

Research

Bacteria Agents of Diarrhea among Children of 5 Years and Younger in Yenagoa, Bayelsa State, Nigeria

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Abstract

This study investigated the prevalence of enteric bacteria in 0 – 5 years children in Bayelsa state, Nigeria. Stool samples from 139 children with diarrhea symptoms were obtained from some private and public health institutions in Yenagoa Metropolis. The samples was analyzed using MacConkey, Eosin Methylene blue agar, Salmonella-Shigella agar under aerobic condition and isolates were identified based on biochemical test. Of the total samples 97.1% contain enteric bacteria, which *Escherichia coli* and *Enterobacter* species was the most prevalent pathogen with incidence rate of 42.2% and 10.4% respectively. Other enteric bacteria isolated include *Shigella*, *Klebsiella* and *Salmonella* species. Based on sex, the prevalence of the *E. coli* were 44.6% and 38.1% for female and male respectively. Intermittent survey of enteric pathogens causing diarrhea in 0 – 5years children should be emphasized to elucidate their epidemiological importance and ease valuable control measures in the study area.

Keywords: Diarrhea; Enteric Bacteria; *Escherichia coli*; Public Health; Sanitation

Introduction

Infectious diarrhea continues to be a health burden (causing diseases and death) across the globe especially in children living in developing nations [1-3]. Diarrhea is one of the major disease conditions that cause morbidity, mortality [1, 4-7] and hospital admissions among children in developing countries including sub-Saharan Africa [7]. Diarrhea is caused by diverse group of organisms including viruses, fungi, bacteria and parasites [5, 7, 8]. According to World Health Organization, diarrhea is the passing of ≥ 3 stools which usually take the shape of the container within a 24 hour period [9]. Typically, a diarrhea condition lasting < 14 days are often referred to as acute diarrhea, while > 14 days are regarded as persistent diarrhea[9].

Diarrhea is transmitted from person-to-person and consumption of foods and water contaminated with human fecal materials with a pathogenic strain[5, 10]. Also, Gautam et al. [5] noted that diarrhea infection occurs mostly in poor or unhygienic sanitary practices. Children, elderly and susceptible travelers are at risk in developing countries [5]. Major symptom of diarrhea is dehydration and it could be fatal, which leads to about 1.8 million deaths annually [2]. Bacteria associated with infantile acute diarrhea in both developing and developed nations include *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia enterocolitica*, *Vibrio cholera*, and diarrheagenic [1]. *Escherichia coli* have been commonly implicated as the main cause of diarrhea in humans including children. According to Ali et al [10]. Diarrheagenic *E.coli* are vital intestinal pathogens that cause several gastrointestinal diseases especially among children in developing nations. *Escherichia coli* strains associated with diarrhea are grouped into six including enteropathogenic *E.coli* (EPEC) (which causes infantile diarrhea); enterotoxigenic *E.coli* (ETEC) (which causes traveller's diarrhea); enteroinvasive *E.coli* (EIEC) (which causes bacillary dysentery-like diarrhea); enterohemorrhagic *E.coli* (EHEC)

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also known as shigatoxigenic *E.coli*(STEC) (which causes bloody diarrhea and hemolytic uremic syndrome); diffusely adherent *E.coli* (DAEC); and Enteroaggregative *E.coli* (EAEC) [7]. Enteroaggregative *E.coli* is known to affect children <5 years than other children >5 years [11]. Also ETEC and EAEC have been reported in a substantial percentage of childhood and travelers' diarrhea, suggesting that some sub-types of these categories could have larger epidemiological importance than others [3]. *Escherichia coli*, a short-rod shaped, gram-negative bacterium commonly found in gastrointestinal tracts [12]. *Escherichia coli* is a typical indicator organism that belongs to the family *Enterobacteriaceae*, and is one of the major causes of both nosocomial and community acquired infections in humans [13]. Other bacteria belonging to the same family with *E.coli* include *Salmonella*, *Shigella*, *Klebsiella*, *Enterobacter species* etc. Diarrhea disease is one of the leading public health importance among children <5 years, causing death of over 315,000 children of preschool age in Nigeria annually [14]. The pathotype of diarrheagenic *E. coli* including prevalence and characterization in children have been carried out in some area in Nigeria including Federal Capital Territory [15 -17], South-western Nigeria [18]. Also authors have variously reported enteric bacteria associated with childhood diarrhea in several locations in Nigeria including Owerri, Imo state [19], Federal Capital Territory, Abuja [16], Abakaliki, Ebonyi state [20], Enugu and Onitsha, southeastern Nigeria [21, 22], Shagari area in Akure South local government of Ondo state [23]. But to the best of our knowledge, information about diarrheagenic bacteria from children is scanty in Bayelsa state. Hence, this study aimed at determining the prevalence of diarrheagenic bacteria among 0 – 5 years children attending public and private hospital in Bayelsa state, Nigeria.

