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The Prevalence of *Shigella* and Enterotoxigenic *E. Coli* Infection amongst Patients Attending a General Hospital, North Bank Makurdi Nigeria

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ABSTRACT
The prevalence of Enterotoxigenic E. coli and Shigella spp infection was conducted at General Hospital Makurdi, Benue State, Nigeria. One hundred and eight (108) fecal specimens from consenting clinically diagnosed patients from both sexes and of all age groups were collected. Each feacal sample was inoculated onto Salmonella Shigella agar (SSA agar) and Triple sugar iron agar (TSIA). Media were incubated at 37ºc for 24 hr. Isolates were identified using standard microbiological techniques. For the study data, Chi-square (χ2) or Fisher's exact test as appropriate and odd ratio analysis were used for analysis. The prevalence of Enterotoxigenic E. coli and Shigella spp from the sample collected was 12.5% each. Favorable factors found to be associated with infection included the wetter seasons of the year and asymptomatic carriage of infection. Gender is not a statistically significant (p> 0.05) risk factor of infection in this study. There was a strong evidence of relationship between bacteria isolate and source of drinking water of the feacal sample. This suggests that it is necessary to drink safe and purified water free from pathogenic organisms as well as washing hands with antibacterial soap after going to the toilet or shaking hands since it can be a source for the transfer of bacteria from one person to the other.
Keywords: *Enterotoxigenic E.coli ,Shigella spp,General Hospital Makurdi*

Introduction

Shigella is an infectious food-borne pathogen which causes a severe diarrhea illness called shigellosis (Grondin *et al*., 2012). *Shigella* is a genus of Gram-negative, facultative anaerobic, non-spore forming, non-motile, rod-shaped bacteria related to *Salmonella* . The genus is named after Kiyoshi Shiga, who first discovered it in 1897 (Yabuuchi, 2002). Four species of *Shigella* exist (*Shigella dysenteriae, Shigella flexneri, Shigella boydii* and *Shigella sonnei*) which cause shigellosis. *Shigella flexneri* is endemic in many developing countries and causes higher mortality than other *Shigella* species (Bennish, 1991). *Escherichia coli* is a Gram-negative, facultative anaerobic, rod-shaped *bacterium* commonly found in the lower intestine of warm-blooded organisms(Singleton, 1999). Most *Escherichia coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination (Vogt

and Dippold, 2005). *Escherichia coli* and other facultative anaerobes constitute about 0.1% of gut flora and fecal–oral transmission is the major route through which pathogenic strains of the *bacterium* cause disease (Eckburg *et al.*, 2005). However, in other parts of the body, *Escherichia coli* can cause serious disease, such as urinary tract infections, bacteraemia and meningitis. (Nataro, 1998). Pathogenic *Escherichia coli* strains are categorized into pathotypes. Six pathotypes are associated with diarrhea and collectively are referred to as diarrheagenic *E. coli* (Center for Disease Control and Prevention, 2006). Shiga toxin-producing *E. coli* (STEC) may also be referred to as Verocytotoxin-producing *E. coli* (VTEC) or *enterohemorrhagic E. coli* (EHEC). This pathotype is most associated with foodborne outbreaks. They include Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAEC), Enteroinvasive *E. coli* (EIEC), Diffusely adherent *E. coli* (DAEC) . *Escherichia coli* encompass an enormous population of bacteria that exhibit a very high degree of both genetic and phenotypic diversity. *Escherichia coli* remain one of the most diverse bacterial species: only 20% of the genome is common to all strains (Lukjancenko, 2010).

Both *Escherichia coli* and *Shigella spp* infections produce potent enterotoxin and shigatoxin respectively which causes severe and life-threatening disease. These species are related and have some similarity in their symptomology, but *Escherichia coli* infections are a serious problem in children (*Wright, 2005).* These infections are responsible for approximately 165 million cases of diarrhea annually, of which 163 million are in developing countries and (Peirano *et al.,* 2006). In developing countries, 69% of all cases are children under the age of 5 years (Peng and Chompook, 2005).

