strains of *E. coli* but the pink colonies utilized sorbitol, mannitol, glucose and arabinose whereas the colorless colonies did not utilize sorbitol. The 47 presumptive *E. coli*O157:H7 isolates (colorless colonies on SMAC medium) did not grow on Cellobiose MacConkey Agar (CMAC) medium revealing the fact that none of them were isolates of *E. hermannii.* These 47 isolates were subjected to serological test. Eleven finally emerged seropositive *E. coli*0157:H7 signifying 3.7% infection rate among the studied population. The isolates were sensitive to gentamicin, naladixic acid, nitrofurantoin, colistin and chloramphenicol (diameter of zone 2.8-7.0 mm) at 25 µg/ml concentration. Twenty three percent of isolates collected from bl oody stool samples were seropositive for *E. coli* O157:H7 signifying the fact that there is a relationship between bloody stool and infection. There is also a relationship bet ween sex and infection at $p<0.05$.
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Keywords: SMAC, CMAC, *E.coli* O157:H7, serotype, *E. hermannii*

1. Introduction

Among the enteric *E. coli*, shiga toxin producing *E. coli* O157:H7 and non-O157:H7 have been identified as aetiological agents for haemorrhagic colitis and haemorrhagic uraemic syndrome (HUS) in humans (Von Baum and Marre, 2005). However, of the two, O157:H7 serotype is considered as being the most significant and has been associated with large food borne outbreaks in North America, Europe and Japan (White *et al* ., 2002). The Centre for Disease Control estimates that *E coli* O157:H7 causes approximately 73,000 illnesses and 61 deaths each year in the USA while non-O157:H7 STEC accounts for additional 37, 740 cases with 30 deaths (White *et al.*, 2002). A total of 9,578 cases of *E. coli* O157:H7 and 11 deaths were documented from outbreaks in Hong kong in 1996 (Hideshi *et al*., 1999). In Canada, reports of *E. coli*O157:H7 enteritis rose to a peak in 1989 and has since been on the decline (Todd, 1997). A large outbreak of bloody diarrhea caused by *E. coli* O157:H7 occurred in Southern Africa (Effler *et al.*, 2001). In 1994, three children died and six others suffered from severe diarrhea caused by *E. coli* O157:H7 after eating hamburgers, koshari and dairy products in Egypt. This pathogen was detected in 6% of unpasteurized milk, 6% of fresh retail beef, 4% of boneless chicken and 4% of lamb meat samples (Abdul-Rauf, 1996). It has been shown that *E. coli* O157:H7 has remained common particularly among the children in Lagos, Nigeria (Olorunshola *et al*., 2000). The prevalence of *E. coli*O157:H7 in 300 fresh beef and 150 roasted beef samples from Kano city of Nigeria was determined and it has been observed that the prevalence rate of *E.coli* O157:H7 in fresh beef and roasted beef were 53% and 25.3% respectively (Dahiru *et al*., 2008). The hide and carcass hygiene of cull cattle at slaughter in four geographically distant regions was examined from July 2005 to April 2006 by measuring the aerobic plate counts and the prevalence and loads of *Salmonella* and *Escherichia coli* O157:H7. The prevalence of *E. coli* O157:H7 were 46.9% and 16.7% on hides and preevisceration carcasses, respectively (Dayna *et al*., 2008). The likely cause of *E. coli* infection is undercooked ground beef. Additionally, chlorinated water, raw milk, cold sandwitches and vegetables have been implicated as source of some outbreaks. Other implicated foods include unpasteurized apple cider and juice, salad dressing containing mayonnaise, home made yoghurt, frozen meats, turkey roll and clams (FAO/WHO, 2003). Agbogu *et al* ., 2006 have reported the incidence of *E. coli* O157:H7 in food and also during outbreaks. Mead *et al* ., 1999 has estimated the incidence of *E. coli* O157:H7 to 50% among EHEC serotype in relation to public health problems. Beef and beef products such as roasted and shredded sun dried beef “Suya” and “Kilishi”, milk and milk

products such as “kindirimo”, “fura da nono” constitute a very high percentage of daily meals for the people in this state. Also water supplies come mostly from wells, rivers and boreholes. This study was aimed at investigating the prevalence of *E. coli* O157:H7 among patients with complains of stomach pain and diarrhea in three major cities in Adamawa State.

