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Comparative Isolation of *Escherichia coli* 0157:H7 from Diarrhoeic and Non-Diarrhoeic Children in Selected Communities in Cross River State, Nigeria

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Abstract: *Escherichia coli* O157:H7 has been considered an emerging foodborne pathogen causing severe diarrheal disease globally especially among children under the age of five years in Sub-Saharan Africa. This study was aimed at conducting a comparative study on the rate of isolation of *Escherichia coli* O157:H7 from diarrhoeic and non- diarrhoeic children in selected communities in Cross River State, Nigeria. Stool samples were collected from children under the age of five yrs and the pathogen isolated and identified using standard microbiological and biochemical procedures. Serological analysis to detect E. coli 0157:H7 serotype was carried out using Enzyme-linked immunosorbent assay (ELISA) and anti-rabbit H7 latex serum agglutination techniques. Out of 367 diarrhoeic children sampled, 70 (19.07%) were positive for *E. coli* 0157:H7 and the prevalence differed significantly ($p<0.05$) with the control. The prevalence of *E. coli* 0157:H7 between diarrhoeal (19.07%) and non-diarrhoeal (1.39%) cases also differed significantly ($p<0.05$) among the sampling areas. Diarrhoeric children below the age of one year had significantly highest prevalence of 26.83% at $p<0.05$ though no significant relationship between the sex of the children and the rate of infection with the organism was observed. Children diarrhoaeic stool therefore serves as a major vehicle in the domestic transmission of *Escherichia coli* O157:H7. Improved personal hygiene and environmental sanitation among parents and care givers can reduce the spread of diarrheal disease caused by this pathogen amongst children under the age of five years.

Keywords: *Escherichia coli* O157:H7, Childhood Diarrhoea, Nigeria

1. Introduction

In developing countries, diarrhea is one of the main causes of morbidity and mortality in children younger than 5 years of age [1]. Twenty-one percent of childhood mortality in children younger than 5 years of age in these countries is associated with diarrheoa, resulting in 2.5 million deaths per year [2]. Diarrhea kills more young children than do AIDS, malaria and measles combined [3, 4].

The different diarrheal syndromes can be caused by bacterial, viral and parasitic infections of either single or multiple aetiology [5]. *Escherichia coli*, is documented as the most-studied bacterium, which colonizes the gastrointestinal tract of most warm-blooded animals within hours or a few days after birth [6]. *Escherichia coli* O157:H7 is a serotype of the bacterial species *Escherichia coli* and is one of the Shiga toxin–producing types [4]. *Escherichia coli* O157:H7 is an important food borne pathogen of public health importance [7, 8] Food pathogens cause more than three-hundred diseases from simple diarrhea to death [9]

Infection with *E. coli* O157:H7 follows ingestion of

contaminated food or water, or oral contact with contaminated surfaces [10] The consumption of contaminated and raw food, including raw milk aids transmission [*11]*. The World Health Organization in 2008 stated that the highest number of diarrheagenic E. coli isolated in their study belonged to the O157:H7 serogroup [4]. In developing countries, the burden of diarrhoea and its mortality in children still exists. Africa and Asia account for 80% of children deaths due to diarrhoea with Nigeria ranking second with an estimated annual total of 151,700 child deaths due to diarrhoea [12]. This comparative study was hereby designed to analyze *Escherichia coli* 0157:H7 from diarrhoeic and non- diarrhoeic children in selected communities in Cross River State, Nigeria.

2. Materials and Methods

2.1. Study Area

This research was carried out in selected highly populated communities in Cross River State. The State is located in the Niger Delta Region, Southern Nigeria and it occupies an area of 299,10km^2 with an estimated population of 3,920,208 as at 2006 Nigeria population Census [13]. It is bounded to the North by Benue State, to the West by Enugu and Abia States, to the East by the Republic of Cameroon and to the South by Akwa Ibom State and the Atlantic Ocean

2.2. Sampling Design

The study area was mapped out according to political senatorial districts i.e Northern, Central and Southern Districts. Each senatorial district was further mapped out into two sampling areas (SA) each comprising of two most populated Local Government Areas (LGAs) namely SA1 (Obudu/Bekwarra LGAs), SA2 (Ogoja/Yala LGAs), SA3 (Ikom/Boki LGAs), SA4 (Obubra/Yakurr LGAs), SA5 (Akamkpa/Biase LGAs) and SA6 (Calabar Municipality/Akpabio LGAs)

2.3. Ethical Approval

Ethical approval was obtained from the Ethical Review Committee of the various health institutions. The parents/guardians were asked for and provided informed consent for participation of their children / wards in this study.

