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PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF *Salmonella* AND *Shigella* SPECIES AMONG CHILDREN AGED 0-5YEARS WITH DIARRHEA ATTENDING GENERAL HOSPITAL MINNA, NIGER STATE, NIGERIA.

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ABSTRACT

Acute diarrhea remains one of the most prevalent diseases affecting young children in developing countries such as Nigeria. The findings of this study revealed that *Salmonella* and *Shigella* sp are among some of the major agents associated with acute diarrhea in children with a combined prevalence of 9.0% (*Salmonella* had a prevalence rate of 7.1% of the cases whereas *Shigella* accounted for a prevalence of 1.9%). Sensitivity test of the selected *Shigella* and *Salmonella* species against some antibiotics showed 100% sensitivity to Azithromycin while also being sensitive to

Introduction:

World Health Organization defines diarrhea as the passage of loose and watery stools at least three times per day, or more frequently than normal for an individual (WHO, 2017), which results to morbidity and mortality in children. Diarrhea can be caused by different agents such as bacteria, parasites and viruses and the common route of infection by these pathogens is through the ingestion of contaminated foods and drinks

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Ceftriaxone, Ciprofloxacin, Cefuroxime, Augmentin and Gentamicin. 100% resistance was observed to Tetracycline and Ampicillin while also being resistant to Metronidazole and Trimethoprim-sulfamethozaxole. Proper environmental cleanliness, good personal hygiene and proper balance diet is recommended for children as well as antibiotics such as Azithromycin, Ciprofloxacin, Ceftriaxone should be used in treatment of acute diarrhea caused by *Salmonella and Shigella* sp.

Keywords: diarrhea, *Salmonella*, *Shigella*, antibiotics, and resistance

(Gebreegziabher *et al.,* 2018). The burden of diarrheal disease seriously affects young children in developing countries whose incidence rates is high due to inadequate safe drinking water, poor sanitation and suboptimal breastfeeding, as well as zinc and Vitamin A deficiency (Peter and Umar, 2018). It has been reported that childhood diarrhea affecting children 5 years old and below accounts for approximately 63% of diarrhea burden (Ugboko *et al.,* 2020).

Among the causative agents of diarrhea, bacteria are responsible for more severe cases of infectious diarrhea especially in children. Children below 5years are the most at risk from foodborne pathogens, such as shiga toxin producing *Eschericchia coli* O157, *Camphylobacter, Shigella, Yersinia, Salmonella* and *Cryptosporidium* (Ugboko *et al.,* 2020). *Salmonella* and *Shigella* species continue to play a major role as the etiology of diarrhea and dysentery in resource-limited environments (Abera *et al*., 2021). They cause mild to severe forms of intestinal tract infection commonly associated with consumption of a variety of foods (Lamboro *et al*., 2016).

About 25% of the overall diarrheal burden has been attributed to diarrhea among children below the age of 5 in Africa, especially Sub-Saharan Africa which has the highest death rate (Ugboko *et al.,* 2020). In Nigeria, there is a regional discrepancy in the distribution of diarrhea. There is an increasing prevalence of diarrheal disease among infants in the Northern part of Nigeria than in the South (Dairo *et al.,* 2017). The prevalence of childhood diarrhea in Nigeria is 18.8% and it accounts for over 16% of deaths, estimated at about 150,000-175,000 annually (Peter and Umar, 2018; Omole *et al.,* 2019). Additionally, the continuous emergence and spread of multiple antimicrobial resistances to antibiotics used in treatment have been described for *Salmonella* and *Shigella* species (Gebreegziabher *et al*., 2018) and this has made antibiotic selection difficult for the treatment of diarrhea caused by this organisms leading to health complications and death (Mamuye *et al*., 2015; Abera *et al*., 2021). This study aims to determine the prevalence and antibiotic susceptibility patterns of *Salmonella* and *Shigella* species among children aged 0-5years with diarrhea attending General Hospital Minna, Niger State. The continuous investigation and surveillance of the antibiotic susceptibility pattern of these diarrhea causing agents will aid in the selection of more effective drugs and establishment of effective management and intervention measures to control diarrhea infection in public health sectors (Lamboro *et al*., 2021).