2.0 Materials and Methods

Study area :
The study area comprises Mubi, Numan and Yola in Adamawa State. Federal Medical Centre, Yola; General Hospital, Mubi and General hospital, Numan were selected for this research work. The stool samples of in and out patients (with complains of stomach pain and diarrhea) were collected from these three hospitals.

Specimen collection :
The samples were collected with the assistance of laboratory staff of respective hospitals and for each sample, sex and age of the patients were recorded. The nature of the stool samples (formed, semiformed, watery and bloody) was also recorded. Screw capped wide mouthed specimen bottles were used for sample collection. All samples were labeled and were cultured using Sorbitol MacConkey agar (SMAC) within two hours of collection.

Isolation of *E. coli* serotype O157:H7 from stool samples*:*
For isolation purpose, 18-20 ml of SMAC medium was poured in sterile petridish for each sample and streak method was employed. Colonies that appeared colorless or pale smooth, circular and shiny were presumptively identified as *E. coli*O157:H7 and the pink colonies were *E. coli* non-O157:H7. All the isolates were further subcultured for identification purpose.

Identification of isolates :
All the isolates were subjected to gram staining and motility tests (Benson, 2002). The isolates were also identified using different biochemical methods like Indole test, Methyl red test, Voges-Proskauer test, Citrate utilization test and sugar utilization tests using glucose, mannitol, maltose, arabinose, sorbitol and cellobiose (Benson, 2002).

Elimination of *E. harmannii:*
Cellobiose MacConkey Agar (CMAC) was used as differential medium to distinguish between *E. coli* O157:H7 and *E. hermannii*which has been shown to cross-react with polycl onal antibodies against *E. coli* O157:H7. All the colorless colonies on SMAC medium were cultured on CMAC medium using streak plate method and the plates were observed for growth.

Serology:
All the 47 colorless col onies on SMAC medium were tested for the O157 antigen using the Latex agglutination test kit (Oxoid). The antiserum was constituted according to the manufacturer’s instructions. Three drops of the antiserum were placed on a clean glass slide (separately). A speck of the test col ony was taken with a sterile loop, placed in the drop in the middle of the slide and mixed well. The positive and negative controls were also mixed with the other two drops of the slide separately. Any agglutination noticed with test sample was compared with the controls before recording positive or negative as the case maybe.

Sensitivity testing :
All the seropositive isolates were used for sensitivity testing. Sensitivity disks containing conventional antibiotics (at 25 µg/ml concentration manufactured by ABTEK BIOLOGICALS LTD, LIVERPOOL and generally used against gram negative bacteria). From each *E. coli* O157:H7 isolate, five representative colonies were touched with a sterile loop and was suspended in sterile distilled water and then was diluted in steps of 1:10 to adjust the suspension to 1x 10^8 density equal to the 0.5 McFarland standards before inoculation (NCCLS, 2002) and was evenly spread on 4% NaCl supplemented Mueller-Hinton agar (MHA) in Petri dishes. Each of antibiotic disks was picked with a pair of sterile forceps and applied to each uniformly seeded area of the plate spaced out so that their centers were at least 2 cm apart, incubated aerobically at 3 ^{0}C for 24h. The antibiogram was read and recorded.

3.0 Results

Description of specimens:
Out of 300 samples, one hundred and fifty five samples were collected from males and one hundred and forty five samples were collected from females. Table 1 shows the distribution of stool samples according to sex in various hospitals. As shown in Table 2, a total of 60 persons had watery stool, one hundred and two had formed and 47 had bloody stool.

Table 1: Distribution of samples according to sex in three hospitals in Adamawa State

Sites	Number of patients		Total (%)
	Males	Females	
Federal medical Centre, Yola	84	74	158 (53)
General Hospital, Mubi	32	38	70 (23)
General Hospital, Numen	39	33	72 (24)
Total	155	145	300 (100)

Table 2: Distribution of samples based on consistency, sex and age

Age (years)	Samples							
	Watery		Semiformed		Formed		Bloody	
	M	F	M	F	M	F	M	F
> 1-10	13	8	-	2	-	-	11	3
11-20	6	6	16	7	11	17	3	5
21-30	6	7	19	18	17	17	-	2
31-40	1	4	6	8	13	17	1	1
41-50	3	1	4	1	2	-	7	7
51-60	2	3	5	5	7	1	2	5
Total	31	29	50	41	50	52	24	23

Table 3: Distribution of *E. coli* O157:H7 and *E. coli* non-O157:H7 in various hospitals in Adamawa State based on presumptive identification.

