Prevalence and Antibiogram of Salmonella and Shigella Species Isolated from Sachet Water in Benin City, Nigeria

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ABSTRACT

Enteric pathogens in portable water constitute a major health problem in developing countries like Nigeria. This study reports the prevalence and antibiotic resistance profile of Salmonella and Shigella species in sachet water sold in Benin City, Nigeria. Sixty randomly selected sachet water samples were cultured and identified biochemically for Salmonella and Shigella spp. and subjected to antibiotic susceptibility testing. Fifty-one enteric isolates comprising of 24 Salmonella spp. and 27 Shigella spp were found. There was no significant difference (P= 0.641) in their occurrence. Both exhibited multiple antibiotic resistances (MAR) against the tested antibiotics. Salmonella spp. were more resistant to cephalexin (91.7%) and nalixidic acid (75%), while Shigella spp. were more resistant to cephalexin and streptomycin (74.1%). The resistance profile of Salmonella spp. was not significantly different from that of Shigella spp (P = 0.172). Ten resistant patterns were demonstrated by both organisms with MAR Index range of 0.1-0.7 (for Salmonella spp) and 0.1-0.9 (for Shigella spp). The MAR index of both organisms showed no significant difference (P = 0.892). The dominant resistance phenotype for Salmonella spp was pef, cep, ofx, na, gen, str and cep, ofx, amc, na, amp, sxt with 4 Salmonella isolates each. Whereas, that for Shigella spp was Pef, cep, cpx, amp, ofx, gen, na, sxt, str with 6 Shigella isolates. Most sachet water available in this metropolis is therefore also a bag of antibiotic resistant organisms. Public health measures are highly required to determine and prevent further contamination of sachet water.

Keywords: Salmonella, Shigella, sachet water, antibiotic, resistance

INTRODUCTION

Salmonella are Gram-negative, motile bacteria belonging to the family Enterobacteriaceae. They are generally non-capsulate and non-spore forming with the exception of *Salmonella typhi*.[1] Salmonella being an enteric pathogen is found almost everywhere with a global distribution that comprises a large number of serovars characterized by different host specificity and distribution. This organism is one of the leading causes of gastroenteritis, bacteraemia all over the world as well as the etiological agent of more severe systemic diseases such as typhoid and paratyphoid fevers.[2,3]

In the year 2000, it was estimated that there are approximately 21.5 million infections and 200,000 deaths from typhoid fever globally each year.[4] In Africa, about 4.36 million cases occur out of an estimated population of 427 million and it is often encountered in tropical countries including Nigeria.[5] The pathogens typically gain entry into water systems through faecal contamination.

Shigella is a group of bacteria that can cause gastroenteritis with dysentery. *Shigella* is typically an inhabitant of the intestinal tract of humans and other primates and is excreted in large numbers in the faeces of infected individuals. It is typically spread by faecal-contaminated drinking water or food or by direct contact with an infected person. In water, Shigella can survive for at least six months at room temperature, and this high survival favours transmission through water.[6,2] 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.[7,8]

The total number of *Shigella* episodes that occur each year throughout the world is estimated to be 164.7 million, including 163.2 million cases in developing countries, 1.1 million of which result in death. Children under 5 account for 61% of all deaths attributable to shigellosis.[9,2]

Diseases caused due to consumption of water such as the ones highlighted above constitute a major health related problem in developing countries like Nigeria. The

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continuous and increasing demand, sale and indiscriminate consumption of sachet water in Nigeria, poses significant public health risks to the citizens especially individuals with compromised immune systems.[10] this is because portable drinking water is inadequate both in quality and quantity.[11] Moreover, most producers of sachet drinking water in Nigeria either just bag and seal pipe water without any form of treatment[12] or do not follow specified standards due to lack of appropriate drinking water treatment technology.[13] This study therefore examines the prevalence and antibiogram of Salmonella and Shigella spp. in sachet water sold in Benin City metropolis of South-South Nigeria.

MATERIALS AND METHOD

Study area

The study was carried out in Benin City, South-South Nigeria. Benin is a city (2006 estimated population 1,147,188) in Edo State, Southern Nigeria. It is a port on the Benin River, situated 322 kilometres by road east of Lagos.

Sample collection

Sixty sachet of water samples of 10 different brands were randomly purchased from different sale points or vendors (e.g. market, Motor Park, institutions of higher learning, etc) in Benin City metropolis, Edo State, Nigeria. The names of the 10 brands were coded as follows; A, B, C, D, E, F, G, H, I and J. Each of the brands was replicated six times using random allocation rule to have a total of 60 samples. Samples from each brand were collected weekly from June-September, 2016 and were examined within 2-4hrs of collection.