MATERIALS AND METHOD

Study area

This study was conducted in General Hospital Minna, Niger State which is attended by most of the population in Minna. Minna which is the capital of Niger State is an area located on latitude $9^0$33N and $9^0$45N and longitude $6^0$34E and $6^0$42E and has a population of about 462,743 according to the World Urbanization Prospects 2021.

Study Population

The study population comprised of children diagnosed with acute diarrhea attending General Hospital, Minna. A well-structured questionnaire and signed consent via a consent form was administered to the children's guardians before admission into this study.

Inclusion criteria

Under five-year-old children (male and female) with acute diarrhea (≥3 watery or loose stools with or without blood or mucus less than 14 days). Written consent from the guardians of the children.

Exclusion criteria

Children who had taken antibiotics 2 weeks before the study, children with incomplete demographic information and children whose parents do not give consent.

Sample Size Determination

Using a prevalence of 16% from a study carried out in Nigeria by Adeh *et al.,* 2019, the sample size was determined using the Fisher's formula:

Equation 1: $n = \frac{z^2pq}{d^2}$

Where: n = Sample Size

z = 1.96 (constant)

p = Anticipated Population Proportion (16%)

d = 0.05 (constant)

q = 1-p

$$n = \frac{z^2pq}{d^2}$$

$$n = \frac{(1.96)^2(0.16)(0.84)}{(0.05)^2}$$

$$n = 207 \text{ samples}$$

Samples to be collected in this study = 210.

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Sample collection

Stool samples were collected from the children with the assistance of their guardians into sterile sample containers and labeled with the sex and age of the patients. The samples were stored in a sterile ice pack and transported to the Laboratory for further analysis.

Isolation and Identification of Isolates

Approximately, 1g of stool specimen was suspended in Selenite F broth in sterile test tube overnight and then inoculated on *Salmonella-Shigella* Agar (SSA) and incubated at 37^0C for 24h. The isolates were then sub-cultured on nutrient agar plates to obtain pure isolates. The pure isolates obtained were identified through their morphological characteristics, Gram staining and biochemical tests such as Triple Sugar Iron test (TSI), Motility test, Indole test, Methyl red test, Voges-proskaeur test and Citrate utilization test (Fawole and Oso, 2011; Aneja, 2014; Zuhair *et al.*, 2014).

Antibiotic Susceptibility Test

The antimicrobial susceptibility testing for the isolates was determined using the Kirby bauer disk diffusion method on Mueller-Hinton agar according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Some commonly used commercial antibiotic discs for the treatment of diarrhea were used for this test and they include: Azithromycin (15µg), Metronidazole (30µg), Cefuroxime (30µg), Ciprofloxacin (5µg), Ceftriaxone (30µg), Trimethoprim-sulfamethoxazole (1.25/23.75µg), Tetracycline (30µg), Gentamicin (10µg), Augmentin (30µg) and Ampicillin (10µg). The isolates were inoculated on Muellar–Hinton agar and the antibiotic discs were placed and incubated at 37°c for 24 hours. The zones were read and interpreted according to the break-points and criteria recommended by the Clinical Laboratory Standards Institute (CLSI, 2018).

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Phenotypic Detection of EBSL producing Isolates

The double disk method will be used to identify extended spectrum β-lactamases (EBSLs) activity. This method will be performed using a combination of cefotaxime (30μg), ceftazidime (30μg), ceftriaxone (30μg), amoxicillin (20μg)/clavunate (10μg). EBSL producers will be identified by reduced zone sizes to third-generation cephalosporins (ceftazidime and ceftriaxone), and expansion of these zones in the presence of the inhibitor (clavunate). Confirmatory tests for EBSL producers will be performed using cephalosporins/ β-lactam inhibitor combinations, namely cefotaxime (30μg)/clavunate (10μg) and ceftazidime (30μg)/clavunate (10μg). Size of the zones will be measured and interpreted according to the CLSI guidelines (Lan *et al.,* 2017).

