

Research Article

# Determination of antibiotics susceptibility profile of *Shigella* species isolated from children with acute diarrhea

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## Abstract

Diarrheal diseases continue to be the major cause of morbidity and mortality among children under 5 years. This study aimed to isolate, identify and determining the prevalence, antimicrobial susceptibility profile of *Shigella* sp associated with acute diarrhea among children in Kano, Northern Nigeria. A cross sectional study was conducted among children less than 5 years diagnosed with acute diarrhea and admitted to paediatric ward of Murtala Muhammad Specialist Hospital Kano. Stool samples from a total of 37 (20 male and 17 female) subjects were used to isolate and identified the pathogen. Antimicrobial susceptibility test was conducted using disc diffusion method. The result showed 12 out of 37 samples were positive for *Shigella* sp which accounted for 32.4%. Higher incidence of *Shigella* sp was found among subjects of age between 2 – 3 years. The isolates were 100% resistant to Ampicillin. High resistance was also observed in Amoxicillin (83.33%), Chloramphenicol (58.33%) and Tetracycline (25%). The isolates are 100% sensitive to ciprofloxacin, 66.7% to Levofloxacin and Gentamicin each and 58.33% to Erythromycin. Three (3) isolates were resistance to Ampicillin and Amoxicillin, 5 isolates were resistance to Ampicillin, Chloramphenicol and Amoxicillin while 2 isolates were resistance to Ampicillin, Chloramphenicol, Tetracycline and Amoxicillin. It is concluded that *Shigella* sp is one of the etiological agent of diarrhea in children. Ciprofloxacin, levofloxacin and Gentamicin are drugs of choice for treating diarrhea caused by *Shigella* sp.

## Introduction

Globally, Diarrheal diseases remain one of the leading causes of morbidity and mortality among children < 5 years of age [1]. About 21% of all deaths in children under the age of five (years) are estimated to be due to diarrhoea and related infections. In Sub-Saharan Africa, it is the second leading cause of death in children under 5 years of age [2,3]. About 37% of all paediatric/childhood deaths in Nigeria are caused by acute diarrhoea, with most of the deaths occurring during the first year of life [4]. Diarrhoea is defined as three or more episodes of watery loose stools in the last 24 hours [4,5]. Diarrhoea can be caused by different agents such as bacteria, parasites and virus [6]. The main etiology of the diarrhoea is related to a wide range of bacteria (such as *Campylobacter jejuni*, *Escherichia coli*, *Salmonella species*, *Shigella species*,

*Vibrio cholera*, *Yersinia enterocolitica*, and *Aeromonas species*), enteroparasites (*Giardia lamblia*, *Cryptosporidium species* and *Entamoeba histolytica*), and viruses (adenovirus, Norwalk virus, and rotavirus) [6]. Among the bacterial causative agents, *Salmonella* and *Shigella* remain the major contributors to acute enteric infections and diarrhoea. The common route of infection by these pathogens is the ingestion of contaminated foods and drinks. The problem of antimicrobial resistance in bacterial pathogens causing diarrheal diseases continues to be alarming [7].

Shigellosis is caused by *Shigella* sp and it is a worldwide problem although more prevalent in developing countries [8]. Shigellosis is common in areas where living standards are very low and access to safe and adequate drinking water and proper waste disposal systems are often very limited, or

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even absent [9]. *Shigella* sp are limited to the intestinal tract of humans and cause bacillary dysentery leading to watery or bloody diarrhea [10]. A variety of raw vegetables, salads, meat, milk, and other dairy products can serve as vehicles for the transmission of *Shigella* sp. Therefore, the most common causes of contamination are unsanitary practices of food handlers and fecally contaminated water [11]. All age groups are affected by *Shigella*, but the age group under 5 years is most susceptible, because of low personal cleanliness and partially developed immunity and absence of past exposures [12].