Many individuals who are infected with *Shigella* expand fever, painful, bloody or mucous diarrhea, and stomach cramps (Mandomando *et al*., 2007) while the main characteristic of Enterotoxigenic

E. coli disease is a watery diarrhea, other symptoms include abdominal cramps, low-grade fever, nausea, headache, muscle ach. Primarily, shigellosis and Enterotoxigenic *E. coli* are transmitted from person to person through the fecal-oral route, but it may also spread indirectly by fecal contamination of water or food (Frank *et al.,* 2011). Little data is available for the prevalence of *Enterotoxigenic E. coli* and *Shigella* infections in Makurdi Metropolis.

Objectives

- To determine the prevalence of *Shigella spp* and Enterotoxigenic *E. coli* infection found in the stool of clinically diagnosed patients at general hospital Makurdi, Benue state.
- Determine the association of the disease with age and sex distribution among clinically diagnosed patient at General Hospital North Bank Makurdi, Benue state.

Materials and Methods

Study Area and Study Population

The study was conducted in the General Hospital, North Bank, one of the oldest and largest tertiary hospitals in Makurdi under North Bank district. The study population consisted of patients of all age group residing in the Makurdi area were the study population irrespective of sex and socio-demographic status presented in Makurdi. The groups considered for this test are: Farmers, civil servants, skilled workers, students, house wives, tailors, hair dressers and traders.

Ethical Consent

Ethical consent was sought from the patients and confidentiality was observed.

Data Collection

Demographic information about each volunteer was obtained using a structured questionnaire. Some such information includes age, marital status, occupation, educational level, gender, source of drinking water.

Sample Collection

A single, fresh, whole stool specimen (at least 3 ml or grams, ideally 10 ml or grams) was collected from One hundred and nine patients who enrolled in General Hospital, Makurdi. A faecal swab was then placed in Cary-Blair medium in a plastic screw top test tube. Stool samples were then transported to the microscopy section for screening common enteric pathogens such as *ETEC E. coli* and *Shigella spp* maintaining standard protocols. Optimal cool temperature was strictly maintained from the point of collection of stool sample to successful submission.

Media Preparation / Plates preparation

According to manufacturers instructions, sixty three grams (63g) of powered salmonella Shigella agar was weighed and dispersed in 1000m/s in distilled water. This was boiled with frequent agitation to dissolve the medium completely and cooled to about $45^{o}c$ or body temperature. It was then mixed and poured into sterile Petri dishes or plate and allowed to solidify.

Sterilization of equipment

The equipment used were washed, and sterilized in the laboratory using a hot-air oven at $160^{o}c$ for 2 hours to avoid contamination.

Preparation of Peptone water

Fifteen grams (15g) of dehydrated peptone water powder was weighed and dissolved in 1000mls of distilled water, this preparation was gently heated to dissolve the medium completely sterilize by autoclaving at 15 psi at $21^{o}c$ for 15mins this was cooled to about $45^{o}c$ or body temperature and 5mls each was dispense into bijou bottle for broth culture preparation.

For ETEC, stool samples were plated onto MacConkey agar and the plates were incubated at 37ºc for 18 hours. Lactose-fermenting individual colonies, morphologically resembling *E. coli*, were tested.

Shigella species were isolated and identified in the enteric microbiology laboratory by using standard biochemical and microbiological methods. Stool specimens were inoculated in *Shigella-Salmonella* agar plates and incubated overnight at 37ºc. Non lactose-fermenting colonies; characteristically resembling *Shigella*, were inoculated into triple sugar iron agar tube.

Triple sugar iron agar (TSIA)

This is used to determine the ability of an organism to attack a specific carbohydrate incorporated into basal growth medium with or without production of gas, along with the determination of possible hydrogen sulphide production.

