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Screening of human and non-human specimens for *Escherichia coli* O157:H7 and Typhoid organisms in Benin City, Nigeria

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***Escherichia coli* O157:H7 and *Salmonella* species have continued to cause gastrointestinal complaints in people in developing countries. 180 stool specimens from 80 undergraduates , 100 food vendors, and 86 specimens from non-human sources were screened for the presence of the pathogens. Fresh stool specimens were inoculated onto Sorbitol MacConkey Agar (Oxoid CM813), Selenite F Broth (Oxoid CM 395), Deoxycholate Citrate Agar (Oxoid CM 0227), MacConkey Agar (Oxoid CM 7) and Blood Agar (Oxoid CM 55). Colonies growing on media after 24 hours incubation at 37^0C were identified biochemically. Latex agglutination test reagents (Oxoid DR 620) were used for serological identification of O157:H7 strains. One (5.6%) specimen from a food vendor yielded *E.coli* O157:H7. 9(5%) specimens yielded *Salmonella* spp. Of the 86 non-human samples, 3 (3.5%) were contaminated with E. coli O157:H7. Thirty (35%) non- O157: H7 strains were also recovered; the highest proportion of 26 (30.2%) being from hawked food items. *Proteus* spp (8.1%) was the next commonly isolated pathogen. Only an isolate each of Salmonella and Shigella spp were recovered from hawked foods. The incidence of *E.coli* O157:H7 and *Salmonella* spp was low in non-human samples; a possible reason for the low incidence observed in humans in this locality.**

Keywords: *Escherichia coli* O157:H7;*Salmonella* spp; Human; Non-human; Specimens; Nigeria.

INTRODUCTION

Gastrointestinal tract diseases commonly afflict people in developing countries where insanitary environmental conditions favour persistence of etiological agents of disease. The faecal-oral route remains the commonest route of transmission of these diseases.Several etiologic agents such as *Vibrio cholerae* (Ogunsanya *et al.,* 1994), *Aeromonas* spp (Ogunsanya *et al.,*1994; Okeke *et al.,* 2000; Akinyemi *et al.,* 1998), *Plesiomonas* spp, (Akinyemi *et al.,* 1998), *Salmonella*, *Shigella, Yersinia enterolytica* (Ogunsanya *et al.,* 1994;), *Campylobacter* sp (Baffone *et al.,* 2001), as well as different strains of *Escherichia coli* (Akinyemi *et al.,* 1998; Agbonlahor and Odugbemi, 1982),have been incriminated in one form of gastrointestinal complaint or the other. Also, various prevalence rates of *Salmonella and Shigella* spp associated with gastrointestinal disorders have been recorded (Okeke *et al*., 2000; Akinyemi *et al.,* 1998; Baffone *et al*., 2001). Typhoid fever has taken its toll on the lives of people in developing countries, especially when the disease condition is mismanaged through wrong laboratory diagnosis and chemotherapy (Isibor and Okoye, 2006).

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Enterohaemorrhagic *E. coli* (EHEC) O157:H7 has severally been isolated in cases of outbreaks of diarrhea in some cities of the world (CDC, 1997; Swinbanks, 1996; Effler *et al*., 2001). Isolated cases have been reported in some Nigerian cities (Okeke *et al*., 2000; Olorunshola *et al*., 2000; Smith *et al*., 2003). Mostly due to cost containment in the management of laboratory services, most laboratories do not routinely seek EHEC together with other enteric pathogens in their isolation methods. For instance, for the differential isolation of EHEC O157:H7 from suspected specimens, the less costly MacConkey agar which does not contain sorbitol, rather than Sorbitol MacConkey Agar (enriched with growth supplements), is used. Fermentation of sorbitol is an important diagnostic feature for the identification of these strains, and EHEC O157:H7 strains are unable to ferment sorbitol.

EHEC O157:H7 is an emerging pathogen of much public health concern and therefore regular screening for the presence of this pathogen in man and his immediate environment is an important proactive measure for early detection of infection and the establishment of necessary control measures, thus avoiding dangerous sequelae such as renal failure, hemolytic uremic syndrome (Siegler *et al*., 1993; Pickering *et al*., 1994; Nataro and Kaper, 1998). Also, effective diagnosis of EHEC infections allows for early commencement of appropriate isolation procedures for infected persons.Such isolation becomes very expedient in institutional settings (Belongia *et al*., 1993) and where outbreaks could result from person – to – person transmission of infection (Bell *et al*., 1994). According to Boyce *et al.* (1995) lack of accurate diagnosis had led to numerous unnecessary and expensive procedures such as exploratory surgery, barium enema, appendectomy etc.

In this study we examined human and non-human specimens in Benin City, Nigeria, for the presence of EHEC O157:H7 and *Salmonella* spp.

MATERIALS AND METHODS

Collection of human specimens

One hundred and eighty (180) stool specimens from 80 undergraduates and 100 food vendors were collected in sterile universal containers (Sterilin) at different time. One hundred had symptoms of gastrointestinal disease, while 80 showed no symptoms (controls).Relevant approval and individual consent were got before screening subjects.