Microbial resistance to antibiotics is one of the most serious health problems threatening human well-being today. Antibiotic resistance is a type of drug resistance where a microorganism is able to survive exposure to an antibiotic [13]. The widespread use of antibiotics both inside and outside of medicine is playing a significant role in the emergence of resistant bacteria [14]. Infections from resistant bacteria are now too common and some pathogens have even become resistant to multiple types or classes of antibiotics (antimicrobials used to treat bacterial infections) [15]. This resistance has complicated the selection of antibiotics for the treatment of enteric bacterial pathogens, particularly to commonly used antimicrobial agents such as ampicillin, tetracycline and trimethoprim-sulfamethoxazole [16]. Since most diarrheal diseases are treated empirically, it is important to know the susceptibility pattern of the prevalent pathogens. There is a great need to establish the identity and antibiotic susceptibility patterns of different bacterial agents which cause enteric infections in order to introduce effective treatment for diarrheal illness [17]. Hence, this study was aimed to isolate, identify and determine the antibiotic susceptibility profile of *Shigella* sp associated with acute diarrhea among children in Kano, Northern Nigeria.

## Materials and methods

### Study area

The study was conducted at paediatric ward of Murtala Muhammad Specialist Hospital Kano. Kano State is one of the states located in Northern Nigeria. It is geographically coordinated at 11° 3' N and 8° 3' E latitude and longitude respectively. It shares borders with Kaduna state to the west, Bauchi state to the South, Jigawa state to the East, Katsina state to the North. It has a total area of 20,131 km<sup>2</sup> (7,777 sqm) and population of 13,405,300 [18].

### Study design

The inclusion criteria for the study participants were being a child of five years of age or less and diagnosed with acute diarrhea. Those having a history of antibiotics use two weeks before enrolment were excluded from the study. A simple structured questionnaire was used to collect participants' demographic information such as sex, age, residential area and socio-economic of the parents.

### Fecal samples collection

Thirty-seven (37) Fecal samples from children (ranges from 1 – 5 years) diagnosed with acute diarrhea admitted to paediatric ward of Murtala Muhammad Specialist Hospital Kano were collected in clean, dry and leak proof sterile bottle from period of January to April 2017. The specimens were immediately transported to Laboratory of Science Laboratory Technology in School of Technology, Kano State Polytechnics for bacterial isolation and identification.

### Isolation and identification of *Shigella* sp

Isolation of *Shigella* sp was conducted according to the method describe by Prescott, et al. [19]. During the process, a sterile wire loop was deep into the fecal sample of the patients and streaked onto the surface of Nutrient agar and MacConkey agar (Life save Biotech, USA). The procedure was repeated for sample and the plates were incubated 37° C for 24 hours. The presumptive colony of *Shigella* sp from each plate was further sub-cultured to obtained pure culture. The pure isolates of *Shigella* sp were preserved in peptone water for further use. The preserved isolates were confirmed as *Shigella* sp using conventional microbiological methods which include Gram staining, lactose fermentation and biochemical (Indole, methyl orange, voges proskauer, nitrate reduction and citrate utilization) tests according to the methods described by Cheesbrough [20].

### Antibiotic susceptibility testing

The *Shigella* sp isolates were subjected to antibiotic susceptibility testing using the agar disc diffusion method as described by Bauer, et al. [21]. Mueller Hinton agar (MHA) plates were inoculated with overnight culture of each isolate by streak plating. The standard antibiotic sensitivity discs were then aseptically placed at equidistance on the plates and allowed to stand for 1 hour. The plates were then incubated at 37 °C for 24 hours. Sensitivity pattern of the isolates to Ampicillin (30 µg), Tetracycline (30 µg), Amoxicillin (30 µg), Erythromycin (10 µg), Chloramphenicol (30 µg), Levofloxacin (10 µg), Gentamicin (30 µg) and Ciprofloxacin (30 µg), produced by Abtek pharmaceutical limited, were determined. Isolates were divided into two groups based on the zone of inhibition produced by the antibiotic disc; susceptible and resistant according to the European committee on antimicrobial susceptibility testing (EUCAST) breakpoint for interpretation of MICs and zone diameters [22]. The experiment was conducted in triplicate and the average zone of inhibition was recorded.

### Ethical approval

Ethical clearance (MOH/off/797/T.I/49) for the study was obtained from Kano State Ministry of Health through Health Service Management Board Kano with the consent of Murtala Muhammad Specialist Hospital Kano ethical committee.



## Result

### Age and sex distribution of the subjects

Table 1 represents the frequency, sex and age of the subjects. A total of 37 participated in the study with 20 males accounted for 54% and 17 females (46%). Subjects within age category 1 – 2 years has the highest frequency with 11 subjects accounted for 29.7%, followed closely by subjects within 2 – 3 years 9 (24.4%). Least frequency was recorded among subjects within age category 4 – 5 years with total frequency of 4 (10.8%).

