Isolation and evaluation of *salmonella* and *shigella* spp in children in Ile-Ife, Nigeria

**Abstract**

Gastroenteritis primarily affects children and those in the developing world. It results in about three to five billion cases and causes 1.4 million deaths a year. One hundred (100) stool samples were collected in sample bottles from three primary health centers in Ile-Ife from children with age bracket 0-5 between August 2013 and March 2014. All samples were inoculated unto the media such as MacConkey (MAC), Selenite F broth, Deocycholate citrate agar (DCA) and *Salmonella* and *Shigella* agar (SSA) and all plates were incubated at 37°C aerobically for 24hrs after which growth were examined and isolates were identified according to Cowan and Steel. Antimicrobial susceptibility test was performed on all isolates as described by NCCLS. Out of 35 samples collected for age group <6 months, only 1 (2.9%) had *Shigella* spp while none had *Salmonella* spp. Age range 6-11 months, had 1 (2.5%) *Salmonella* spp and 1 (2.5%) *Shigella* spp. Age range 1-2 years, 2 (10.5%) had *Shigella* spp while 1 (5.3%) had *Salmonella* spp. Age range 3-4 years had none of the organisms. Sachet water, 2 (5.7%) had *Salmonella* spp and 2 (5.7%) also had *Shigella* spp. Well water had no *Salmonella* and *Shigella* spp. Tap water had only 1 (3.8%) *Shigella* spp with zero *Salmonella* spp. Rain water had only 1 (3.8%) *Shigella* spp while none had *Salmonella* spp. Watery stools had 3 (5.2%) *Shigella* spp and 2 (3.4%) *Salmonella* spp; while mucoid stool had 1 (3.4%) *Shigella* spp without *Salmonella* spp and formed stool had none of the organisms. Parent that complete primary school only had 1 (3.3%) *Shigella* spp and none *Salmonella* spp from parent that completed modern 3, and from Parents that completed SSCE, had 1 (3.7%) *Shigella* spp and 1 (3.7%) *Salmonella* spp. Parents that have post-secondary education, had 2 (6.9%) *Shigella* spp while 1 (3.4%) had *Salmonella* spp. Parent with level of income of <$3,000 and 3,000-85,000 had no *Salmonella* nor *Shigella* spp, Parents with level of income between 96,000-10,000 had 2 (14.3%) *Shigella* spp while none had *Salmonella* spp. Parents with level of income >10,000, had 1 (4.3%) each for *Shigella* spp and *Salmonella* spp. Parents with no stable amount of income, had 1 (2%) *Shigella* spp and 1 (2%) *Salmonella* spp. All the strains showed 100% susceptibility to Ofloxacin, Ciprofloxacin and Gentamicin, and resistance to Augmentin and Ampicillin.

**Keywords:** isolation, evaluation, *salmonella*, *Shigella*, children; Nigeria

**Abbreviations:** DCA, deocycholate citrate agar; SSA, *Salmonella* and *Shigella* agar; MAC, macconkey

**Introduction**

Worldwide in 2004 approximately 2.5 billion cases of diarrhea occurred which results in 1.5 million deaths among children under the age of five. Greater than half of these were in Africa and South Asia. This is down from a death rate of 4.5 million in 1980 for gastroenteritis. Gastroenteritis or infectious diarrhea is a medical condition characterized by inflammation ("-itis") of the gastrointestinal tract that involves the stomach ("gastro")- and the small intestine ("entero"), resulting in some combination of diarrhea, vomiting, and abdominal pain and cramping. Gastroenteritis primarily affects children and those in the developing world. Worldwide, 3-5 billion cases of acute gastroenteritis and nearly 2 million deaths occur each year in children under 5 years. In children, bacteria are the cause in about 15% of cases, with the most common types being *Escherichia coli*, *Salmonella* species, *Shigella* species, and *Campylobacter* species. If food becomes contaminated with bacteria and remains at room temperature for a period of several hours, the bacteria multiply and increase the risk of infection in those who consume the food. Shigella is a group of germs (bacteria) that can cause gastroenteritis with dysentery. Shigella is highly contagious, requiring less than 100 organisms to cause infection. The pathogenesis of Shigella is via invasion of colonic epithelium and production of enterotoxins. Infection with *Shigella* spp may be caught by drinking water contaminated with infected faeces, eating food prepared using contaminated water or by close contact with someone who has the infection. Infection may occur in people who have travelled to countries with poor sanitation. Transmission may occur via consumption of contaminated water. In places with wet and dry seasons, water quality typically worsens during the wet season, and this correlates with the time of outbreaks. In areas of the world with seasons, infections are more common in the winter. Bottle-feeding of babies with improperly sanitized bottles is a significant cause on infection. Transmission rates are also related to poor hygiene, especially among children, in crowded households, and in those with pre-existing poor nutritional status. After developing tolerance, adults may carry certain organisms without exhibiting signs or symptoms, and thus act as natural reservoirs of contagion. While some agents (such as Shigella) only occur in primates, others may occur in a wide variety of animals (such as Giardia).
Zinc supplementation benefits children suffering from diarrhea in developing countries, but only in infants over six months old. This supports the World Health Organization guidelines for zinc, but not in the very young Lazzerini et al.11 Probiotics reduce the duration of symptoms by one day and reduced the chances of symptoms lasting longer than four days by 60%. The probiotic lactobacillus can help prevent antibiotic associated diarrhea in adults but possibly not children Kale-Pradhan et al.12 The study is aimed at investigating the prevalence of Salmonella and Shigella spp in gastroenteritis cases in children in Ile-Ife, detect and isolate Salmonella and Shigella spp and to assess the effectiveness of antibiotics on Salmonella and Shigella -induced gastroenteritis in children.