Sites	Total no. of isolates	*E.coli* non O157:H7	*E. coli* O157:H7
Federal Medical Centre, Yola	154	120	34
GH, Mubi	66	60	6
GH, Numan	60	53	7
Total	280	233	47

Cultural characteristics of isolates

Out of 300 stool samples cultured on SMAC medium, 233 samples (77.7 %) yielded pink colonies and 47 samples (15.7%) produced colorless colonies. Twenty samples (6.6%) did not produce any growth. The col orless colonies are presumptive *E. coli* O157:H7 isolates. Table 3 shows the distribution of *E. coli* O157:H7 and *E. coli* non-O157:H7 in various hospitals in Adamawa State based on presumptive identification.

Results of Biochemical Tests :

Gram stain experiments revealed that all the isolates were gram negative and motile. All isolates were found to be indole and methyl red positive but Voges-Prskauer and citrate negative. Sugar utilization test shows that the pink colonies utilize sorbitol, mannitol, glucose and arabinose but not maltose and cellobiose. The colorless colonies (presumptive *E. coli* O157:H7) utilized only maltose, glucose and arabinose of all the sugars tested.

Elimination of *E. harmannii:*
All the presumptive E. coli O157:H7 (colorless colonies on SMAC medium) did not grow on CMAC medium indicating the fact that *E. harmannii* was not involved.

Results of Serotyping :
Out of 47 isolates, 11 were found to be seropositive showing the fact that 3.7% were infected with *E. coli* O157:H7. Three percent males are infected and 0.7% females were infected. Distribution of *E. coli* O157:H7 based on serotyping is shown in Table 4. Table 5 shows the distribution of infection cases based on stool consistency and age of patients Biostatistical analysis shows that there is an association between infection and sex (x^2 calculated 3.95 and x^2 tabulated 3.84 at $p<0.05$).

Table 4: Distribution of *E. coli* O157:H7 based on serotyping

Sites	A	B	C	D
Federal Medical Centre,Yola	34	6	5	1
General Hospital, Mubi	6	3	2	1
General Hospital, Numan	7	2	2	-
Total	47	11	9	2

A- Total no. of presumptive *E. coli* 157:H7; B- Total no. of seropositive is olates; C- Seropositive isolates from males; D- Seropositive isolates from females

Table 5: Distribution of infection cases based on stool consistency and age of patients

Age	Watery		Semiformed		Formed		Bloody	
(yrs)	A	B	A	B	A	B	A	B
<1-10	21	-	4	-	-	-	14	1
11-20	17	-	23	-	28	-	8	3
21-30	13	-	37	-	36	-	2	-
31-40	3	-	14	-	30	-	2	1
41-50	3	-	5	-	2	-	14	3
51-60	3	-	8	-	6	-	7	3
Total	60	-	91	-	102	-	47	11

A- Total no. examined; B- Total no. infected

Results of antibiotic sensitivity Test :
Nitrofurantoin showed definite strong antimicrobial activity (diameter of zone in the range of 6.5-7.8 mm) followed by gentamicin (diameter of zone in the range of 5.8-7.5 mm).

Table 6 shows the diameter of zones produced by various antibiotics against the 11 *E. coli*O157:H7 isolates.

Table 6: Results of susceptibility tests

Antibiotics	Diameter of zone (mm)										
	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11
Gentamicin	7.0	6.5	7.2	7.5	5.8	5.8	6.2	7.0	7.5	6.6	7.0
Ampicillin	-	-	-	-	-	-	-	-	-	-	-
Penicillin	-	-	-	-	-	-	-	-	-	-	-
Naladixic acid	3.6	3.0	3.5	3.0	3.6	3.5	3.2	3.0	3.4	3.5	3.1
Nitrofurantoin	6.5	6.7	6.9	7.2	7.0	7.0	7.5	7.0	7.8	7.5	7.0
Colistin	2.0	1.8	2.0	1.8	2.2	2.0	1.9	2.1	2.0	2.2	2.1
Streptomycin	5.0	5.2	5.0	5.1	4.9	5.0	4.7	4.5	5.1	5.0	4.7
Chloramphenicol	2.8	2.5	2.5	2.7	2.3	2.8	2.9	2.3	2.3	2.1	2.8
Erythromycin	-	-	-	-	-	-	-	-	-	-	-
Tetracycline	-	-	-	-	-	-	-	-	-	-	-
Cloxacilin	-	-	-	-	-	-	-	-	-	-	-
Cortimoxazole	-	-	-	-	-	-	-	-	-	-	-