RESULTS

Demographic and Socio-economic characteristics

With regards to the guardian's information, majority 198 (94.3%) of the guardians were married, more than half of them 150 (71.4%) completed secondary school with 127 (60.5%) of them being unemployed.

Table 1: Socio-demographic data of the guardians in General Hospital Minna, Niger State, Nigeria from May to August 2022.

Variable		Frequency	Percent %
Marital status	Single	12	5.7
	Married	198	94.3
Educational status	Illiterate	13	6.2
	Primary school	32	15.2
	Secondary school	150	71.4
	College	15	7.1
Occupation	Unemployed	127	60.5

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	Employed	30	14.3
	Self-employed	53	25.2

Among the 210 under-five aged children, 158 (75.2%) were female and 52 (24.8%) were male resulting in a female-to-male ratio of 3:1. More than half 159 (63.0%) of the patients were between the age group of 25 to 60 month.

Table 2: Socio-demographic data of under-five children with diarrhea

Variable		Frequency	Percent %
Age in month	0-12	12	5.7
	13-24	95	45.2
	25-60	103	49.0
Gender	Male	52	20.0
	Female	158	75.2
fever	Yes	69	32.9
	No	141	67.1
Vomiting	Yes	12	5.7
	No	198	94.2
Dehydration	Yes	6	2.9
	No	204	97.1
Stool appearance	**Watery**	**103**	**49.0**
	Mucoid	**72**	**34.2**
	Bloody	**35**	**16.7**

Distribution and prevalence of the Isolates

Among the 210 examined stool samples, *Salmonella* sp had a prevalence rate of 7.1% (15) whereas *Shigella* sp accounted for a prevalence of 1.9% (4).

Table 3: Distribution and Prevalence of *Salmonella* and *Shigella* in under-five children with diarrhea in General Hospital Minna, Niger State, Nigeria from May to August, 2022.

	0-12 months	13-24 months	25-60 months	Total
Salmonella sp	4	6	5	15
Shigella sp	1	2	1	4
Total	5	8	6	19

Antibiotic Sensitivity Patterns

Among the 19 samples subjected to antimicrobial susceptibility testing, the four (4) isolates of *Shigella* were resistance to ampicillin and tetracycline, showed strong sensitivity to ciprofloxacin, cefuroxime, azithromycin, gentamycin while some showed slight sensitivity to ceftriaxone and trimethoprim-sulfamethozaxole. The *Salmonella* isolates also all showed resistant to ampicillin and tetracycline but were sensitive to azithromycin, ciprofloxacin and cefuroxime with some isolates showing slight sensitivity to trimethoprim-sulfamethozaxole and metronidazole.

Table 4: Antimicrobial susceptibility patterns of *Salmonella* and *Shigella* isolates among under five years old children in General Hospital Minna Niger state, Nigeria.

Antibiotics	*Salmonella sp* (15)			*Shigella sp* (4)			Total (19)		
(Number & Percentage)	S	I	R	S	I	R	S	I	R
Augmentin (AU)	**9(60.0)**	**4(26.7)**	**2(13.3)**	**0(0.0)**	**2(50.0)**	**2(50.0)**	**9(47.4)**	**6(31.6)**	**4(21.1)**
Azithromycin (AZN)	**15(100)**	**0(0.0)**	**0(0.0)**	**4(100)**	**0(0.0)**	**0(0.0)**	**19(100)**	**0(0.0)**	**0(0.0)**
Gentamycin (CN)	**8(53.3)**	**5(26.7)**	**2(13.3)**	**3(75.0)**	**1(25.0)**	**0(0.0)**	**11(57.9)**	**6(31.6)**	**2(10.5)**

