Bacterial Agents Associated With Infantile Diarrhea and Their Antibiotics Susceptibility Pattern in Port Harcourt, South-South, Nigeria

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Abstract
A study of the bacterial agents associated with infantile diarrhea along with their antibiotics susceptibility pattern were carried out in Port Harcourt, Rivers State, Nigeria. Out of the 100 infants with diarrhea (cases) sampled, at least one bacterial isolate, probably pathogenic, was detected in 49 (49.0%) and no bacterial agent was detected in 51 (51%). Among the 20 infants without diarrhea (controls), 4 (20.0%) had a bacterial isolate while 16 (80%) had no isolate. Bacteria detected among cases arranged in order of their decreasing prevalence included; Escherichia coli (23.0%), Salmonella species (10.0%), Shigella species (8.0%), Yersinia enterocolitica (5.0%), Vibrio cholera (3.0%). Infants between the ages of 0-12 months recorded the highest frequency of bacterial isolates which decreased with increase in age. All the bacterial isolates were subjected to antibiotics sensitivity screening in which the percentage of sensitive isolates were recorded and it was observed that all the isolates showed more than 70% susceptibility to Ofloxacin, Gentamicin and Ciprofloxacin except Vibrio cholera, with Shigella species showing 100% susceptibility to the three antibiotics mentioned earlier and Yersinia species and Vibrio cholera showing 100% sensitivity to Ofloxacin and Gentamicin only. All the bacterial isolates showed 100% resistance to Septrin while only Escherichia coli, Shigella species, Yersinia enterocolitica and Vibrio cholera showed 100% resistance to Augumentin. Mothers of the diarrheal infants at home where some samples were collected were educated on the major routes of diarrheal transmission and how to prevent and control it as well as the impact of ORS in diarrhea control.

Introduction
In medicine, diarrhea, from the Greek word “diarrhoia” meaning “a flow through”, (Medterms dictionary) also spells diarrhea as the condition of having frequent loss of fluid from the bowel.

Infants in medicine are also referred to as children that are still very young, below the age of 5 years. Therefore infantile diarrhea can now be said to be the frequent loss of fluid from the bowel of very young individuals. Acute diarrhea is a common cause of death in developing countries like Nigeria and the second most common cause of infant mortality worldwide (WHO, 2013).
About 1.7 to 5 billion cases of diarrhea occur per year (WHO, 2013, Doyle, 2013). The loss of fluid through diarrhea can cause severe dehydration which is one of the causes of death in people with diarrhea.

Infections are the major cause of severe morbidity and mortality among children worldwide (lawn et al., 2005). Diarrheal illness stands as an important cause of infectious morbidity in children, which is exceeded by respiratory tract infections, and mortality is currently associated with cases that evolve in infants without proper feeding or rehydration care, invasive diarrheas with extra intestinal or systemic involvement, or persistent diarrheas that occur especially in infants from low level socioeconomic groups, who suffer previous deficiencies and develop severe enteric infection (Torres et al., 2001).

Despite much progress in the understanding of the pathogenesis and of the management, diarrheal illness still remains one of the most important causes of global childhood mortality and morbidity, this is largely because the etiology and pathogenesis of persistent diarrhea are usually multifactorial and sometimes cannot be identified (Garcia et al., 1994). Reports from different parts of the world have implicated various pathogens such as Parasites like; Giardia lamblia, Entamoeba histolytica, Bacteria like; Escherichia coli, Salmonella species, Klebsiella species, Enterobacter species e.t.c., and Viruses like the Rotavirus with the outbreak of infantile diarrheal disease (Chatterjee et al., 1998; Ali et al., 2005; Olesen et al., 2005; Diniz-Santos et al., 2005; Parashar et al., 2006; Vernacchio et al; 2006). However in the developing countries like Nigeria, infantile diarrhea disease is grossly under-reported and the incidence under-estimated, this is attributed to poverty and ignorance among the affected group who constitute up to 80% of the population of the area (Synder and Merson, 1982).