**Materials and methods**

**Sample collection**

Stool samples were collected in sample bottles from three primary health centers in Ile-Ife from children with age bracket 0-5 between August 2013 and March 2014. Questionnaires were filled which include the age of the child, the age of the mother, the occupation of the mother, the level of education of the mother, the level of income of the mother, the type of toilet system used, source of drinking water The samples were taken to the Microbiology laboratory for analysis.

**Macrosopic examination**

The stool sample was examined whether it is formed, loose or watery; the color whether it is greenish, yellowish or any other; it is checked if it is blood stained or mucous.

**Isolation characterization**

All samples were inoculated onto the media such as MacConkey (MAC), Elecysite F broth, Desoxycholate citrate agar (DCA) and Salmonella and Shigella agar (SSA) within 10minutes after collection and all plates were incubated at 37°C aerobically for 24hrs after which growth were examined and isolates were identified by colony characteristics Gram Stain and standard biochemical procedures according to Cowan and Steel. Disc diffusion method of antimicrobial susceptibility test was performed on all isolates as described by NCCLS

**Data analysis**

The statistical analysis of the data was done using SPSS.

**Results**

Out of the 100 samples collected, 6(6%) were positive for Salmonella and Shigella spp. Two of the samples were positive for Salmonellas pp (2%) and four were positive Shigella spp (4%) as shown in Table 1. The age range of the children whose samples were tested for Salmonella and Shigella spp were grouped as, 6-11 months, 1-2years and 3-5years. Out of the total of 35 samples tested for age group <6 months, only 1(2.9%) had Shigella spp while none had Salmonella spp. Out of the total of 40 samples collected for age range 6-11 months, 1(2.5%) Salmonella spp and 1(2.5%) Shigella spp. Out of the total of 19 samples collected for age range 1-2years, 2(10.5%) had Shigella spp while 1(5.3%) had Salmonella spp age range 3-4years had none of the organisms. As shown on Table 2. From the total of 47 male children, 3(6.4%) had Shigella spp while 1(2.1%) had Salmonella spp while out of the total of 53 female children, 1(1.9%) had Shigella spp and 1(1.9%) also had Salmonella spp see in Table 3.

Out of 35 samples gotten from children that take sachet water, 2(5.7%) had Salmonella spp and 2(5.7%) also had Shigella spp. 32 samples were gotten from children taking well water and none had Salmonella and Shigella spp. 29 samples were gotten from children that takes tap water had only 1(3.8%) Shigella spp with zero Salmonella spp. 29 samples were gotten from children that take rain water, only 1(3.8%) had Shigella spp while none had Salmonella spp. Out of the total of 53 samples gotten from children with zero water, only 1(3.7%) had Shigella spp while none had Salmonella spp from parent that completed primary school none was also positive for Salmonella and Shigella spp, only 1(5.3%) had Shigella spp while none had Salmonella spp from parent that completed modern 3, and from 27 samples collected from parents that completed SSCE, only 1(3.7%) had Shigella spp and 1(3.7%) Salmonella spp. 29 samples were collected from parents that have post-secondary education, 2(6.9%) had Shigella spp while 1(3.4%) had Salmonella spp. (Table 6).