Materials and Methods

Study area

Yenagoa metropolis is the capital of Bayelsa State, Nigeria. Majority of the residents Yenagoa metropolis are civil servants [24]. Bayelsa state is within the sedimentary basin and fishing is a major occupation of the indigenous people of the area. Urban flooding frequently occurs in the area almost at annual basis. Flooded water has the potential to enter the ground water aquifer causing contaminations. The area has high water table. Surface water is major dumpsite in the area. Also pier toilet systems are built in surface water, which are also used for domestic purpose by some families in the area especially the ones

aligning the water ways. Due to these inherent problems, potable water from groundwater is a challenge in the region. Some families also reside in poor sanitary condition in houses lacking toilet system.

Study Population

A total of 139 children with symptoms of diarrhea were recruited for this study. The age of the children ranged from 0 – 5 years. Stool samples were collected from the children who presented with clinical symptoms of gastroenteritis such as fever, dysentery (with mucus or blood), diarrhea, and abdominal pains attending 3 public and 5 private health institutions in Yenagoa, Bayelsa State, Nigeria. Informed consent was obtained from the parents/guardian of the children prior to specimen collection. Ethical clearance for the study was obtained from Bayelsa State Ministry of Health.

Method of Specimen Collection and Culture

Nurses and the mothers of the children recruited, assisted in the collection of the stool samples into clean, wide-mouthed containers with tight-fitting leak-proof lids, containing no disinfectant or detergent residue. The specimens were streaked in several differential and selective media including MacConkey agar, Eosin Methylene blue agar and Salmonella-Shigella agar and incubated at 37°C for 24 hours.

Identification of the Isolates

Emergent colonies was subcultured in basal medium (Nutrient Agar) and incubated at 37°C at 24 hours. The resultant pure colonies were identified based on colonial and morphological appearance using the scheme of Cheesbrough [25], Benson [26]. The biochemical test carried out for the identification include Gram stain, indole production, citrate utilization, motility test, carbohydrate fermentation test, urease, methyl red, Voges pastuer, catalase, etc. The resultant characteristics were compared with those known taxa using the scheme of Holt et al [27] and the isolates were presented in Table 1.

Statistical Analysis

Frequencies and percentages were calculated for these study variables. The data obtained subjected to chi-square and Fisher's exact tests statistics. Test statistics were carried out at P<0.05 and was considered to be statistically significant.

Table 1: Characteristics of the bacteria agents

Isolates	Gram reaction	Indole	Motility	Citrate	Urease	Lactose	Mannitol	Glucose	Slope	Butt	Hydrogen sulphide production	Gas production
<i>E.coli</i>	-	+	-	-	-	+	-	-	Y	Y	-	+
<i>Samonella</i> species	-	-	+	-	-	-	-	-	R	Y	+	-
<i>Shigella</i> species	-	-	-	-	-	-	-	-	Y	Y	-	+
<i>Enterobacter</i> species	-	-	+	+	-	+	-	-	Y	Y	-	+
<i>Klebsiella</i> species	-	-	-	+	+	+	NA	NA	NA	NA	NA	NA

NA=Not applicable; Y = Yellow; R=red; += positive; -=negative

Results and Discussion

Table 2 presents the sex distribution of children with diarrheagenic bacteria isolated from patients from selected public and private health centres in Yenagoa, Bayelsa state for medical attention. Of the 139 samples collected from patient with symptoms of diarrhea, 135 (97.1%) had enteric bacteria. The Diarrhea prevalence rate in this study is higher than previous studies of 64% in Owerri, Imo state [19], 68.5% Federal Capital Territory, Abuja [16], 83.1% (associated with enteropathogenic microorganisms) in Abakaliki, Ebonyi state, Nigeria [20], 25.10% in Enugu and Onitsha, southeastern Nigeria [22], 38.0% in Shagari area in Akure South local government of Ondo state [23]. The variation in prevalence rate from this study when compared to other studies in Nigerian cities could be associated to differences in infrastructural and socioeconomic indices [16]. The non isolation of bacteria from the entire children with diarrhea symptom suggest that other causative agents such as parasites and viruses could be main cause of diarrhea or the children are on drugs in the rest 2.9%. Authors have variously reported that diarrhea is caused by parasites such as protozoans, viruses, fungi and bacteria. Again it could be due to gram positive microorganisms such as *Staphylococci* which have been reported to cause diarrhea [16, 19] and parasite such as *Entamoeba histolytica* [28]. On parasite perspective, Dada et al. [23] reported the prevalence of intestinal protozoan as 31.6% from Shagari area in Akure South local government of Ondo state (*Entamoeba histolytica* 19.2% and *Giardia lamblia* 12.4%) from school children. Ogunlesi et al [29] reported parasite prevalence rate of 23.3% diarrheagenic patients ≤5 years in Ilesa, Nigeria mainly caused by helminthes (6%), protozoans (17.3%) including ova of *Ascaris lumbricoides* (18.6%), cysts and trophozoites of *Entamoeba histolytica* (65.75%), cysts of *Entamoeba coli* (1.4%), *Giardia lamblia* (7.1%), *Nector americanus* (1.4%) and