In Nigeria, available reports indicate that more than 315,000 deaths of preschool age children are recorded annually from diarrheal disease (Alabi et al., 1998; Babiniyi, 1999). Nevertheless, despite the public health implication and enormous burden imposed on the primary health care delivery system by infantile diarrhea illness in the country, there is still paucity of information on the epidemiology and etiology of infantile diarrhea in many regions including the South-South part of the country. Since pathogens responsible for diarrhea infections employ ingenious mechanisms to establish disease, regional variation in the microbiological profile may exist even in the same country (Synder and Merson, 1982; Thaper and Sanderson, 2004). A study carried out by many researchers also shows that there has been a rising trend of resistance to antimicrobials used in the treatment of diarrheal infections in the area. Considering all these problems mentioned earlier, it is necessary to add to the body of existing knowledge of the epidemiology, prevalence, etiology and the sensitivity pattern to antimicrobials, of the bacterial etiology of infantile diarrhea in Port Harcourt, Rivers State which is one of the major cities in the South-South Nigeria, it is an urban area surrounded by few rural areas, it is also made up of low, average and high income earners in a decreasing order. This study will provide adequate scientific information required for effective public health interventional efforts and adequate management of the disease. It will also help mothers to know what diarrhea and its management entails and to help medical personnel in their prescribing policies of antimicrobial agents.
Materials and Method

Study Area

This study was conducted in Port Harcourt, a city in the South-South Nigeria; it has a population of about 1,382,592 according to the 2006 Nigerian census. It is approximately 140 square miles (360 Km²) in area. It lies along the Bonny River and it is located in the Niger Delta. The area lies approximately between Longitude 7°E and Latitude 4.75°N. The climate is tropical monsoon with an annual rainfall of about 1600mm and average atmospheric temperature of 25°C - 28°C.

There are two distinct seasons in the area, the lengthy and heavy rainy season (Wet) and very short Dry season, the former occurs between April and October while the later occurs from November to March. This seasonal change affects the epidemiology of diarrhea. The area comprises of mostly average income earners.

Ethical Considerations

Throughout this research, individuals’ anonymity were maintained, good laboratory practice/quality control were ensured and every finding was treated with utmost confidentiality and for the purpose of the research only.

Study Population, Sample Size and Sample Collection

Infants aged 0-60 months with diarrheal cases which were noticeable from the type of faeces they produced were examined. 100 samples of diarrheic feces, with 20 samples of non-diarrheic faces (control) were analyzed. The samples were collected from clinics, hospitals, medical laboratories, day care centers and homes, all within Port Harcourt.

About 5-10 grams or its equivalence in milliliters each of the different diarrheic stool samples were collected for the study. The samples were collected from their diapers or container in which they defecated. The samples were collected with small sterile plastic spoons and transferred into a universal sterile specimen bottle aseptically and covered. The samples were labeled properly, transported to the laboratory and analyzed immediately.

Materials used

Proper laboratory materials were used and sterilization of the necessary materials used for the work was done using the autoclave, bunsen burner and alcohol to prevent contamination. Appropriate culture media for culturing and sub-culturing such as MacConkey Agar, TCBS Agar, DCA, SSA, EMBA, NA and Selenite F broth were prepared and used according to the manufacturer’s (Oxide, Biotech e.t.c) prescription after which sterility testing was done on the media contained in the petri dishes collected at random.

Laboratory Analysis

The method for the laboratory analysis was adopted from the work of Ogbu et al. (2008) for the bacteriological analysis of the samples collected and also standard methods were used for the isolation and identification of bacterial pathogens (Cheesbrough, 2000).
First, the **Macroscopy** of the diarrheic stool samples were done to check for characteristic colour, presence of blood and mucus, which its presence can act as a presumptive test for a suspected bacterial agent causing the diarrheal disease.

The stool samples were cultured and the growths from the samples were sub-cultured to get a pure culture for necessary characterization and identification.