Sample analysis

Six millilitres from each sample was introduced into 15ml pre-enrichment broth (phosphate peptone water) and was incubated for 18-24hrs at 35-37^{o}C. After incubation, 1.5ml of pre-enrichment culture was transferred aseptically into 10ml enrichment broth; selenite cysteine (for evaluation of Shigella spp) and tetrathionate broth (for evaluation of Salmonella spp.). These were incubated aerobically for 24hrs at 37^{o}C. A loopful of culture from each broth was streaked unto selective agar (Salmonella-Shigella agar) and was incubated for 18-24hrs at 37^{o}C.

Identification of isolates

All the pale and dark centred colonies were considered presumptive *Salmonella spp,* hence were purified on Nutrient agar. Other isolates and presumptive Salmonella spp were identified by the conventional biochemical methods such as motility, indole and reaction on KIA.[14, 15]

The Antibiotic Susceptibility Testing

The antibiotic susceptibility of the bacterial species isolated was performed on Mueller Hinton agar plates by disk diffusion method as described by the Clinical and Laboratory Standards Institute.[16] The commercially available discs containing the following antibiotics were used; Peflacine (pef, 10ug), nalidixic acid (na, 30ug), cephalexin (Cep, 10ug), amoxicillin-clavulanic acid (amc, 30ug), gentamycin (gen, 10ug), ciprofloxacin (cpx, 10ug), ofloxacin (ofx, 10ug), streptomycin (str, 30ug), ampicillin (amp, 30ug) and co-trimoxazole (sxt, 30ug) (Himedia Laboratories pvt Limited, Mumbai, India). The discs were aseptically placed on the surfaces of the sensitivity agar plates with a sterile forceps and were incubated at 37°C 16-18 hours. Zones of inhibition after incubation were observed and the diameters of inhibition zones were measured in millimetres. The interpretation of the measurement as sensitive and resistant was made according to the CLSI standard zone size interpretative table.

Determination of Multiple Antibiotic Resistance (MAR) Index

Multiple antibiotic resistance (MAR) index was determined using the formula $MAR=x/y$, where x is the number of antibiotics to which test isolate displayed resistance and y is the total number of antibiotics to which the test organism has been evaluated for sensitivity.[17]

Statistical analysis:

Non-parametric Mann-Whitney statistics was used to test for significant difference in all the data obtained. All statistical analyses were carried out using the SPSS 22.0 (IBM) window based program. Significant difference and non-significant difference were defined when $p< 0.05$ and $p\geq 0.05$ respectively.

RESULTS

Fifty-one enteric isolates comprising of 24 Salmonella spp and 27 Shigella spp. were isolated from 60 sachet water samples (Table 1). Although the number of Shigella spp. were more than Salmonella spp., there was no statistically significant difference (P= 0.641). The antibiogram showed that all Salmonella spp. were susceptible to ciprofloxacin, but were more resistant to cephalexin (91.7%) and nalidixic acid (75%). In the same vein, Shigella spp. were more resistant to cephalexin and streptomycin (74.1%) and least to ciprofloxacin (51.9%) (Table 2). The resistance profile of Salmonella spp. was not significantly different from that of Shigella spp. (P=0.172).

The antibiotic resistant pattern showed 10 resistant patterns for both Salmonella and Shigella spp. with MAR index range of 0.1 - 0.7 (for Salmonella spp) and 0.1 - 0.9 (for Shigella spp.). The MAR index of both organisms showed no significant difference statistically (P = 0.892) (Table 3 and 4).

The most abundant resistance phenotype for Salmonella spp. are Pef, cep, ofx, na, gen, str and Cep, ofx, amc, na, amp, sxt with 4 Salmonella isolates each (Table 3). Also, the most dominant resistance phenotype for Shigella spp. are Pef, cep, cpx, amp, ofx, gen, na, sxt, str with 6 Shigella isolates (Table 3).

Table 1: Prevalence of *Salmonella* and *Shigella* species in sachet water

Brand Code	No. of sample analysed	No. (%) of sample +ve for Salmonella	No. (%) of sample +ve for Shigella
A	6	2(33.3)	2(33.3)
B	6	1(16.7)	3(50)
BB	6	4(66.7)	2(33.3)
C	6	4(66.7)	1(16.7)
D	6	2(33.3)	4(66.7)
E	6	3(50)	2(33.3)
F	6	3(50)	1(16.7)
G	6	1(16.7)	5(83.8)
H	6	1(16.7)	3(50)
I	6	3(50)	4(66.7)
Total	60	24(40)	27(45)

Table 2: Resistance profile of *Salmonella* and *Shigella* spp

Antibiotics	***Salmonella* spp[a]** No (%) Resistant	***Shigella* spp[a]** No (%) Resistant
Ciprofloxacin	0	14(51.9)
Gentamycin	11(45.8)	18(66.7)
Amoxicillin -clavulanic acid	12(50.0)	16(59.3)
Ampicillin	12(50.0)	16(59.3)
Septrin	12(50.0)	17(62.9)
Streptomycin	15(62.5)	20(74.1)
Tarvid	17(70.8)	17(62.9)
Perflacine	13(54.2)	18(66.7)
Ceporex	22(91.7)	20(74.1)
Nalixidic acid	18(75.0)	18(66.7)

a = not significantly different (P = 0.172)