Metronidazole (MET)	1(6.7)	7(46.7)	7(46.7)	2(50.0)	1(25.0)	1(25.0)	3(15.8)	8(42.1)	8(42.1)
Cefuroxime (CXM)	10(66.7)	5(26.7)	0(0.0)	3(75.0)	1(25.0)	0(0.0)	13(68.4)	6(31.6)	0(0.0)
Ciprofloxacin (CPX)	12(80.0)	3(20.0)	0(0.0)	4(100)	0(0.0)	0(0.0)	16(84.2)	3(15.8)	0(0.0)
Ceftriaxone (CTR)	12(80.0)	0(0.0)	3(20.0)	0(0.0)	3(50.0)	1(25.0)	12(63.2)	3(15.8)	4(21.1)
Trimethoprim-sulfamethozaxole (SXT)	2(13.3)	4(26.7)	9(60.0)	0(25.0)	2(50.0)	2(50.0)	3(15.8)	6(31.6)	10(52.6)
Tetracycline (TET)	0(0.0)	0(0.0)	15(100)	0(0.0)	0(0.0)	4(100)	0(0.0)	0(0.0)	19(100)
Ampicillin (PN)	0(0.0)	0(0.0)	15(100)	0(0.0)	0(0.0)	4(100)	0(0.0)	0(0.0)	19(100)

Keys: S=sensitive, R=resistant, I=intermediate

Multiple Antibiotic Resistance Index (MARI) of *Salmonella* and *Shigella* Isolates

It was observed that *Salmonella* and *Shigella* species showed multiple antibiotic resistance to the antibiotics used in the study. Among *Salmonella* isolates, 9 of them (60.0%) showed resistance to two to three antimicrobials, 3 of them (20.0%) were resistant to four antimicrobials and then 1(6.7%) & 2(13.3%) of the *Salmonella* isolates were resistant to four and five antimicrobials respectively. Meanwhile for *Shigella* isolates, 2(50.0%) of them showed multidrug resistance to two antimicrobials, 1(25.0%) isolate showed resistance to three antimicrobials and 1 (25.0%) *Shigella* isolate was resistant to five antimicrobials. Therefore, multidrug resistance (MDR) was seen in all of the isolates as shown in table 5&6. The formula used to calculate the MAR index for each isolate is given below

$$MAR\ index = \frac{\text{Number of antibiotics to which isolate is resisitant}}{\text{Total number of antibiotics against which isolate was tested}} \quad (4.1)$$

Table 5: MAR index of *Salmonella* sp. Isolates

Isolate number	S1	S2	S3	S4	S5	S7	S8	S9	S10	S14	S15	S16	S17	S18	S19
(a)	4	3	4	3	6	2	3	2	6	4	3	2	5	2	2
MAR index= (a)/b	0.4	0.3	0.4	0.3	0.6	0.2	0.3	0.2	0.6	0.4	0.3	0.2	0.5	0.2	0.2

(a) = No. of antibiotics to which isolate was resistant **b =** the number of antibiotics to which the isolate was exposed (n=10)

Table 6: MAR index of *Shigella* spp. Isolates

Isolate number	No. of antibiotics to which isolate was resistant (a)	MAR index= a/b
S6	5	0.5
S11	2	0.2
S12	3	0.3
S13	2	0.2

b = the number of antibiotics to which the isolate was exposed (n=10)

Table 7: Phenotypic Detection of EBSL producing Isolates

This table shows the result of some isolates being resistance to some third generation cephalosporins thereby signifying the production of Extended Broad Spectrum Betalactamases.