### Identification of *Shigella* sp

The result for identification of *Shigella* sp is presented in table 2. The isolate was identified based on Gram staining, cultural characteristics and biochemical test. The result showed that the isolate is Gram negative bacteria, positive for MR, nitrate reduction and indole tests but negative for VP, urease and citrate utilization test. The isolate cannot ferment lactose and produce transparent colourless colony in both MacConkey and *Salmonella-Shigella* agar.

### Prevalence of *Shigella* sp

The Prevalence of *Shigella* sp in the fecal sample of the subjects with acute diarrhoea is presented in table 3. The result showed 12 out of 37 samples were positive for *Shigella* sp which accounted for 32.4%. Higher incidence of *Shigella* sp was found among subjects of age between 2 – 3 years.

### Antibiotic sensitivity testing

The result for antibiotic susceptibility testing is presented in table 4. From the result, the percentage resistance of the isolates to the tested antibiotics is 37.5%. The result showed that all *Shigella* sp were resistant to Ampicillin. High resistance was also observed in Amoxicillin (83.33%), Chloramphenicol (58.33%) and Tetracycline (25%). The isolates are 100%

**Table 1:** Age and sex distribution of the Subjects.

Age (year)	Male (n)	Female (n)	Frequency (n)	Percentage (%)
Less than 1	4	3	7	18.9
1 – 2	7	4	11	29.7
2 – 3	5	4	9	24.4
3 – 4	2	4	6	16.2
4 – 5	2	2	4	10.8
Total	20	17	37	100

**Table 2:** Biochemical Characterization of *Shigella* sp.

S/N	Test	Inference
1	Gram staining	-
2	Indole	+
3	Methyl-red	+
4	Voges Proskauer	-
5	Citrate utilization	-
6	Nitrate reduction	+
7	Urease	-
8	Lactose fermentation	-
9	Salmonella-Shigella agar growth	Transparent colorless colony

**Table 3:** Prevalence of *Shigella* sp in the faecal sample of the subjects.

Age (year)	Frequency (n)	<i>Shigella</i> sp + (n)	Prevalence (%)
Less than 1	7	2	5.4
1 – 2	11	3	8.1
2 – 3	9	4	10.8
3 – 4	6	2	5.4
4 – 5	4	1	2.7
Total	37	12	32.4

**Table 4:** Antibiotic Susceptibility testing of the isolates against some antibiotics.

Antibiotics	Concentration (µg)	Sensitive	Intermediate	Resistant
Ampicillin	30	0 (%)	0 (%)	12 (100%)
Tetracycline	30	3 (25%)	6 (50%)	3 (25%)
Amoxicillin	30	0 (0%)	2 (16.67%)	10 (83.33%)
Chloramphenicol	30	2 (16.67%)	3 (25%)	7 (58.33%)
Gentamicin	30	8 (66.67%)	3 (25%)	1 (8.33%)
Levofloxacin	10	8 (66.67%)	2 (16.67%)	2 (16.67%)
Ciprofloxacin	30	9 (75%)	3 (25%)	0 (%)
Erythromycin	10	7 (58.33%)	4 (33.34%)	1 (8.33%)

**Table 5:** Multi-drug Resistance Pattern of the Isolates.

S/N	No. of Antibiotics resisted	Antibiotics resisted	No. of isolates
1	2	Amp, Amox	3
2	3	Amp, Chlo, Amox,	5
3	4	Amp, Chlo, Amox, Tetr.	2

Key: Amp: Ampicillin; Chlo: Chloramphenicol; Amox: Amoxicillin; Tetr: Tetracycline

sensitive to ciprofloxacin. Other antibiotics that are effective against the isolate include Levofloxacin and Gentamicin with sensitivity of 66.7% each and Erythromycin (58.33%).

### Multidrug resistance pattern of the isolates

The multi-drug resistance pattern of the isolates is presented in table 5. The result showed that 3 isolates were resistance to Ampicillin and Amoxicillin, 5 isolates were resistance to Ampicillin, Chloramphenicol and Amoxicillin while 2 isolates were resistance to Ampicillin, Chloramphenicol, Tetracycline and Amoxicillin.