Streak plate method was used aseptically and the plates were covered and incubated. After 18-24 hours the plates were observed for growth and colonial appearance. After overnight incubation, growth on Selenite F broth was sub-cultured on DCA/SSA to look for *Yersinia species* and growth on Peptone water with 3% NaCl was sub-cultured on TCBS to look for *Vibrio species* (LeClair et al., 1970). Other growths on other plates were sub-cultured on freshly prepared Nutrient Agar plate and further incubated for 18-24 hours to obtain a pure culture of the bacterial isolates. Some samples yielded more than one bacterial pathogen. Pure cultures were stored in agar slant.

**Characterization and Identification**

Standard inoculum of the isolates, $10^6 – 10^8$ cfu/ml were prepared, isolates’ colonial appearances were observed; Gram staining were done and subsequently subjected to the biochemical tests for proper identification and characterization (Cheesbrough, 2000). Other forms of tests and staining done were; **Motility test** to determine presence of locomotive organs, **Spore Staining** to determine isolates’ ability to produce endospores with the method of Ugbogu et al. (2004).

**Antibiotic Susceptibility Test**

The following antibiotics were employed in this study based on the local prescribing policies and availability; Ofloxacin, Gentamicin, Ciprofloxacin, Ampicillin, Augumentin and Septrin. Kirby-Bauer’s Disk Diffusion method was used to assess the susceptibility pattern of the bacterial isolates to these antibiotics.

The Inhibition Zone Diameter in (mm) (IZD) which is a measure of the susceptibility of the isolates were referred to the standard interpretative chart reporting the zone sizes of each antimicrobial in the book of Cheesbrough, (2000).

**Statistical Analysis Used**

Chi-square Goodness of Fit test at 5% level of significance, was used to analyze the work and to make inferences on the frequency of occurrence of the bacterial pathogens associated with infantile diarrhea and to show that there is a significant difference in the prevalence of bacterial infection in relation to age of infants with diarrhea in Port Harcourt, South-South Nigeria.

**Results**

After the research the following results were obtained.
Frequency of occurrence of the bacterial agents isolated from the diarrheic stool of infants in Port-Harcourt, South-South Nigeria.

From a total of 100 samples collected and analyzed, 49 samples had bacterial pathogens causing infantile diarrhea isolated from them in varying frequencies of occurrence. Figure 1 shows a pie chart of the different species of bacteria isolated and their frequencies of occurrence. This outcome was also statistically analyzed using the Chi-square analysis to check if there is a significant difference in the frequencies of occurrence of the bacterial pathogens associated with infantile diarrhea in Port Harcourt, South-South Nigeria when compared with similar works carried out in other places (using Houston, USA as a case study). The result was tested at 5% confidence level of significance and degree of freedom equals 2 ($\chi^2(0.05)$). At $\chi^2(0.05)$, critical value 5.99 was greater than the calculated value 2.44. Therefore the Alternative Hypothesis ($H_1$) was rejected in favor of the Null Hypothesis ($H_0$) showing that there was no significant difference in the frequency of occurrence.

Prevalence of bacterial agents among Infants with diarrhea (Cases) and Infants without diarrhea (Controls) in Port Harcourt, South-South Nigeria.

Of the 100 infants with diarrhea cases, at least one bacterial pathogen was detected in (49.0%) of them, while amongst the 20 infants without diarrhea cases (Controls), only 4 (20%) had a bacterial pathogen. *Shigella species*, *Yersinia enterocolitica* and *Vibrio cholera* were not found in the controls but were implicated in the diarrheic stool samples. Figure 2 shows the relative prevalence of the bacterial pathogen in infantile diarrhea as well as prevalence in infants without diarrhea (Controls).
bacterial agents of infantile diarrhea and their antibiotics susceptibility pattern

Figure 2: a bar chart representing the percentage prevalence of Bacterial agents among infants with Diarrhea cases and Non Diarrhea cases in Port Harcourt, South-South Nigeria.