Out of 100 total samples collected, 7 samples were collected from parent with level of income of <#3,000 none had both Salmonella and Shigella spp, and none from parents with level of income between #3,000-#5,000 had both Salmonella and Shigella spp (Table 7). Parents with level of income between #6,000-10,000, 2(14.3%) had Shigella spp while none had Salmonella spp. Parents with level of income >10,000, had 1(4.3%) each for Shigella spp and Salmonella spp. Parents with no stable amount of income, had 1(2%) Shigella spp and 1(2%) Salmonella spp. All the strains showed 100% susceptibility to Ofloxacin, Ciprofloxacin and Gentamicin revealing high degree of sensitivity to the antibiotics. All the strains showed resistance to Augmentin and Ampicillin while they showed 83% resistance to Cefuroxime and Cefaluzidine which implies that the antibiotics are ineffective against the organisms (Figure 1).

| Table 1 | The frequency of salmonella and shigella spps |
|--------|---------------------------------------------|
| Isolates     | Frequency | Percentage |
| Salmonella spp | 2          | 2%         |
| Shigella spp  | 4          | 4%         |
| Total        | 6          | 6%         |
| Table 2 | Distribution of salmonella and shigella spp in relation to age |
|---------|-------------------------------------------------------------|
| Results | Positive to shigella spp (%) | Positive to salmonella spp (%) | Negative to both (%) | Total |
| <6month | 1(2.9%) | 0 | 34(97.1%) | 35 |
| 6-11 month | 1(2.5%) | 1(2.5%) | 38(95%) | 40 |
| 1-2 years | 2(10.5%) | 1(5.3%) | 16(84.2%) | 19 |
| 3-5 years | 0 | 0 | 6(100%) | 6 |
| Total | 4 | 2 | 94 | 100 |

| Table 3 | Prevalence of salmonella and shigella spp in relation to gender |
|---------|---------------------------------------------------------------|
| Results | Positive to shigella spp (%) | Positive to salmonella spp (%) | Negative to both (%) | Total |
| Sex | | | | |
| M | 3(6.4%) | 1(2.1%) | 43(91.5%) | 47 |
| F | 1(1.9) | 1(1.9%) | 51(96.2%) | 53 |
| Total | 4 | 2 | 94 | 100 |

| Table 4 | Distribution of salmonella and shigella spp in relation to source of drinking water |
|---------|-----------------------------------------------------------------------------------|
| Results | Positive to shigella spp (%) | Positive to salmonella spp (%) | Negative to both (%) | Total |
| Source of water | | | | |
| sachet water | 2(5.7%) | 2(5.7%) | 31(88.6%) | 35 |
| Well water | 0 | 0 | 32(100%) | 32 |
| Tap water | 1(3.8%) | 0 | 28(96.6%) | 29 |
| Rain water | 1(25%) | 0 | 3(75%) | 4 |
| Total | 4 | 2 | 94 | 100 |

| Table 5 | Distribution of Salmonella and Shigella spp in relation to stool information |
|---------|---------------------------------------------------------------------------|
| Results | Positive to shigella spp (%) | Positive to salmonella spp (%) | Negative to both (%) | Total |
| Stool info | | | | |
| Watery | 3(5.2%) | 2(3.4%) | 53(91.4%) | 58 |
| Mucoid | 1(3.4%) | 0 | 28(96.6%) | 29 |
| Formed | 0 | 0 | 13(100%) | 13 |
| Total | 4 | 2 | 94 | 100 |

| Table 6 | Distribution of salmonella and shigella spp in relation to level of education |
|---------|--------------------------------------------------------------------------------|
| Results | Positive to shigella spp (%) | Positive to salmonella spp (%) | Negative to both (%) | Total |
| Level of Education | | | | |
| No formal education | 0 | 0 | 4(100%) | 4 |
| Modern 1 | 0 | 0 | 3(100%) | 3 |
| completed primary school | 0 | 0 | 18(100%) | 18 |
| modern 3 | 1(5.3%) | 0 | 18(94.7%) | 19 |
| completed SSCE | 1(3.7%) | 1(3.7%) | 25(92.6%) | 27 |
| Post-Secondary | 2(6.9%) | 1(3.4%) | 26(89.7%) | 29 |
| Total | 4 | 2 | 94 | 100 |
Isolation and evaluation of salmonella and shigella spp in children in Ile-Ife, Nigeria

From 40 samples collected for age range 6-11 months, 1(2.5%) was tested positive for Salmonella spp while 1(2.1%) was tested positive for Shigella spp. Prevalence of diarrhea was found to be highest in children between age group 0-2 years. This result agrees with the study conducted by Banerjee et al., which showed diarrheal disease was significantly higher among children of age group 6-24 months.