Trichuris trichuria (5.7%). Out of the enteric bacteria isolates, *E.coli* was the most prevalent pathogen recovered (42.2%) while *Enterobacter* species were least prevalent (10.4%). The higher prevalence rate of *E.coli* could be due to the fact that is the predominant cause of diarrhea in children. The prevalence of the isolates has similar trend with the finding of Ajero et al. [19] that reported *E.coli* (75%), *Enterobacter* species (28.12%), *Klebsiella* species (13.02%), *Salmonella* species (39.06%) from 192 diarrhea patients attending Federal Medical Center, Owerri seeking for medical attention. Nweze [22] reported *E.coli* (47%), *Salmonella* species (15.79%), *Shigella* species (15.35%), *Klebsiella* species (9.21%) from 0- 45 years old patient with cases of acute gastroenteritis attending both private and public hospitals/ medical laboratories in Enugu and Onitsha in southeastern Nigeria. Again the findings of this study are far from the work of some authors, though showing similar trend. Ifeanyi et al. [16] also reported the prevalence rate 62.8% (*Escherichia coli*), 3.1% (*Salmonella typhi*), 8.7% (*Klebsiella pneumoniae*), and 1.8% (*Enterobacter cloacae*) from 277 diarrhea children attending various health institution for treatment in the Federal Capital Territory, Abuja. Akinnibosun and Nwa for [14] reported the enteric bacteria causing diarrhea in preschool age children (≤5 years) attending University of Benin Teaching Hospital to include *E.coli* (62.58%), *Enterobacter* species (2.16%), *Salmonella* species (2.88%), *Shigella* species (1.44%), *Klebsiella pneumoniae* (8.63%). But higher than the findings of Dada et al. [23] that reported prevalence of bacteria isolates from fecal samples to include; *E.coli* (21.2%), *Enterobacter* species (1.6%), *Salmonella* species (1.6%), *Shigella* species (2.0%), *Klebsiella pneumoniae* (1.6%) from school children in Shagari area of Akure South local government of Ondo state. The variation could again be due to socio-economics, infrastructure and general life pattern of the people of a given demography.

Table 2: Sex distribution of children with diarrheagenic bacteria isolated from patient attending public and private health centres in Yenagoa, Bayelsa state, Nigeria

Bacterial isolates	No. of isolates tested (%)	No of isolates from male (%)	No of isolates from Female (%)
<i>Escherichia coli</i>	57 (42.2)	24 (38.1)	33 (44.6)
<i>Salmonella</i> species	26 (19.3)	12 (19)	14 (18.9)
<i>Shigella</i> species	21(15.6)	12 (19)	9 (12.2)
<i>Klebsiella</i> species	17 (12.6)	7 (11.1)	10 (13.5)
<i>Enterobacter</i> species	14(10.4)	6 (9.5)	8 (10.8)
Total	135	61 (77.7)	74(100)