Sixty five grams of dehydrated triple sugar iron agar was weighed and dissolved in 1000mls of distilled water. It was gently heated to dissolve the medium and completely distributed into tubes, sterilized by autoclaving at 10psi $(115^{o}c)$ for 15minites. Triple sugar iron agar slant was inoculated with the test organism by stabbing the butt and streaking the surface of the slant. This was incubated at $37^{o}c$ for 24 hours. Glucose is fermented only when butt appear yellow and the slant is red. Also, when glucose, lactose and/or sucrose is fermented, both butt and slant appears yellow and when there is no action, butt and slant appears red in color. Hydrogen supplied production was indicated by the presence of black precipitate (CDC, 2009).

Inoculation

The prepared SSA where dried in hot air oven at $37^{o}c$ to remove excess moisture, the plate where labeled 1-108 and the stool sample culture was inoculated in the respective plate with the aid of a sterile wire loop. The plates were incubated for 24hrs at $37^{o}c$.

Morphological Characteristics

After incubation time of the plates were observed morphologically for color, odour, obesity, consistency, size, shape, e.t.c

Characterization and identification of isolates

Morphological descriptions of colonies were carried out, physical observation and other biological tests.

Gram staining

A drop of distilled water was placed on a clean glass slide, a wire loop was flamed and used to pick a small portion of the colony from the cultured plate to make a thin smear, which heat-was fixed to dry. These smears were flood with crystal violet for 30-60 seconds and washed with tap water and flood with Lugol's iodine for another 30-60 seconds and washed with tap water and decolourized with alcohol (ethanol) for 20 seconds and washed with tap water and lastly counter stained with safranine for 20 seconds, washed with tap water and kept to air dry. These were later examined under a light microscope using an oil immersion and the morphological characteristics were observed and recorded.

Biochemical Identification

All biochemical test were done as described in Cheesbrough (2000) .Biochemical reactions of each bacterial isolate were verified using the different biochemical tests. For each sample, discrete colonies of the bacterial isolates were picked with a wire-loop and sub-cultured for biochemical analysis. The following tests were carried out:

Indole test

Indole test was used in the identification of enterobacteria. Most strains of *Escherichia coli* species break down the amino acid tryptophan with the release of indole.

The test organism was inoculated in a bijou bottle containing 3 ml of sterile tryptone water and inoculated at 35-37°c for 48hours. This was done by adding 0.5 ml of kovac's reagent and shaked gently and observed for a red colour in the surface layer within 10minutes. The appearance of a red colour in the surface layer of the bijou bottle confirmed a positive indole and no colour appearance confirmed a negative indole.

Motility test

This is used to determine if an organism is motile or non motile. Motility test agar (nutrient agar) was inoculated with a Small bit of the test organism and stab to the bottom of the bottle of the semi solid nutrient agar using a sterile straight wire. The straight wire is pulled out in the same line from the rest agar and incubated aerobically at 37°c for 48hours. The motile bacteria spreads throughout the medium (true motility), while the non motile bacteria grow only at the line of the stab.

Hydrogen Sulphide Production

Few colonies each of suspected organisms where transferred into TSIA and incubated at 37°c for 24hrs. it was then obsereved for presence of blacking Hydrogen Sulphide) and gas production. TSIA was obsereved fo Maltose, sucrose and lactose fermentation. Shigella shows red slope, Yellow booth and negative gas production. *E. coli* shows yellow booth. No hydrogen sulphide but gas production. The evidence of gas production shows crack.

Questionaire

Questionaires were used to get information on the sources of the patients' sources of drinking water, age and gender.

Statistical Analysis

Descriptive analytic technique (percentages) was used. Data obtained were analyzed using Chi-square ($\chi 2$) or Fisher's exact test as appropriate and odd ratio analysis using the statistical software INSTAT. A P-value of less than 0.05 (i.e. $P < 0.05$) was considered to be statistically significant, while P -value more than 0.05 ($P> 0.05$) was considered to be statistically not significant. Statistical Package for Social Sciences (Version 21) was used for data entry and subsequent analysis.