Age related prevalence of bacterial infection among infants with diarrhea in Port Harcourt, South-South Nigeria

The prevalence of the bacterial infection was found to be more in infants of 0-12 months old than in the older infants. Figure 3 shows a chart representing the age related prevalence of bacterial infection among Infants with diarrhea in Port Harcourt, South-South Nigeria.

This outcome was subjected to Chi-square analysis; Table 1 shows the Chi-square value for the observed and expected frequencies of occurrence of bacterial infection in relation to age of infants with diarrhea in Port Harcourt, South-South Nigeria.

Figure 3: a bar chart representing the Age related prevalence of bacterial infection among Infants with Diarrhea in Port Harcourt, South-South Nigeria.
Table 1: Chi-square value for the Observed and Expected frequencies of Occurrence of Bacterial infection in relation to age of Infants with Diarrhea in Port Harcourt, South-South Nigeria.

<table>
<thead>
<tr>
<th>Age (Months)</th>
<th>Observed Frequency ($f_o$)</th>
<th>Prevalence (%)</th>
<th>Expected Frequency ($f_e$)</th>
<th>$f_o - f_e$</th>
<th>($f_o - f_e)^2$</th>
<th>($f_o - f_e)^2 / f_e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-12</td>
<td>19</td>
<td>61.3</td>
<td>30.0</td>
<td>-11</td>
<td>121</td>
<td>4.03</td>
</tr>
<tr>
<td>13-24</td>
<td>14</td>
<td>53.8</td>
<td>26.0</td>
<td>-12</td>
<td>144</td>
<td>5.54</td>
</tr>
<tr>
<td>25-36</td>
<td>9</td>
<td>42.9</td>
<td>21.0</td>
<td>-12</td>
<td>144</td>
<td>6.86</td>
</tr>
<tr>
<td>37-48</td>
<td>4</td>
<td>36.4</td>
<td>18.0</td>
<td>-14</td>
<td>196</td>
<td>10.89</td>
</tr>
<tr>
<td>49-60</td>
<td>3</td>
<td>27.3</td>
<td>13.0</td>
<td>-10</td>
<td>100</td>
<td>7.69</td>
</tr>
</tbody>
</table>

$x^2 = \sum \left( \frac{(f_o - f_e)^2}{f_e} \right) = 4.03 + 5.54 + 6.86 + 10.89 + 7.69 = 35.01$

Equation 1: formula for the Chi-square calculated value

Since the $x^2$ calculated is $> x^2$ tabulated (critical value) at 5% confidence level of significance and degree of freedom equal 2, it shows that there is a significant difference in the frequency of occurrence of bacterial infection in relation to age of infants with diarrhea in Port-Harcourt, South-South Nigeria.

**Age-related prevalence of the individual bacterial agents among infants with diarrhea in Port Harcourt, South-South Nigeria.**

The frequency of occurrence of the bacterial pathogens generally decreased with increase in the age of the infants with diarrhea. It was found out that infants aged 0-12 months old recorded the highest frequency of bacterial pathogens followed by those aged 13-24 months old. Among these age categories, *Escherichia coli* (32.3% versus 23.1%), *Salmonella species* (12.9% versus 11.5%) and *Shigella species* (12.9% versus 7.7%), but the reverse was the case in the other two bacteria pathogens where there were no isolates of the bacteria in infants aged 0-12 months but was found in older infants.
Table 2: Age-related prevalence of the individual bacterial agents among infants with Diarrhea in Port Harcourt, South-South Nigeria