Table 3: Resistance Pattern of *Salmonella* and *Shigella* spp

Salmonella spp Resistant pattern	**No. of antibiotics**	**No. of isolates**	**Shigella spp** Resistant Pattern	**No. of antibiotics**	**No. of isolates**
Amc,	1	1	Na	1	1
Sxt, ofx, str	3	1	Cep, amc, cpx	3	1
Pef, na, cep	3	2	Pef, na, cep	3	2
Pef, cep, amp, gen, str	5	2	Cpx, na, str, amp	4	2
Pef, cep, amc, gen, sxt	5	2	Amc, gen, amp, str	4	2
Cep, amc, ofx, na, sxt, str	6	2	Pef, amc, gen, na, str	5	2
Pef, cep, of x, na, gen, str	6	4	Cep, amc, ofx, amp, sxt, str	6	3
Cep, ofx, amc, na, amp, sxt	6	4	Pef, cep, amc, cpx, ofx, gen, amp, sxt	8	3
Cep, amc, ofx, na, sxt, amp, str	7	3	Pef, cep, cpx, amc, ofx, gen, na, sxt, str	9	5
Pef, cep, ofx, na, amp, gen, str	7	3	Pef, cep, cpx, amp, ofx, gen, na, sxt, str	9	6

Key: pef= Peflacine (10ug), na = nalixidic acid (30ug), Cep = ceprox (10ug), amc = amoxicillin-clavulanic acid (30ug), gen = gentamycin (10ug), cpx = ciprofloxacin (10ug), ofx = tarvid (10ug), str = streptomycin (30ug), amp = ampicillin (30ug) and sxt = septrin (30ug).

Table 4: multiple antibiotic resistances (MAR) index of *Salmonella* and *Shigella* spp

MAR Index	***Salmonella* spp (%)^{b}**	***Shigella* spp (%)^{b}**
0.1	1(4.2)	1(3.7)
0.2	0	0
0.3	3(12.5)	3(11.1)
0.4	0	4(14.8)
0.5	4(16.7)	2(7.4)
0.6	10(41.7)	3(11.1)
0.7	6(25.0)	0
0.8	0	3(11.1)
0.9	0	11(40.7)
Total	24	27

b = not significantly different (P=0.892)

DISCUSSION

According to the recommended standards for drinking water, there is no tolerable lower limit for pathogens in water intended for consumption, whether for preparing food, drinking or for personal hygiene, i.e. it should contain no bacteria pathogenic to humans.[2] This study, however show varying proportion of Salmonella and Shigella isolated in the samples. It would appear virtually all the brands are contaminated but there are no indications of an outbreak of these diseases in the area. However, the frequent high titre of disease related serological tests erroneously attributed to wrong interpretation of methodology might not be unconnected with actual high rate of infection. Infections associated with Salmonella and Shigella is among the leading global public health problems. Salmonella and Shigella cause mild to severe forms of intestinal tract infection commonly associated with consumption of a variety of foods and water.

The results of this study are comparable to previous studies where Salmonella, Shigella and Aeromonas or Pseudomonas spp. were reported as potential bacterial pathogens in packaged drinking water.[18,19] Another study similar to this showed that out of nine genera of bacteria isolated from water source, Shigella spp. had the highest frequency of occurrence followed by Salmonella and *E.coli* spp.[20] The isolates in this study are important human pathogens associated with a variety of infectious diseases such as gastroenteritis, typhoid fever, dysentery, etc.

The finding of this study showed that both Salmonella and Shigella spp exhibit multi-drug resistance (MDR) phenotype. This is comparable to previous report of prevalence and antibiogram on Salmonella and Shigella in Lettuce and red pepper.[21] Resistance of Salmonella and Shigella isolates to specific drugs could possibly be due to dissemination of drug resistance in the environment arising from the misuse of antibiotics among the general population. Also, the presence of antibiotic resistant bacteria in sachet water is especially significant because of the danger in promoting multiple antibiotic resistant organisms in humans through possible colonization of the gastrointestinal tract and conjugal transfer of such antibiotic resistance to the normal gut flora. This can lead to more complicated multiple antibiotic resistant organisms.[22] in this study, Salmonella has MAR index of 0.1 to 0.7, while Shigella spp. has MAR index of 0.1 to 0.9. The MAR indices of large proportion of these organisms are higher than 0.2 which indicate that a very large proportion of the bacterial isolates have been exposed to several antibiotics from human, environmental and agricultural sources.

The incidence of MDR Salmonella and Shigella spp. in supposedly treated and packaged water from the study area is worrisome and need to be addressed by appropriate authorities. Consequently, public health measures are highly required in the study area to determine and prevent further contamination of sachet water.

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