Results

The results from the total of one hundred and eight (108) stool samples shows the morphological characteristics of the isolated bacteria namely; (*Escherichia coli, Salmonella spp, Shigella spp, Proteus spp*) (Table 1)

Table 2 shows the biochemical characteristics of bacteria isolated from the samples collected.

Table 3 shows Gram reaction, shape of cell and arrangement of *bacteria* isolated from stool sample

Table 4 shows the different types of bacteria isolated from stool specimens of clinical diagnosed patient screened in this study. On the whole, one hundred and one (101) stool specimens yielded no growth of microbial colonies. Seven (7) specimens yielded microbial growth. The highest prevalence of bacterial growth was recorded for *salmonella spp* (4, 50%) next to *Proteus* spp(2,25%) which is more than *Escherichia coli* and *Shigella* that is our interest.

TABLE 1: MORPHOLOGICAL CHARACTERISTICS OF THE ORGANISMS ISOLATED

S/NO	COLONY CHARACTERISTICS	PROBABLE ORGANISM
1	Pink to rose-red colonies, may have bile precipitate. LF	*Escherichia coli*
2	Colorless colonies with black centers. NLF	*Salmonella typhimurium*
3	Colorless colonies. NLF	*Shigella flexneri*
4	Colorless, transparent.	*Proteus spp*

NLF-Non-lactose fermenter

LF-Lactose Fermenter

TABLE 2: BIOCHEMICAL CHARACTERISTICS OF BACTERIA ISOLATED FROM STOOL SAMPLE.

S/no	Motility	Indole	Hydrogen production	sulphide	Probable organism
1	+ve	+ve	+ve		*Shigella flexneri*
2	+ve	+ve	+ve		*ETEC Escherichia coli*

KEY

+ve-positive

-ve-Negetive

TABLE 3: GRAM REACTION AND SHAPE OF CELL OF GRAM NEGATIVE BACTERIA ISOLATED

S/No	GRAM REACTION	SHAPE OF CELL	ARRANGEMENT	PROBABLE ORGANISM
1	-ve	Rods	Clusters	*E. coli*
2	-ve	Rods	Scattered	*Shigella spp*

KEY

+ve-positive

-ve-Negetive

TABLE 4: DISTRIBUTION OF ORGANISMS ISOLATED FROM STOOL SPECIMENS IN GENERAL HOSPITAL NORTH BANK

S/No	Organisms	Frequency	Percent (%)
1	*Escherichia coli*	1	12.5
2	*Shigella spp*	1	12.5
3		2	25
4	*Proteus*	4	50
	Salmonella spp		
	Total	8	100

The prevalence of infection with *E. coli* and *Shigella spp* by source of drinking water among clinical diagnosed patients within General Hospital Makurdi districts of Benue State, though out of the organisms of research interest, *Salmonella* spp and *Proteus* spp were found as shown in Table 5. Out of the 108 persons screened, 1(0.9%) were infection gotten from drinking stream water while 2(1.9%) and 4(3.7%) where borehole water infection. No infection was found among persons that drank from rivers and well water. Here, the significance level (0.000) for the Chi-Square value (35.668) is less than the 0.05 cut off, and hence, there is a significant relationship between the source of drinkable water by bacteria isolated and infection with the pathogen.

Table 6 shows the observed frequencies of infection which were compared with the various age groups of patients. A cursory look at the table reveals that 1(0.9%) persons in the 21-30 age group were infected with *E. coli* and *Shigella spp*, while 1(0.9%) and 1(0.9%) persons within the 21-30 and 31-40 age bracket were infected with *proteus* and *salmonella* respectively, The percentage distribution of persons infected shows that there is moderate difference in the proportion of people living with *E. coli* and *Shigella spp* by person's age.