2.4. Environmental/Human Factor Investigation on Escherichia coli 0157:H7 Transmission

A standard structured questionnaire was issued to parent/guardian of each subject to obtain vital demographic, clinical and environmental data such as age, sex, occupation of parents, feeding habits sources of drinking water, diarrhoeal type, animal contact etc. of the subject.

2.5. Sample Collection

Stool specimens were obtained from children of both sexes age between 0-5yrs that had not been on any antibiotic treatment for at least 48 hours prior to sample collection. A total of 309 fresh diarrhoeric samples with the following distribution:69, 60, 63, 65, 52 and 58 collected from sampling areas 1, 2, 3, 4, 5, and 6 respectively and 216 non-diarrhoeric samples (36 from each sampling area) were collected from both out-patients and in-patients in pediatric wards and those on post-natal visits. Hospitals and Health Centers located within each sampling area were used as referral centers.

A case of diarrhoea was defined as a history of more than one stool of liquid consistency or three or more stools of loose consistency, during the previous 24 hrs. Severe diarrhoea was defined as more than three watery stools plus signs of dehydration (reduced consciousness, sunken eyes, dryness of mucous membranes, thirst and skin turgor) [14]. Samples were collected in clean, leak-proof screw-capped plastic containers containing Amies transport medium. All samples collected were transported within 24 hrs in an ice cold box at 4°C to the microbiology laboratory for analysis.

2.6. Sample Processing

About 1.0 ml of each stool samples suspension from transport medium was introduced in to 10ml of buffered peptone water containing cefixime (0.05mg/l) and vancomycin (8.0 mg/l) (BPW-CV) and vigorously vortexed for 30 seconds to homogenize the mixture. It was then incubated at 37°C for 24hrs for enrichment.

The Enzyme-linked immunosorbent assay (ELISA) technique was used for the qualitative detection of *E. coli* 0157 antigens in all enriched samples [15]. Two drops of each sample was introduced into separate wells until all the required number of wells (excluding the control wells) were used depending on the number of enriched samples to be assayed. Two drops of the positive and negative control solutions were also dropped into their respective wells. The contents were incubated at 37°C for 30 min and 2 drops of the enzyme conjugate added to each well. After a period of 30 min incubation, the contents were washed thrice using deionized water and 2 drops of chromogen added to each with gentle shaking. The results were compared to those of the positive and negative control wells. A significant colour change to yellow indicated the presence of the *E. coli* 0157 antigen bound by the anti-*E. coli* 0157 antibodies impregnated in the wells. An optical density (OD) reading greater than 0.15 also confirmed a positive result.

All enriched samples with positive ELISA results were analysed using the standard *E. coli* 0157:H7 culture technique as recommended by [16]

All enriched, ELISA positive stool samples were serially diluted to 10^{-3} using physiological saline (0.85%w/v NaCl). Approximately 0.1ml of 10^{-2} and 10^{-3} dilutions were spread plated on sorbitol MacConkey agar supplemented with cefixime (0.5mg/l) and potassium tellurite (2.5mg/l) (SMAC-CT). All cultured samples were incubated overnight at 42°C for 24 hr. sorbitol-negative colonies that appeared colourless to grey on SMAC-CT were considered positive for *E. coli*

0157:H7. Three randomly selected suspected colonies were isolated on each plate and separately subcultured on nutrient agar slants and stored at 4°C in a refrigerator.

All positive colonies isolated on nutrient agar slants were further inoculated into test tubes containing *E. coli* with MUG (*E. coli*-MUG) medium and incubated at 42°C for 18-24 h. The broth cultures were then observed under ultraviolet (uv) light of long wavelength (650nm) to detect the inability of *E. coli* 0157:H7 to cleave MUG (about 92% of *E. coli* other than *E. coli* 0157:H7 produce the enzyme glucoronidase which cleaves MUG to produce a blue fluorescent product). Positive isolates were considered as those that fermented lactose (yellow broth), produced gas (collected at the tip of the immersed durham tubes) and did not produce any fluorescence. Other confirmatory biochemical tests typical to *E coli* such as indole, methyl red, voges proskauer, citrate and lysine decarboxylase were also performed on the isolates.