Collection of specimens from domestic animals

Using sterile hand gloves and wooden spatulas, a small portion of faecal material from each animal observed defecating, was collected into a sterile universal container Animals sampled included goats, cows, chickens, turkeys and ducks. Various sections of the abattoir were swabbed, while pieces of beef meat were transferred into sterile containers using sterile forceps. Allsamples were immediately taken to the laboratory for processing.

Collection of hawked food specimens

Samples of some food commonly hawked in the open places were randomly selected and put in sterile containers and cultured in the laboratory.

Cultural and isolation methods

A loopful of each stool specimen was inoculated and streaked on Sorbitol MacConkey Agar (Oxoid CM 0981) supplemented with cefixime and tellurite (Oxoid SR 0172), MacConkey Agar (Oxoid CM 7), Blood Agar (Oxoid CM 55) and Selenite F broth (Oxoid CM 395). All inoculated media were incubated at 37^0C for 18 – 24 hours. The selenite F broth culture was then subcultured onto Deoxycholate Citrate Agar and plates were incubated at 37^0C for 18 – 24 hours.Swabs were inoculated directly onto the culture media. About 1 gm of each food sample was suspended in 3 ml of nutrient broth (Oxoid CM 67) and incubated for 6 hours. The suspension was then streaked onto sorbitol MacConkey agar and plates were incubated at 37^0C for 18 – 24 hours.

Identification of Isolates

Non-sorbitol fermenting were identified serologically (using O157 agglutination latex reagent) and biochemically (using dulcitol, raffinose and cellubiose fermentation, as well as β-glucuronidase reaction).Non-lactose fermenters growing on Deoxycholate Citrate Agar were also identified (Cowan and Steel, 1993).

RESULTS

The organisms isolated from the human subjects are shown in Table 1. In all, 8 different organisms were isolated from human stools. Of the human subjects screened, 1(0.6%) specimen yielded *E. coli* O157:H7, while 9 (5%) yielded *Salmonella* sp.

Table 2 shows the types and numbers of microbial isolates in relation to the ages of the human subjects. The only EHEC isolate was from a food vendor within the age group of 39-42 years. All the 9 isolates of *Salmonella* sp were recovered from the stools of subjects within the age range 23-42 years. All subjects had other strains of *E. coli* and *Enterococcus faecalis* in their stools.

Table 1: Microbial isolates from human stool specimens.

Isolates	No (%) of Microbial Isolates	
	From students	From food vendors
Salmonella sp	3(1.7)	6(3.3)
EHEC O157:H7	0(0)	1(5.6)
Proteus sp	12(6.7)	16(8.9)
Klebsiella aerogenes	8(4.4)	7(3.9)
Non O157 *E.coli*	41(22.8)	14(7.8)
Enterococcus faecalis	20(11.1)	16(8.9)
Pseudomonas aeruginosa	7(3.9)	8(4.4)
Candida sp	7(3.9)	3(1.7)
Total	107(59)	73(41)

Table 2: Distribution of organisms among human subjects according toage groups.

	Number (%) of isolates in age groups									
Age group (years)	***Salmonella* sp**	**EHEC O157:H7**	***Proteus* sp**	***Klebsiella aerogenes***	**Non O157 *E. coli***	***Staph aureus***	***Enterococcus faecalis***	***Pseudomonas aeruginosa***	***Candida albicans***	**Total No(%) of isolates**
15-18	0(0)	0(0)	2(1.1)	0(0)	6(3.3)	2(1.1)	3(1.7)	0(0)	0(0)	13(7.2)
19-22	0(0)	0(0)	4(2.2)	1(5.6)	3(1.7)	1(5.6)	4(2.2)	0(0)	0(0)	13(7.2)
23-26	1(5.6)	0(0)	2(1.1)	0(0)	6(3.3)	0(0)	4(2.2)	2(1.1)	0(0)	15(8.3)
27-30	6(3.3)	0(0)	4(2.2)	0(0)	8(4.4)	2(1.1)	6(3.3)	4(2.2)	6(3.3)	36(20)
31-34	0(0)	0(0)	1(5.6)	2(1.1)	9(5.0)	0(0)	2(1.1)	0(0)	0(0)	14(7.8)
35-38	1(5.6)	0(0)	1(5.6)	0(0)	5(2.8)	3(1.7)	3(1.7)	7(3.9)	2(1.1	22(12.2)
39-42	1(5.6)	1(5.6)	0(0)	1(5.6)	3(1.7)	1(5.6)	3(1.7)	0(0)	2(1.1)	12(6.7)
43-46	0(0)	0(0)	3(1.7)	4(2.2)	2(1.1)	0(0)	1(5.6)	0(0)	0(0)	10(5.6)
47-50	0(0)	0(0)	5(2.8)	3(1.7)	2(1.1)	2(1.1)	3(1.7)	2(1.1)	0(0)	17(9.4)
51-54	0(0)	0(0)	4(2.2)	0(0)	6(3.3)	0(0)	5(2.8)	0(0)	0(0)	15(8.3)
55-58	0(0)	0(0)	2(1.1)	4(2.2)	5(2.8)	0(0)	2(1.1)	0(0)	0(0)	13(7.2)
Total	**9**	**1**	**28**	**15**	**55**	**11**	**36**	**15**	**10**	**180**

Table 3 reflects the number of subjects infected with EHEC and *Salmonella* sp alone, in relation to their symptoms. Most of the organisms isolated were recovered from subjects with symptoms of gastrointestinal complaints. The relative percentages of microbial isolates from hawked foods, abattoirs and animal faecal materials are shown in Table 4.