TABLE 5: CONTINGENCY TABLE OF OBSERVED FREQUENCY OF INFECTION OF BY SOURCE OF DRINKING WATER IN GENERAL HOSPITAL MAKURDI (N= 108)

	Shigella spp/ E. coli		*Proteus*	*Salmonella*	Total	Chi-Square	P-Value
	No. Infected	(%) Not Infected					
Source of Water							
Borehole	0(0.0%)	95(88.0%)	2(1.9%)	4(3.7%)	101(93.5%)		
Stream	1(0.9%)	2(1.9%)	0(0.0%)	0(0.0%)	3(2.8%)		
Well	0(0.0%)	3(2.8%)	0(0.0%)	0(0.0%)	3(2.8%)	35.668	0.00*
River	0(0.0%)	1(0.9%)	0(0.0%)	0(0.0%)	1(0.9%)		
Total	1(0.9%)	101(93.5%)	2(1.9%)	4(3.7%)	108(100.0%)		

* Statistically significant $p < 0.05$

TABLE 6: CONTINGENCY TABLE OF OBSERVED FREQUENCY OF INFECTION BY AGE OF PATIENTS IN GENERAL HOSPITAL MAKURDI (N= 108)

	Shigella spp/ E. coli		Proteus	Salmonella	Total	Chi-Square	P-Value
	No.(%) Infected	Not Infected					
0-10	0(0.0%)	25(23.1%)	0(0.0%)	1(0.9%)	26(24.1%)		
11-20	0(0.0%)	12(11.1%)	0(0.0%)	0(0.0%)	12(11.1%)		
21-30	1(0.9%)	37(34.3%)	1(0.9%)	1(0.9%)	40(37.0%)		
31-40	0(0.0%)	14(13.0%)	1(0.9%)	1(0.9%)	16(14.8%)	31.959	0.022
41-50	0(0.0%)	9(8.3%)	0(0.0%)	0(0.0%)	9(8.3%)		
51-60	0(0.0%)	0(0.0%)	0(0.0%)	1(0.9%)	1(0.9%)		
60-above	0(0.0%)	4(3.7%)	0(0.0%)	0(0.0%)	4(3.7%)		
Total	1(0.9%)	101(93.5%)	2(1.9%)	4(3.7%)	108(100.0%)		

* Statistically significant $p < 0.05$

The observed frequencies of the *bacteria* in relation to gender of persons are given in Table 7, which reflects the Fisher's exact test values for the two nominal variables ("sex of persons" and *E. coli* and *Shigella spp* infection") as well as the P-value and Odds ratio and 95% confidence interval. Since the 2-sided P-value (P = 0.50) is greater than 0.05 cut off, there is not a significant association between the two tested variables.

TABLE 7: FREQUENCIES OF *E. COLI* AND *SHIGELLA SPP* INFECTION IN RELATION TO GENDER OF PATIENTS SCREENED

Gender	Number Screened	Number Infected	(%)	Odd Ratio	95% Limit	Confidence	P-Value
Male	54	1.90%		0.981	0.946 - 1.018		0.5
Female	54	0.00%					

* Statistically significant $p < 0.05$

Discussion

Shigella spp and ETEC are the known major etiologic agents that are responsible for deaths particularly in children (Parashar, 2003). There is a significant relationship between sources of drinking water and bacteria isolated. Out of a total of one hundred and nine (108) stool samples, only 1(0.9%) were found infected with *Enterotoxigenic E. coli* and *Shigella spp.* The low infection rate may be attributed to the dry season. Unseasonable heavy rain falls may cause flooding and contamination of sources of drinking water.

In Table 6 the observed frequencies of infection with *Enterotoxigenic E. coli* and *Shigella spp* were compared with the various age groups of patients screened. Since the significance level 0.022 is greater than the P< 0.05 cut off, it can be concluded that *Enterotoxigenic E. coli* and *Shigella spp* infection are dependent of person's age. According to Slutsker *et al.,* 1997, age-specific isolation proportions from fecal specimens in adults are equivalent to population based incidence rates, although their findings suggest that infections were seen more in adults 50 years and above. In another study, however, the age specific annual incidence rate was highest for children younger than 5 years of age (Ostroff *et al.,* 1989). It can be inferred from the result obtained in Table 7 that since the P- value of 0.5 is greater than 0.05 cut off, there is no significant relationship between the sex of persons and their infection with the organism. Su and Brandt (1995) have also reported in their studies that *Enterotoxigenic E. coli* and *Shigella spp* infection

generally affects both sexes equally. Thus it can be deduced that persons' gender does not constitute a risk factor for *Enterotoxigenic E. coli* and *Shigella spp* infection.