A1-A11- seropositive isolates

4.0 **Discussion**

The three hundred stool samples from the study area yielded 47 presumptive *E. coli* O157:H7 isolates and 233 *E. coli* O157:H7 isolates. Although according to March and Ratnam (1986) that the detection of *E. coli*157:H7 in SMAC medium has a sensitivity of 100%, a specificity of 85% and accuracy of 86%, these isolates were regarded as presumptive because *E. hermannii* is known to thrive on the SMAC medium. None of the isolates grew on CMAC medium although *E. hermannii* was said to grow on it, so it may be concluded that none of the isolates was *E. hermannii* . In the IMViC test, all the isolates were indole and methyl red positive and VP and citrate negative. The pink colonies utilize sorbitol, mannitol, glucose and arabinose whereas colorless col onies did not utilize sorbitol. Out of 47 presumptive *E. coli* O157:H7 isolates, 11 isolates were seropositive indicating 3.7% isolation rate among the sampled Adamawa State population. The isolation rate was lowered than those obtained in Lagos and the provincial southwestern Nigeria (Olorunshola *et al.*, 2000). This low isolation rate could be due to two factors namely (1) the stool consistency was not considered and (2) the fact that Lagos was urbanized than the sampled areas in question. Effler *et al* ., 2001 reported that infection with *E. coli* O157:H7 in urbanized area occurred as a result of eating mass prepared food, drinking contaminated municipal water, eating undercooked beef and beef products, milk, apple cider, person to person transmission in nursing homes, day care centres, swimming in contaminated swimming pool water and eating at fast food joints.

It has been shown that this infection has an association with sex namely among men the infection is at higher rate compared to women. This is in contrast with Su and Brandt (1995) who reported that this infection affects both sexes equally. This could be due to the life st yle or cultural behavior of the people of Adamawa State. Here women are less exposed to eating outside the home than their male

counter parts who are used to eat suya , Kilishi and balangwu (sun dried and roasted beef) in restaurants and local eating places (bukkas). Abdullahi *et al*, 2005 studied the microbiological quality of three local meat products namely balangwu, tsire and Kilishi sold in Zaria, Nigeria. The general evaluation of microbial hazards showed the aerobic plate count ($\log_{10}$) for balangwu, tsire and Kilishi are 5.30, 6.27 and 4.76 respectively.

This research work agrees with the fact that stool consistency is an important factor in determining rate of isolation of *E. coli* O157:H7 from humans (Su and Brandt, 1995). The results in Table 5 show that out of 300 samples 47 were bloody stool samples and out of 47 samples eleven (twenty three percent) were seropositive. The other samples (formed, semiformed and watery) had no single seropositive isolate. The isolates were sensitive to gentamicin, naladixic acid, nitrofurantoin, colistn .streptomycin and chloramphenicol. As a rule, resistance levels in *E. coli* are usually high for broad spectrum penicillins and trimethoprim and low for third generation cephalosporins and nitrofurantoin (Von Baum and Marre, 2005). There is no consensus as to whether antimicrobials should be recommended for treatment of *E. coli* O157:H7 infections in humans. The major concern is that antimicrobial treatment of *E. coli* O157:H7 may worsen the disease by inducing the release of Shiga toxin (the cause of HUS) and also enhances the transfer of virulence factors in vivo (White *et al*., 2002). In Japan, it has been shown that antimicrobial therapy (fosfomycin) significantly reduces the number of infected children that develop HUS. Isolation of multi- drug resistant *E. coli* O157:H7 from food, animals and humans has been documented (Von Baum and Marre, 2005). The most frequently reported resistance phenotype of *E. coli* O157:H7 and O157:NM isolates being to streptomycin- sulfisoxazole-tetracycline, which accounts for over 70% of the resistant strains. Increasing resistance to fosfom ycin, the drug of choice for intestinal infections due to STEC infections in Japan, has also been documented (White *et al*, 2002). Strict measures should be recommended so that the problem of haemorrhagic colitis, haemolytic uremic syndrome and thrombotic thrombocytopenic purpura due to *E. coli* O157:H7 can be tackled effectively.

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