Antigen typing was performed using standard *E. coli* 0157:H7 antisera (Difco Laboratories, Detroit, Mich.) produced from rabbits and preserved with glycerol using 1:2 dilution. Slide agglutination technique was used to test resuscitated colonies directly from sorbitol MacConkey agar (SMAC) as recommended by [3]. Colonies that agglutinated rapidly with the *E. coli* 0157:H7 antisera were considered as confirmed positive *E. coli* 0157:H7 colonies.

3. Results

The various percentage prevalence of *E. coli* 0157:H7 in diarrhoeal and non-diarrhoeal cases from the various sampling areas are presented in Table 1. The highest percentage prevalence of 30.77% from diarrhoeal cases was obtained in sampling area 4 (Obubra/Yakurr Local Government Areas), followed by sampling area 3 (Ikom/Boki Local Government Areas) with 25.40% while the least value of 11.54% was obtained in sampling area 5 (Akamkpa/Odukpani Local Government Areas). Significant difference ($p<0.05$) was observed in the prevalence of *E. coli* 0157:H7 in diarrhoeal cases among the various sampling areas. Non-diarrhoeal samples had prevalence of 2.78% from SA1 (Obudu/Bekwarra LGAs), SA4 (Obubra/Yakurr LGAs) and SA6 (Calabar Municipality/Akpabio LGAs) while no pathogen was isolated from SA2, SA3 and SA5. No significant difference ($P>0.05$) was observed in the percentage prevalence in non-diarrhoeal cases while the values between the diarrhoeal and non-diarrhoeal cases differed significantly ($P<0.05$).

Male diarrheal samples had highest prevalence of 28.57% obtained from sampling area 4 followed by sampling area 3 with 21.21%. The least percentage was 10.34% obtained from sampling area 2 while the overall percentage prevalence was 17.51%. In female diarrheal cases, the highest percentage prevalence was 30.00% obtained from sampling area 3 followed by 27.27% from sampling area 4. The least percentage of 10.00% was obtained from sampling area 5 while the overall percentage prevalence obtained was 18.95%. No significant difference ($p>0.05$) was observed in the percentage prevalence between both sexes as presented in table 2.

The occurrence of *E. coli* 0157:H7 in diarrhoeic children according to various age range is presented in Table 3. The age range <1year had the highest percentage prevalence of 26.83% while the age range 3-4yrs had the least value of 16.78%. The percentage prevalence of *E. coli* 0157:H7 among the various age ranges was significantly different ($P<0.05$).

In the evaluation of the impact of environmental and behavioural practices by diarrhoeic children in the transmission of *E. coli* 0157:H7, environmental factors considered with high frequency of occurrence include; drinking water (surface water: 72.86%), toilet facility (open: 62.86%) and animal contact (ruminants: 78.57%). Behavioral practices that obtained high frequencies of occurrence include eating habits (home dishes: 92.86%), Occupation of parent/guardians (farming: 77.14%), parent /guardians education background (none: 44.29%) and diarrhoeal macroscopy (bloody: 81.43%). The various frequencies of occurrence among all the factors considered had significant differences at $p<0.05$. Table 4 shows the frequency of occurrence of each factor considered while a sample of the structured questionnaire used to obtain the data.

Table 1. *Prevalence of Escherichia coli 0157:H7 in diarrhoeal and non-diarrhoeal samples from various sampling areas.*

Sampling Areas oeal	Total No. diarrhoea of samples	No. of positive diarrhoeal samples (%)	No. of non-diarrhoeal samples	No. of positive non-diarrh samples (%)
SA1	69	13 (18.84)	36	1 (2.78)
SA2	60	7 (11.67)	36	0 (0.00)
SA3	63	16 (25.40)	36	0 (0.00)
SA4	65	20 (30.77)	36	1 (2.78)
SA5	52	6 (11.54)	36	0 (0.00)
SA6	58	8 (13.79)	36	1 (2.78)
Total	367	70 (19.07)	216	3 (1.39)

Table 2. *Prevalence of Escherichia coli 0157:H7 in diarrhoeic samples from children according to various sexes.*