<table>
<thead>
<tr>
<th>Bacterial Agents</th>
<th>Age in Months (Number of cases)</th>
<th>No (%) of Bacterial Agents Detected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-12(31)</td>
<td>10(32.3)</td>
</tr>
<tr>
<td></td>
<td>13-24(26)</td>
<td>6(23.1)</td>
</tr>
<tr>
<td></td>
<td>25-36(21)</td>
<td>4(19.0)</td>
</tr>
<tr>
<td></td>
<td>37-48(11)</td>
<td>2(18.2)</td>
</tr>
<tr>
<td></td>
<td>49-60(11)</td>
<td>1(9.1)</td>
</tr>
<tr>
<td></td>
<td>Total (100)</td>
<td>25</td>
</tr>
<tr>
<td>E. coli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salmonella spp</td>
<td></td>
<td>4(12.9)</td>
</tr>
<tr>
<td>Shigella spp</td>
<td></td>
<td>3(12.9)</td>
</tr>
<tr>
<td>Yersinia spp</td>
<td></td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Vibrio cholera</td>
<td></td>
<td>0(0.0)</td>
</tr>
</tbody>
</table>

Sex related prevalence of bacterial infection among infants with diarrhea in Port Harcourt, South-South Nigeria.

It was discovered that the prevalence of infection in male and female were approximately 49%, although the samples were collected at random where the total male infants sampled were 49 infants and that of female infants were 51.

Table 3: Sex-related prevalence of the bacterial infection among infants with Diarrhea in Port Harcourt, South-South Nigeria

<table>
<thead>
<tr>
<th>Sex</th>
<th>No (%) Infected</th>
<th>No (%) Uninfected</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>24 (48.98)</td>
<td>25 (51.02)</td>
<td>49</td>
</tr>
<tr>
<td>Female</td>
<td>25 (49.02)</td>
<td>26 (50.98)</td>
<td>51</td>
</tr>
</tbody>
</table>

Antibiotic susceptibility pattern of the bacterial pathogens associated with Infantile diarrhea in Port Harcourt, South-South Nigeria.

Table 4 shows the antibiotic susceptibility pattern of the bacteria isolates. Results indicated that all the isolates showed more than 70% susceptibility to Ofloxacin, Gentamicin and Ciprofloxacin except Vibrio cholera with Shigella species showing 100% susceptibility to the three antibiotics mentioned earlier. Yersinia enterocolitica and Vibrio cholera showed 100% sensitivity to Ofloxacin and Gentamicin. All bacterial isolates showed 100% resistance to Septrin while only Escherichia coli, Shigella species, Yersinia enterocolitica and Vibrio cholera showed 100% resistance to Augumentin.
Table 4: Antibiotics susceptibility pattern of the bacterial pathogens associated with infantile diarrhea in Port Harcourt, South-South Nigeria.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>% Sensitivity of Bacterial Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E.coli</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>90.0</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>80.0</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>90.0</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>10.0</td>
</tr>
<tr>
<td>Augumentin</td>
<td>0.0</td>
</tr>
<tr>
<td>Septrin</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Conclusion

Infantile gastroenteritis (Diarrhea) of microbial origin is said to still constitute a problem, with high mortality rate in developing countries and high impact on health cost in industrialized countries (Guarino et al., 1998). In this study, it was established that as high as 49% of cases of infantile diarrhea in Port Harcourt, South-South Nigeria were associated with enteropathogenic bacterial agents and was considerably higher than the frequency of the pathogens in the control (20%). This was comparable to the outcome of studies in Calcutta, India (Chatterjee et al., 1989), Sao Paulo, Brazil (Souza et al., 2002), Santiago, Chile (Saelzer et al., 1989), Houston, USA (Martha et al., 1970) and in a recent carried out in Abakiliki, South-Eastern Nigeria, bacterial agents in this investigation and other non-bacterial agents of infantile diarrhea may have been responsible for the outstanding 51.0% diarrheic cases.

Although the spectrum of bacterial isolates implicated in acute childhood Diarrhea varies regionally, *Escherichia coli*, *Salmonella species* and *Shigella Species* were the commonest bacteria pathogens isolated in this investigation and this is in conformity with findings from other parts of the world such as Brazil and Guinea Bissau. Both *Escherichia coli* and *Salmonella species* are reported to be very commonly associated with enteric diseases in developing countries and are more important to the epidemiology of diarrhea in poorer areas (Notario et al., 1996; Gomes et al., 1991; Ansaruzzman et al., 2000). Among the control samples, 3 had *Escherichia coli* in their stool but no diarrhea.