Also, this study has revealed that 1 patient (0.9%), although asymptomatic, had *Enterotoxigenic E. coli* and *Shigella spp* isolated from their stool specimens. According to Okeke *et al*., 2000 and Opintan *et al*., (2010) healthy carriage of enteric pathogens in general and *Enterotoxigenic E.coli* in particular is high in many African studies. It is not surprising, therefore, to isolate this organism from apparently healthy individuals, as human infection caused by *E.coli* O157:H7 can present a broad clinical spectrum ranging from asymptomatic cases to death (Lim *et al*., 2010). Because asymptomatic cases can occur in outbreaks, there has been concern that persons with such infections could unwittingly spread infection to others. The existence of such asymptomatic cases during outbreaks has been well documented (Pavia *et al*., 1990; Belongia *et al.*, 1993). Asymptomatic infections have also been demonstrated in family members and other close contacts of persons with hemolytic uremic syndrome or symptomatic *E. coli* O157:H7 (Marsh *et al.*, 1992).

The rate of infection in relation to the total numbers of seasonal isolation of *Enterotoxigenic E. coli* and *Shigella spp* were documented by previous research to be high during the wet seasons of the year i.e. May through October of the four-year period of study. Only one confirmed *Enterotoxigenic E. coli* and *Shigella spp* isolations were made during this study which was conducted during the dry seasons. These findings agree with other studies (Boyce *et al.,* 1995, Chapman, 2000) which show that human infections associated with *Enterotoxigenic E. coli* and *Shigella spp* seem to be more common during the summer months, with the majority of cases occurring during the months of May through September. Ostroff *et al.* (1989) suggested that the increased shedding of *Enterotoxigenic E. coli* and by cattle during summer months may contribute to a similar seasonal pattern of *Enterotoxigenic E. coli*

associated food borne illness in humans. Thus it can be inferred that most food- borne and water-borne infections are commonest during the wet seasons of the year. Especially in Benue State, insanitary market places, over- flooded drainages and living environment as well as contaminated abattoir-work environment, could serve as sources of several bacterial infections.

Conclusion

The data from this present study indicate that certain predisposing factors such as source of drinking water, wet seasons of the year and asymptomatic carriage of infection can contribute to *Enterotoxigenic E. coli and Shigella spp* infection. Personal and environmental hygiene and adherence to good food handling practices represent the last line of defense for assuring prevention of *Enterotoxigenic E. coli and Shigella spp* infection.

Recommendations

Since the infection primarily occurs via fecal-oral route, drinking chlorine- treated water could be very useful and food hygiene measures like proper cooking of meat, consumption of pasteurized milk, washing fruits and vegetables especially those to be eaten raw There is therefore need to adopt modern sanitary methods which are discussed below to prevent the spread of *Enterotoxigenic E. coli and Shigella spp* infection, which include the following:

- It is recommended to maintain the health education activities on prevention of *Enterotoxigenic E. coli and Shigella spp* infection, which include both food industry and members of the general public
- Subsequent studies should be carried out in other university institutions since this study was limited to General Hospital Makurdi, Benue State
- Establish collaboration of overseas public health authorities in exchange of information and alert the food trade

promptly once there is incident of food contamination with Enterotoxigenic *E. coli and Shigella spp.*

- Maintaining the surveillance program of *Enterotoxigenic E. coli and Shigella spp* infection in local markets and slaughterhouse
- Personal hygiene practices such as washing of hands after visiting the toilet and after eating food.
- Similar work should be carried out during the rainy season of the year as this was carried out during the dry season.

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