

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 1.4million 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 onto the media such as MacConkey (MAC), Selenite F broth, Deoxycholate 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 6-11months, had 1(2.5%) *Salmonella* spp and 1(2.5%) *Shigella* spp. age range 1-2years, 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(5.3%) *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- #5,000 had no *Salmonella* nor *Shigella* spp, Parents with level of income between #6,000-10,000 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

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Abbreviations: DCA, deoxycholate citrate agar; SSA, salmonella and shigella agar; MAC, macconkey

Introduction

Worldwide in 2004 approximately 2.5billion cases of diarrhea occurred which results in 1.5million deaths among children under the age of five. Greater than half of these were in Africa and South Asia.¹ This is down from a death rate of 4.5million in 1980 for gastroenteritis.² 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.³ Gastroenteritis primarily affects children and those in the developing world. Worldwide, 3-5billion cases of acute gastroenteritis and nearly 2million deaths occur each year in children under 5years.⁴ 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*.⁵ 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.²

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.^{6,7} 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.⁸ 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 a global scale.⁹ Transmission rates are also related to poor hygiene, especially among children, in crowded households,¹⁰ 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*).²

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 Lazzarini 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.

Macroscopic 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 into the media such as MacConkey (MAC), Selenite F broth, Deoxycholate citrate agar (DCA) and *Salmonella* and *Shigella* agar (SSA) within 10 minutes 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 sp*. Two of the samples were positive for *Salmonella spp* (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, had 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* Table 4. 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 Table 5. Four samples were collected from parent with no formal education and none was positive for the 2 organisms; 3 samples were gotten from parent with education level up to modern 1 and none of the 2 organisms was gotten from these samples; out of 18 samples gotten 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 Ceftazidime which implies that the antibiotics are ineffective against the organisms (Figure 1).

Table 1 The frequency of salmonella and shigella spp

Isolates	Frequency	Percentage
<i>Salmonella spp</i>	2	2%
<i>Shigella spp</i>	4	4%
Total	6	6%

Table 2 Distribution of salmonella and shigella spp in relation to age

		Results			Total
		Positive to shigella spp (%)	Positive to salmonella spp (%)	Negative to both (%)	
Age	<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			Total
		Positive to shigella spp (%)	Positive to salmonella spp (%)	Negative to both (%)	
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			Total
		Positive to shigella spp (%)	Positive to salmonella spp (%)	Negative to both (%)	
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

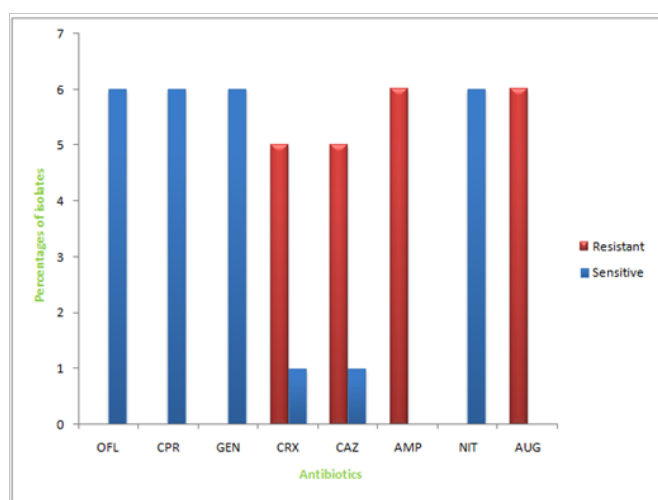
		Results			Total
		Positive to shigella spp (%)	Positive to salmonella spp (%)	Negative to both (%)	
Stool info	Watery	3(5.2%)	2(3.4%)	53(91.4%)	58
	Mucoid	1(3.4%)	0	28(96.6%)	29
	Formed	0	0	13(100%)	13
Total		4	2	94	100

Table 6 Distribution of salmonella and shigella spp in relation to level of education

		Results			Total
		Positive to shigella spp (%)	Positive to salmonella spp (%)	Negative to both (%)	
Level of Education	No formal education	0	0	4(100%)	4
	Modern 1 completed primary school	0	0	3(100%)	3
	modern 3 completed SSCE	1(5.3%)	0	18(94.7%)	19
	Post-Secondary	1(3.7%)	1(3.7%)	25(92.6%)	27
		2(6.9%)	1(3.4%)	26(89.7%)	29
	Total	4	2	94	100

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

Level of income (Naira)	Results			Total
	Positive to <i>shigella</i> (%)	Positive to <i>salmonella</i> (%)	Negative to both (%)	
<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, ceftazidime; AMP, ampicillin; NIT, nitrofurantoin

Discussion

Diarrhea caused by enteric infections is a major factor in morbidity and mortality worldwide. An estimated 2-4 billion episode of infectious diarrhea occurs each year and is especially prevalent in infants. In developing countries, enteric bacterial pathogens and parasites are the leading cause of infectious diarrhea.¹³ 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 <6months, 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 6-11months, 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. Prevalence of diarrhea was found to be highest in children between age group 0-2years. This result agrees with the study conducted by Banerjee *et al.*¹⁴ which showed diarrhoeal that the frequency of diarrhoea was significantly higher among children of age group 6- 24months.

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 47male children, 3(6.4%) were tested positive for *Shigella* spp while 1(2.1%) was tested positive for *Salmonella* spp. Out of the total of 53female children, 1(1.9%) was tested positive for *Shigella* spp and 1(1.9%) was also tested positive for *Salmonella* spp. The prevalence of diarrhea was found to be higher in males than females. This agrees with Molbak¹⁵ who stated that the boys are more vulnerable to diarrhea than females and gives a biological explanation to the fact that boys during infancy have to build up a larger muscle mass than girls. 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 Ceftazidime which implies that the antibiotics are ineffective against the organisms. This agrees with a study by Juan-Pablo *et al.*¹⁶ 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.*¹⁷ 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

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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