The prevalence of diarrheagenic bacteria based on sex indicates that female are apparently affected with diarrhea (100%) compared to male (77.7%). This value is close to the findings of Ifeanyi et al. [16] who reported prevalence of 45.5% and 54.5% for male and female respectively from Diarrhea children in the Federal Capital Territory, Abuja, Nigeria. Also Nweze [21] reported the prevalence rate of 47.81% and 52.19% males and females in southeastern (Onitsha and Enugu), Nigeria. But far from the findings of Dada et al. [23] that reported the prevalence of enteric bacteria in male (20.4%) and female (17.6%) from school children from school children in Shagari area of Akure South local government of Ondo state. Also Akinnibosun and Nwafor [14] reported a dissimilar trend in the sex distribution (male 58% and female 42%) of enteric bacteria causing Diarrhea in Teaching Hospital, University of Benin, Nigeria. Again the variation may be associated with life pattern in the given location. In both sex, *E.coli* were the highest 38.1% for males and 44.6% for female, while *Enterobacter* species were least with prevalence rate of 9.5% for male and 10.8% for female. Statistically, there is no significant variation ($P>0.05$) among the bacteria prevalence for both sex. The sex distribution of the various isolates in this study has some similarity with the report of authors. Nweze [21, 22] reported sex distribution of patients attending private and public hospitals in Enugu and Onitsha south eastern Nigeria as 47% and 55% for male and respectively (*E.coli*), 12% (male), 24% (female) (*Salmonella* species), 23% (male) and 12% (female) (*Shigella* species) and 11% (male) and 10% (female) (*Klebsiella* species). The age distribution of bacteria pathogens isolated from the study population is shown in Table 3. The prevalence of *E. coli*, *Salmonella* and *Shigella* species were significantly affected by age of the children ($P<0.0001$, $P=0.0156$ and $P=0.0102$). *Escherichia coli* was most prevalent in the 0-1 age group (95.8%) and the prevalence decreased with increasing age. However, for *Salmonella* and *Shigella* species the prevalence increased with increasing age. The prevalence of *Klebsiella* and *Enterobacter* species were not significantly different ($P=0.1218$ and $P= 0.1525$) among the various age group. The *E.coli* prevalence rate decreases as age increases. This suggested that *E. coli* is the predominant enteric bacteria causative agent diarrhea in children ≤ 1 years. Also, *Salmonella*, *Shigella* and *Klebsiella* species prevalence increases with age and was 0.0% in children ≤ 1 year old. This could be attributed to the protection obtained from their mothers, which is usually secreted through breast milk [22]. Again, the higher

occurrence of *E.coli* in 0 -1 year children compared to 2 – 5years provides information about immune status of the patients. It is most likely that 5years or younger children do not have fully developed immune system, which could have contribute to their higher rate of susceptibility to diarrheagenic micro-organisms.

Table 3: Age distribution of children with diarrheagenic bacteria isolated from patient attending selected private and public health centres in Yenagoa, Bayelsa state, Nigeria

Bacterial isolates	0-1 n= 24(%)	2-3 n= 40(%)	4-5 n= 71(%)
<i>Escherichia coli</i>	23 (95.8)	19 (47.5)	15 (21.1)
<i>Salmonella</i> species	1 (4.2)	5 (12.5)	20 (28.2)
<i>Shigella</i> species	0	4 (10)	17 (23.9)
<i>Klebsiella</i> species	0	6 (15)	11 (15.5)
<i>Enterobacter</i> species	0	6 (15)	8 (11.3)

n is number tested

In the study area, the higher prevalence of other bacteria may be connected to the hygienic, sanitation level. Akinnibosun and Nwafor [14] reported the prevalence of age distribution of enteric bacteria causing diarrhea in preschool children attending Teaching Hospital, University of Benin, Nigeria for treatment as 18%, 34%, 22%, 12%, 4% and 10% for 0- 6, 7- 12, 13 – 24, 25 – 36, 37 – 48, 49 – 60 months respectively. Ifeanyi et al. [17] reported the prevalence of age distribution of children 0 -5 years with diarrhea from federal capital territory as 41.3% (6–12 months), 29.0% (13–24 months), 7.3% (25–36 months), 4.3% (37–48 months) and 6.0% (49–60 months). Sang et al. [7] studied the prevalence of diarrhea in Kenya children as reported that out of the 651 patients screened, 115 (17.7%) have diarrhea and the associated bacteria are pathogenic *E.coli* (11.2%) [Enterotoxigenic (8.9%), enterotoxigenic (1.2%), enteroinvasive (0.6%), shigatoxigenic (0.5%)], *Salmonella* (3.5%), *Shigella* (2%) and *Vibrio cholera* (0.7%). In Iran, Klantar et al. [30] reported the distribution of acute diarrhea episodes in different age group in Kurdistan province as 26.3% (1-12 months), 37.4% (13-24 months), 14.1% (25-36 months), 8.1% (37 – 48 months) and 14.1% (>48 months). The differences with the result of this study from previous works indicate the importance of regional differences in the prevalence. Generally, the epidemiological importance *E.coli* (a major causative agent of diarrhea in children)

category in childhood diarrhea varies with the geographical area [30].

Conclusion

This study evaluated the enteric bacteria causing Diarrhea in children 5 years. Five bacteria isolates including *E.coli*, *Klebsiella*, *Salmonella*, *Shigella* and *Enterobacter* species were the isolates found in the children of 5 years and younger. *Escherichia coli* had the highest prevalence rate of the in the study area. Females harbor the enteric bacteria causative agent than their male counterpart in the study area. Generally, diarrhea in children is dangerous to dehydration and child mortality. Basic sanitation is a public health problem in Nigeria, hence this could increase diarrhea cases in children. As such, there is the need for intermittent survey of enteric pathogens causing diarrhea in children to elucidate their epidemiological importance and ease valuable control measures in the study area.

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