Sampling Area	Male		Female	
	No. of samples	No. of positive samples (%)	No. of samples	No. of positive samples (%)
SA1	32	5 (15.63)	37	8 (21.62)
SA2	29	3 (10.34)	31	4 (12.9)
SA3	33	7 (21.21)	30	9 (30.00)

Sampling Area	Male		Female	
	No. of samples	No. of positive samples (%)	No. of samples	No. of positive samples (%)
SA4	28	8 (28.57)	33	9 (27.27)
SA5	25	4 (16.00)	30	3 (10.00)
SA6	30	4 (13.33)	28	4 (14.29)
Total	177	31 (17.51)	190	36 (18.95)

Table 3. *Prevalence of Escherichia coli 0157:H7 in diarrhoeric samples from children in study area according to age range.*

Age (yrs)	No of samples	No of positive samples	Prevalence (%)
<1	41	11	26.83
1-2	67	15	22.39
3-4	143	24	16.78
5	116	20	17.24
Total	367	70	p<0.05

Table 4. *Impacts of environmental and behavoural practices on the transmission of Escherichia coli 0157:H7 in diarrhoeic stool from children in the study area.*

Factors	sampling area						Total N=70	Frequency of occurrence (%)
	SA1	SA2	SA3	SA4	SA5	SA6		
	N=13	N=7	N=16	N=20	N=6	N=8		
Environmental								
1. DRINKING WATER *								
Surface	11	7	10	12	7	4	51	72.86
Spring	2	1	2	2	1	2	10	14.29
Borehole	3	1	5	7	1	2	19	27.14
								p<0.05
2. TOILET FACILITY *								
Open	9	5	11	13	4	2	44	62.86
Pit	4	2	4	9	2	5	26	37.14
Water system	0	0	1	0	0	1	2	2.86
								p<0.05
3 ANIMAL CONTACT *								
Ruminants	12	0	13	17	3	3	23	78.57
Pigs	2	5	4	5	2	0	13	18.57
Dogs	5	0	4	6	1	1	21	30.00
Cats	1	3	1	2	4	0	7	10.00
Chicken	10	1	14	16	3	2	50	71.43
None	0	6	0	2	0	4	6	8.57
								p<0.05
Behavioural Practices								
1. EATING HABITS *								
Exclusive breast feeding	1	0	1	1	0	1	4	5.71
Home dishes	12	7	14	18	6	7	65	92.86
Hawkers	6	4	11	17	4	5	47	67.14
Restaurant	2	2	5	11	2	6	8	40.00
Infant formulas	1	2	4	3	1	0	2	15.71
								p<0.05
2. OCCUPATION OF PARENTS/GUARDIAN*								
Farming	1	0	1	2	0	1	5	7.14
Civil (office) service	11	6	14	17	4	2	54	77.14
Business	6	2	5	6	2	5	26	37.14
Artisans	2	1	2	3	0	1	9	12.86
								p<0.05
3. PARENTS/GARDIAN EDUCATION BACKGROUND								
Primary	4	2	9	7	2	2	22	31.43
Secondary	2	1	1	4	1	3	12	17.14
ertiary	2	0	1	1	0	1	5	7.14
None	5	4	5	8	3	2	31	44.29
								p<0.05
4. DIARRHOEA MACROSCOPY								
Bloody	10	5	14	17	5	6	57	81.43
Non-bloody	3	2	2	3	1	2	13	18.57

N = No of diarrhoeal samples positive for *Escherichia coli* 0157:H7

* = A sample positive for more than one factor is counted as positive for each factor

4. Discussion

This study confirmed the presence of *E. coli* 0157:H7 among children in selected communities in Cross River State, Nigeria. The prevalence of *E. coli* 0157:H7 between diarrhoeal (19.07%) and non-diarrhoeal (4.17%) cases differed significantly ($p<0.05$) among the sampling areas. Also, the prevalence among the diarrhoeal cases had significant difference ($p<0.05$) while that for non-diarrhoeal cases was insignificant ($p>0.05$). A Higher prevalence was obtained in sample areas (SA4 and SA3) which are within the Central Senatorial Districts with congested populations, high livestock farming and relatively low hygienic standards. Some communities in this location have been shown to be endemic to diarrhoea [17]