Out of the 100 samples collected and tested for presence of Salmonella and Shigella spp, 47 samples were from male children while 53 were collected from female children. From the total of 53 female children, 1(1.9%) was tested positive for Salmonella spp. While 1(5.3%) was tested positive for Shigella spp while 1(1.9%) was tested positive for Salmonella spp. Prevalence of diarrhea was found to be highest in children between age group 0-2 years. This result agrees with the study conducted by Banerjee et al., which showed diarrhea prevalence was significantly higher among children of age group 6-24 months.

Discussion

Diarrhea caused by enteric infections is a major factor in morbidity and mortality worldwide. An estimated 2.4 billion episode of infectious diarrheal disease occurs each year and is especially prevalent in infants. In developing countries, enteric bacterial pathogens and parasites are the leading cause of infectious diarrheal disease. In this study, a total of 100 stool samples were collected in from three primary health centers in Ile-Ife from children with age bracket 0-5 between August 2013 and March 2014. Out of the samples, 6(6%) were positive for Salmonella and Shigella spp. Two of the samples were positive for Salmonella spp (2%) and four were positive for Shigella spp (4%). The prevalence of Salmonella and Shigella spp (6%) is low which may be due to low sample number. The data collected was subjected to statistical analysis using SPSS software package. Out of the total of 35 samples tested for <6 months, only 1(2.9%) was tested positive for Shigella spp while none was positive for Salmonella spp. From the 40 samples collected for age range 6-11 months, 1(2.5%) was tested positive for Salmonella spp and 1(2.5%) was also tested positive for Shigella spp. From 19 samples collected for age range 1-2 years, 2(10.5%) was tested positive for Shigella spp while 1(5.3%) was tested positive for Salmonella spp.

Fluoroquinolones are the most frequently employed antibacterial drugs for infectious diarrhea in adults. In a placebo controlled study ciprofloxacin was shown to be effective in decreasing the duration of diarrhea in patients with severe community acquired diarrhea. Norfloxacin, ofloxacin and levofloxacin are other fluoroquinolones identified including younger age, lack of access to pure water due to contamination, lack of proper sanitation, poor hygiene practices and low parental education. Consequently, boys might have increased demands for macronutrients and are therefore more at risk of a negative balance including lack of vitamin A and Zinc. This vulnerability place boys as weaker sex regarding infection.

All the strains isolated showed 100% susceptibility to Ofloxacin, Ciprofloxacin and Gentamicin revealing high degree of sensitivity to the antibiotics. All the strains showed resistance to Augmentin and Ampicillin while they showed 83% resistance to Cefuroxime and Ceftazidine which implies that the antibiotics are ineffective against the organisms. This agrees with a study by Juan-Pablo et al., that Fluoroquinolones are the most frequently employed antibacterial drugs for infectious diarrhea in adults. In a placebo controlled study ciprofloxacin was shown to be effective in decreasing the duration of diarrhea in patients with severe community acquired diarrhea. Norfloxacin, ofloxacin and levofloxacin are other fluoroquinolones that have shown to reduce the duration of diarrhea and other symptoms. This also agrees with Gray et al. that Ciprofloxacin is often administered at the first sign of severe gastroenteritis.

Conclusion

In conclusion a number of risk factors for diarrhea have been identified including younger age, lack of access to pure water due to contamination, lack of proper sanitation, poor hygiene practices and low parental education. From this study, Ofloxacin, Ciprofloxacin

Table 7 Distribution of salmonella and shigella spp in relation to level of income

| Level of income (Naira) | Positive to shigella (%) | Positive to salmonella (%) | Negative to both (%) | Total |
|------------------------|--------------------------|----------------------------|---------------------|-------|
| <3,000                 | 0                        | 0                          | 7(100%)             | 7     |
| 3,000-5,000            | 0                        | 0                          | 7(100%)             | 7     |
| 6,000-10,000           | 2(14.3%)                 | 0                          | 12(85.7%)           | 14    |
| >10,000                | 1(4.3%)                  | 1(4.3%)                    | 21(91.3%)           | 23    |
| No stable source of income | 1(2%)                  | 1(2%)                      | 47(96%)             | 49    |
| Total                  | 4                        | 2                          | 94                  | 100   |

Figure 1 showing the distribution of antibiotics susceptibility.

Abbreviations: AUG, augmentin; OFL, ofloxacin; CPR, ciprofloxacin; GEN, gentamicin; CRX, cefuroxime; CAZ, ceftazidine; AMP, ampicillin; NIT, nitrofuratoin.
and Gentamicin can be used as antibiotics against Salmonella and Shigella-induced diarrhea.

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None.

Conflict of interest
The author declares no conflict of interest.

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