DISCUSSION

Escherichia coli O157:H7 has emerged as an important agent of public health concern, with many outbreaks and sporadic cases. The incidence of enteric pathogens such as *Salmonella* and *Shigella* spp, in persons with gastrointestinal complaints has been previously reported in some Nigerian communities (Okeke *et al.,* 2000*;* Akinyemi *et al.,* 1998). *Salmonella* spp have played a leading role in typhoid fever and food poisoning (Isibor and Onwuzuruigbo*,* 1999; Sandt *et al.,* 2007*).* There have been reported outbreaks of EHEC in some African countries: Swaziland (Effler *et al.,* 2001), Central African Republic (Germanii *et al.,* 1997), and Cameroon (Cunin *et al.,* 1999*),* a country close to the eastern border of Nigeria.

This study has recorded the presence of EHEC O157:H7

Table 3: Distribution of EHEC O157:H7 and *Salmonella* sp among subjects with and without symptoms.

		Bacterial Isolates	
Class of subjects.	**No. of subjects.**	**No (%) of EHEC O157:H7**	**No (%) of *Salmonella* sp**
With symptoms	100	1(1.0)	7(7.0)
Without symptoms	80	0(0)	2(2.5)
Total	**180**	**1(5.6)**	**9(5.0)**

Table 4: Microbial isolates recovered from non-human specimens.

Isolates	Hawked food	Abatoirs	Animals	Total (%)
Klebsiella aerogenes	2(2.3)	4(4.7)	9(10.6)	15(17.4)
EHEC O157:H7	1(1.2)	1(1.2)	1(1.2)	3(3.5)
E.coli	26(30.2)	0(0)	4(4.7)	30(34.9)
Proteus sp	7(8.1)	2(2.3)	2(2.3)	11(12.8)
Pseudomonas aeruginosa	5(5.8)	0(0)	4(4.7)	9(10.5)
Staph aureus	4(4.7)	2(2.3)	4 (4.7)	10(11.6)
Salmonella sp	1(1.2)	0(0)	0(0)	1(1.2)
Shigella sp	1(1.2)	0(0)	0(0)	1(1.2)
Candida sp	2(2.3)	0(0)	4(4.7)	6(7.0)
Total	**49(57)**	**9(10.5)**	**28(32.6)**	**86(100)**

and *Salmonella* sp in subjects with gastrointestinal complaints. Amongst the human subjects, a greater proportion of *Salmonella* sp (3.3%) was isolated from the stools of food vendors than University students (1.7%) (Table 1). Food vendors and restaurants attendants infected with *Salmonella* sp can serve either as active source of infections or as healthy carriers. Routine screening of this category of individuals, especially in communities where insanitary lifestyle prevails, is very necessary in order to forestall possible outbreaks of infection.

The only EHEC isolate among the human subjects was from a food vendor. There have been no recorded outbreaks of EHEC O157:H7 infections in our area of study. Other workers have reported varying rates of isolation within Nigeria. A zero prevalence rate was recorded by Akinterinwa and Paul (1982) and Dosunmu – Ogunbi *et al.* (1983). On the other hand, a prevalence rate of 7 (8.4%) out of 83 isolates of *E. coli* group was recorded by Akinyemi *et al.*(1998) in Lagos, Nigeria.

The non-O157 strains of *E. coli* isolated in this study were however not typed into their pathotypes. Other microbial isolates could go as normal intestinal flora, except in immunocompromised patients where any of the groups could initiate an infection.

The *Salmonella* organisms isolated were from subjects within ages 23-42 years, while the only EHEC O157:H7 isolate was from the age group 39 – 59 years. Although children were not screened in this study, other studies (Okeke *et al.*, 2000; Ryan *et al.,* 1986) have shown that adults might be more exposed to infections than children. There was a significant association between *E. coli* O157:H7 and *Salmonella* sp infection and gastrointestinal complaints ($P < 0.05$) (Table 3).

In the category of non-human specimens investigated, EHEC O157:H7 was recovered from hawked foods, specimens from abattoir and from domestic animals (3.5%). The frequencies of microbial isolates from non-human source were in the following decreasing order: 57%, 32.6% and 10.5% for hawked foods, animal faecal materials and abattoirs respectively (Table 4).

As shown in Table 4, nine (9) different organisms were isolated from the food items screened. This is not surprising because in this part of Nigeria, food hawkers usually convey their foodstuffs in open trolleys. Where such items are displayed on tables, they are usually exposed and fall short of standard sanitary regulations. We suggest that thorough and effective health awareness programmes be mounted to enhance personal and environmental health, in order to forestall outbreaks of preventable diseases.

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