The prevalence of (19.07%) of *E. coli* 0157:H7 among children with diarrhoeal in this study agrees with the findings of [18] who conducted a similar study from Hospitalized Children in various hospitals in South- South Nigeria with an incidence rate of 20% for *E. coli* O157: H7 strains. A higher prevalence (45%) of *E. coli* O157: H7 pathotypes was obtained among diarrhoea patients who were children below five (5) years in Zaria, Kaduna State, Nigeria [19]. [20] reported a lower prevalence of 5.00% among patients with diarrhoea in some parts of Plateau State, Nigeria. In a study conducted by [21] in Edo State, Nigeria, a low prevalence of 2.7% was obtained from diarrhoeic patients. In Zahedan, Islamic Republic of Iran, [22] reported that out of 322 stool samples examined from children with diarrhoea, 21 colonies of sorbitol negative *E. coli* strains (6.5%) were isolated. Serotyping revealed 4 strains positive for *E. coli* O157: H7, out of which only 2 (0.6%) strains showed positive reaction with anti-H7 and were identified as *E. coli* O157:H7. Discrepancies in the prevalence values from different studies may be due to differences in geographical locations. Also, [23] reported the use of Loop-mediated Geothermal Amplification (LAMP) and Whole Genome Sequencing (WGS) as current diagnostic methods for the detection of *E. coli* O157:H7 to reduce discrepancies of results due to analytical methods used.

Different reports have demonstrated that *E. coli* 0157:H7 is significantly associated with childhood diarrhoea [1, 17]. This tallies with the significant difference obtained in its isolation between diarrhoeal and non-diarrhoeal cases in this study. The difference in the prevalence among diarrhoeal cases in the study areas may be due to behavioral practices by the parents or care givers. It could also be due to environmental factors.

A prevalence of 4.17% was reported from non-diarrhoeal cases in this study. This finding demonstrates that *E. coli* O157: H7 enteric infection can be found in asymptomatic individuals without the evidence of diarrhoea. This agrees with the findings of. [24, 25].

In relation to the sex of the children, there was no significant relationship between the sex of the children and their infection with the organism. It can be inferred from the result obtained in Table 2 that gender does not constitute a risk factor for *E. coli* O157:H7 infection for children under the age of five. This concurs with the findings of [26] who also reported in their studies that *E. coli* O157:H7 generally affects both sexes equally. However, [18] working with human subjects in some parts of Plateau State, Nigeria reported a significantly higher prevalence of *E. coli* O157:H7 in males with 3.43% higher than 1.57% in females though his study was not limited to children.

Diarrhoeric children below the age of one year had the highest prevalence of 26.83% at ($p<0.05$). This is in agreement with [27] who reported that the infection rate of diarrhoeal *E. coli* was found to decrease with age. Lower age groups were also reported by [28], [18], [29] to have highest prevalence among human subjects from parts of Northern Nigeria. The high rate of isolation within lower age groups may be due to the fact that the immune system is still developing or exposure to unhygienic environments. Immunocompromised subjects, such as those with HIV/AIDS have also been shown to have high prevalence of *E. coli* O157:H7 infection [29]

Older children within the age group of 1-5 years had a lower prevalence and this may be associated with the development of immunity or loss of receptors for some specific adhesion molecules [30-32]

Table 4 shows that the source of drinking water, contact with animals and poor toilet facilities poses a great risk to diarrhoeral infections and this agrees with the report of [10] These factors have also been implicated recently with the transmission of shiga toxin-producing *E. coli* O157:H7 in Africa [33]. Water used by the inhabitants of most of the studied communities for drinking and other domestic use were observed to be mostly untreated surface water or unprotected underground water. Most households had free-ranged domestic animals and poor sewage disposal systems. The implication of these factors as risk factors in the transmission of the pathogen in this study was therefore not surprising.

5. Conclusion

The prevalence rate of E. coli in this study was high. The findings of this study also reveals that *E. coli* 0157:H7 is responsible for infectious diarrhoea among children. Since the infection primarily occurs via fecal-oral route, food hygiene measures like consumption of pasteurized milk and drinking chlorinated water could reduce transmission rate. Personal hygiene such as the habit of hand-washing with soap and clean water after visiting toilet and before feeding the child by the mothers and caretakers will significantly reduce the spread of diarrhea caused by *E. coli* O157:H7 in children.

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