The presence of *Escherichia coli* in apparently healthy children has been well documented, previous studies have shown that 8-12% of the apparently healthy children in this age group excrete *Escherichia coli* (Feldman et al., 1970) but its rate of isolation is low and the organism was not a predominant flora in the stool.

Other bacterial pathogens isolated in this study apart from *Escherichia coli*, *Salmonella species* and *Shigella species* which had 23%, 10% and 8% prevalence respectively, were *Yersinia enterocolitica* (5%) and *Vibrio cholera* (3%).

*Yersinia enterocolitica* implicated in this study may have been isolated from infants inhabiting homes where there was improper animal and pest control which is a situation common in most areas of developing countries of the world, since this bacterium is a common flora of birds and animal
feces. Infants can easily be infected when they play in soil or with sand contaminated by this organism.

*Vibrio cholera* was isolated from infants aged 36 months and above, this is obtainable due to the fact that this age group had started developing an attribute if independency, they can now drink water at their own volition, when these water are not properly treated, the child could be infected by this pathogen which is a common contaminant of untreated water, and this causes Cholera. Other bacteria like the *Salmonella species*, *Shigella species* and *Escherichia coli* are associated with diary products, fecal contaminated food or water, hence proper hygiene and sanitation must be practiced to reduce infection by these pathogen.

The result from this study showed that the majority of the enteric bacterial pathogens were detected among the cases who were 24 months of age and less, which may be said to correspond to the period when the children’s contact with the environmental pathogens increases dramatically. This is in agreement with findings from previous studies in Brazil, Denmark and Turkey (Olesen *et al.*, 2005; Diniz-Santos *et al.*, 2005; Karadag *et al.*, 2005). This high frequency of occurrence can also be attributed to the incompetence of the immune system of infants at that age group. Exclusive breast-feeding can be of help to shield infant from these pathogens.

Because specific antimicrobial treatment may be required to supplement supportive anti-dehydration treatment which is the cornerstone of therapy of acute infantile diarrhea, selective use of antimicrobial agents therefore cannot be overemphasized. This is vital especially in the developing countries where inadequate health services, inadequate drug supplies, non-adherence to treatment strategies and dubious drug quality all favor the emergence of microbial resistance (WHO, 2002). Therefore, it is worth noting that one outcome of the increased availability and usage of antimicrobial agents for symptomatic treatment of illness has been the emergence of antimicrobial resistance. This was clearly evident from this study where up to 80% resistance was observed in two to three antibiotics (Seprin and Augumentin) by *Escherichia coli, Salmonella species, Shigella species, Yersinia enterocolitica* and *Vibrio cholera*. This is of particular concern to developing countries in the world including Nigeria because less affordable, appropriate and effective treatment options such as Ciprofloxacain, Ofloxacain which from this work were the most effective chemotherapeutic agent because they showed activity of more than 70% are readily available in most rural and urban communities. It has become increasingly important to monitor patterns of resistance as the antimicrobial susceptibility of bacterial pathogens which contribute significantly to the burden of infantile diarrhea is declining. The susceptibility of some isolates of different bacteria to the antibiotics and the variable reaction of different isolates to biochemical tests indicate that different strains of a particular organism can cause diarrhea in infants.

In conclusion, it is important to state that the inability to screen for other enteric pathogens particularly viruses and parasites is a limitation of this study. Nevertheless, the present study has provided insight into the burden of infantile diarrhea in Nigeria. As a public health measure to reduce the disease burden, an integrated package of immunization services and other childcare programmes need to be implemented in addition to well focused health education messages to improve treatment–seeking behavior for childhood diarrhea as well as improved personal and environmental hygiene.
References