## Discussion

Acute diarrhoea might strike at any age, but it is mostly a children disease affecting those younger than 5 years. The period of weaning is a major factor to favor the occurrence of diarrhoea, while many other risk factors such as low socioeconomic level, poor hygiene habits, unsavory dwelling, elevated environmental exposure to entero-pathogenesis and poor nutritional status may be considered [23]. In table 2, the isolate was tested for gram staining and biochemical test. The isolate produces colourless and transparent colony on MacConkey and *Salmonella-Shigella* agar medium; hence cannot ferment lactose. In the present study, *Shigella* sp was found as one of the bacteria associated with acute diarrhoea in children. Out of 37 samples examined, 12 were positive for *Shigella* sp which accounted for 32.4%. Higher incidence of *Shigella* sp was found among subjects of age between 2–3 years. Several studies were conducted to characterize bacteria associated with gastro-intestinal infection and found *Shigella*



sp as one of the bacteria associated with diarrhea. The finding of this study was in conformity with that of Elmanama and Abdelateef [24] in Palestine and Sheikh, et al. [25] in Iran who both found *Shigella* in stool sample of Children diagnosed with diarrhea as 16.7% and 13.2% respectively. Similar study was conducted by Obi, et al. [26] on enteric bacterial pathogen in stools of residents of urban and rural regions of Nigeria, the results shows the most frequently encountered pathogens in rural area are *E. coli*, followed by *Salmonella*, *Shigella*, *Enterobacter* and *Campylobacter*. A study conducted by Lopez, et al. [27] on Entero-pathogenic agents isolated in persistent diarrhoea, the result shows that *Salmonella*, *E. coli* and *Shigella* as the major enteric bacteria responsible for gastric disorder. This finding supported the present study.

On the susceptibility of the isolates against the antibiotics used, some of the antibiotics were active against the isolates while resistance to others. The isolates were 100% resistant to Ampicillin. High resistance was also observed in Amoxicillin (83.33%), Chloramphenicol (58.33%) and Tetracycline (25%). The isolates are 100% sensitive to ciprofloxacin, 66.7% to Levofloxacin and Gentamicin each and 58.33% to Erythromycin. This finding showed *Shigella* is sensitive to  $\beta$ -lactam drugs (Amoxicillin and ampicillin) while sensitive to Fluroquinolones such as ciprofloxacin and Levofloxacin. Isolates were also sensitive to Gentamicin. This finding correlate with that of Gebreegziabher, et al. [7] and Ameye, et al. [9] who both found resistivity of *Shigella* to Amoxicillin and Ampicillin but sensitive to Fluroquinolones such as Ciprofloxacin. High resistance to Amoxicillin and Ampicillin may be attributed to the fact that these antibiotics have been used for long in the country ampicillin and because of their easy availability and potential for misuse. *Shigella* isolates produce R plasmids that code for several resistance genes and can confer multiple antibiotic resistances [28]. Similarly, analyses of antibiotics resistance genes of *Shigella* isolates causing diarrhea in children under age of 5 years in South eastern Africa revealed the presence of oxa-1-like  $\beta$ -lactamases for ampicillin, dfrA1 genes for trimethoprim-sulfamethoxazole/co-trimoxazole, tetB genes for tetracycline and Chlactyltransferase (CAT) activity for chloramphenicol [4]. Isolates were resistance to Ampicillin and Amoxicillin, 5 isolates were resistance to Ampicillin, Chloramphenicol and Amoxicillin while 2 isolates were resistance to Ampicillin, Chloramphenicol, Tetracycline and Amoxicillin.

## Conclusion

Acute diarrhoea remains as one of the most prevalent diseases affecting young children in developing countries. The findings of the study revealed that *Shigella* sp is one of the bacteria associated with acute diarrhoea in children. It is highly prevalent (32.4%) among children diagnosed with acute diarrhea. Sensitivity test of *Shigella* sp against some antibiotics showed sensitivity to ciprofloxacin, Levofloxacin, Erythromycin and Gentamicin and resistant to Ampicillin and

Amoxicillin. Proper environmental cleanliness, good personal hygiene and use balance diet is recommended for children as well as antibiotics such as ciprofloxacin, Gentamicin and Levofloxacin should be used in treatment of acute diarrhoea. It is also recommended that; further studies on genetic makeup of the isolated should be conducted to determine the resistant genes.

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