Isolate Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Cefotaxime 30μg (CTX)	*	+	*	+	+	+	+	+	*	+	+	*	+	+	*	*	+	*	*
Ceftazidime 30μg (CAZ)	+	+	+	*	+	+	*	*	+	+	*	*	+	+	+	+	+	*	*

Ceftriaxone 30µg (CTR)	*	*	*	*	+	+	*	*	*	+	*	*	*	*	*	*	+	*	*

*= Negative, + = positive

Discussion

This study intended to examine the prevalence, and antimicrobial susceptibility status of *Salmonella* and *Shigella* species isolated from under-five children with diarrhea attending general hospital in Minna Niger state. The overall prevalence of *Salmonella* and *Shigella* infection was 9.0%, and there was a high rate of multidrug resistance, among the isolates. While the highest resistance observed was against Ampicillin and Tetracycline, the least was for Gentamicin, Augmentin, and Ceftriaxone. The overall prevalence of *Salmonella* and *Shigella* infection was less than the findings from studies carried out by Egbuim, *et al.,* 2018 (13.2%), Ali *et al.,* 2020 (16.7%) and Mamuye *et al*., 2015 (13.1%). The lower *Shigella* and *Salmonella* isolation might be due to other potential enteric pathogen causes of diarrhea such as Rotavirus, *Escherichia coli*, and protozoans.

In this study, the majority 189 (90.0%) of participants used unprotected water for drinking and 168 (80.0%) of the care givers had poor hand washing practices. Around 126 (60.0%) of the care givers had a good hygienic status and 321 (78.1%) of them did not clean utensils for their child. Only 17.2% of mothers/caregivers used ventilated improved toilets while the remaining 82.7% made use of open defecation or local toilets. Associated factors related to child diarrhea were assessed, and it was found that unprotected drinking water source, poor hand washing practice of caregivers and poor cleaning of utensil for child feeding were statistically significant for the presence of enteropathogens in stool samples.

This study shows that the isolates *Salmonella* and *Shigella* sp were multi resistant to different classes of antibiotics ranging from 2-6 antibiotic classes. They showed complete resistance to Ampicillin and Tetracycline and high resistance to Trimethoprim-sulfamethozaxole. However, the isolates were completely sensitive to Azithromycin and showed high sensitivity towards Ceftriaxone, Ciprofloxacin, Gentamicin and Cefuroxime. This finding showed that *Shigella* and *Salmonella* are resistant to the β - lactam drug Ampicillin while sensitive to Fluroquinolones such as ciprofloxacin and cephalosporins like Cefuroxime and Ceftraixone. This findings correlates with studies carried out by Tosisa *et al*., 2020, Eyamo *et al*., 2020 and Assefa & Girma 2019 who recorded high resistance to Ampicillin and Tetracyclines while high sensitivity was observed towards Ciprofloxacin, Cefuroxime, Ceftriaxone and Gentamicin by both *Salmonella* and *Shigella* species. High resistance to Ampicillin and Tetracycline may be attributed to the fact that these antibiotics have been used for long in the country and because of their easy availability and potential for misuse. Also, the public health implication of such high resistance could be associated with the suboptimal water and sanitation conditions and inadequate sewage disposal systems. This could be further complicated by the hand hygiene practice of caregivers and/or mothers.

CONCLUSION

In conclusion, antibiotic resistance has increased in *Salmonella* and *Shigella* sp. due to misuse and overuse of antibiotics. The high prevalence of multi-drug resistance in *Salmonella* and *Shigella* isolates increases the concerns about dissemination of the antibiotic-resistant isolates in this bacterium. Avoiding the distribution of antibiotic resistance and the spread of the integrons in both *Salmonella* and *Shigella* sp. is an immediate issue. Therefore, regular monitoring programs to prevent further spread of MDR are essential.

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This study also revealed that the enteric pathogen infection was significantly associated in poor hand washing practice, drinking unprotected water and usage of unclean utensils for feeding of children. Therefore, improving hygiene status of under five children and implementation work on identified associated factors with regular drug susceptibility test is important to reduce the problem. The government should imply and ease the supply of getting protective water and improve the sanitary conditions of the peoples.

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