Received: 18th March, 2020

Accepted: 19th June, 2020

Prevalence, Risk Factors and Antibiotic Susceptibility Pattern of *Shigella* spp Isolated from Gastroenteritis Patients attending some Hospitals in Kano State, Nigeria

*Sulaiman, M.A., Aminu, M., Ella, E.E. and Abdullahi, I.O.

Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University, Zaria

Correspondence address: +2348038937309/ masulaiman@abu.edu.ng

Abstract

Shigella spp are among the major causes of gastroenteritis, some of which have become multidrug resistant (MDR), making the infection a public health threat. The study was aimed at determining the prevalence, risk factors and antibiotic susceptibility pattern of *Shigella* spp isolated from gastroenteritis patients. A total of 540 stool samples were collected, involving 450 from gastroenteritis patients (GEPs) and 90 from apparently healthy individuals (AHIs). The isolates were identified based on conventional microbiological techniques and their susceptibility patterns were determined by using Kirby-Bauer method. The patients' information and demographics were obtained by administering questionnaire. The overall prevalence recorded was 0.9%, with 1.1% and 0% in GEPs and AHIs respectively (Odd ratio= 2.246; 95%CI= 0.1225-40.7708 Significance Level = 0.5873). The highest (2.4%) and lowest (0%) prevalence was recorded among patients who presented with diarrhoea and formed stool respectively ($p=0.0487$). The prevalence was higher among those who presented with bloody stool (23.1%), compared to those whose appeared normal (0.23%) ($p=0.00001$). Additionally, prevalence of 4.8% and 0% was observed among those who experienced fever and headache respectively, and the observed differences were significant ($P=0.0097$). However, neither age nor gender was found to be a risk factor. All the isolates were susceptible to augmentin, and 60% of the isolates showed significant Multiple Antibiotic Resistance (MAR) index. It was concluded that the prevalence of shigellosis was comparatively low and most of the patients presented with bloody diarrhoea and fever. Augmentin was the drug of choice and a possible sign of inappropriate use of antibiotics was observed among the subjects.

Key Words: Gastroenteritis, Shigellosis, Antibiotics Susceptibility Pattern, Kano state

INTRODUCTION

Gastroenteritis is an illness caused by infection or inflammation of the digestive tract, characterized by nausea, vomiting, diarrhoea and stomach cramp (CDC, 2019). *Shigella* is an organism (bacteria) associated with serious gastroenteritis. The identification of the pathogen remained a challenge, as the organism is fragile outside the human hosts (Abdullahi *et al.*, 2010). There are four subgroups of *Shigella* represented as letters A, B, C and D, being *S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei* respectively (Aranda *et al.*, 2004; Murray *et al.*, 2007). All these subgroups are intracellular parasite that can induce the Peyer's patch cells to ingest them after being phagocytosed thereby disrupting the phagosome membrane and get released into the cytoplasm where they reproduce (Willey *et al.*, 2008). After the reproduction, they invade adjacent mucosal cells where they initiate the inflammatory reaction (Willey *et al.*, 2008; Baker and The, 2018). The watery stools is often bloody, mucoid, and may contain pus cells, while in severe cases lesion is developed in the colon (Hyma *et al.*, 2005; Willey *et al.*, 2008; Helmy *et al.*, 2013). Most of the cases of shigellosis are from developing countries due to poor social amenities,

overcrowded living conditions, poor personal hygiene and improper sewage disposal (Hussen *et al.*, 2019).

The emergence of multidrug resistant strains of *Shigella* is on increase, as strains were reported to be highly resistant to cotrimoxazole, chloramphenicol, ampicillin and norfloxacin (Ngoshe *et al.*, 2017; Abdullahi *et al.*, 2010). The organism is mainly transmitted via fecal-oral route and it is more prevalent among children 1-4 years of age (Murray *et al.*, 2007). The infectious dose of the bacteria is 10 to 100 cells and the incubation period usually ranges from 1 to 3 days (Willey *et al.*, 2008).

The prevalence of shigellosis in Nigeria was reported as 5.6% in Kano (Abdullahi, 2017), 8% from North-eastern Nigeria (Ngoshe *et al.*, 2018) and 23.3% was reported in vegetable from Ile-Ife (Olaniyi *et al.*, 2019). Kano as the most populous state of Nigeria, can give a reflection of a burden of an infection in the country, thereby guiding for the policy making in the control of the disease. The study was aimed at determining the prevalence of *Shigella* spp among gastroenteritis patients in relation to some symptoms, risk factors and their susceptibility pattern to some commonly used antibiotics.

MATERIALS AND METHODS

Study design

The study was a hospital based cross-sectional study carried out in Kano state involving 6 hospitals across the 3 senatorial zones of the state. Ethical approval was secured from Kano State Hospitals Management Board (Ref:Off/797/T.I./128).

Population for the study comprised of all patients with gastroenteritis of all ages and sexes attending the selected hospitals. Only patients sent to the microbiology laboratories of the selected hospitals, for suspected cases of gastroenteritis who consented were included in the study, while non-gastroenteritis patients and those that did not consent were excluded. Individuals with no apparent gastroenteritis were used as the control population.

An informed consent was obtained from the patient, guardian/caregiver in the case of children prior to sample collections from the subjects. A structured questionnaire was administered and filled by the patients, guardian/ care giver to obtain information related with the infection.

The sample size was calculated according to the formula of Zubair (2012) using the prevalence of 0.078% reported by Abdullahi (2017).

$$n = Z^2pq/L^2$$

$$n = \frac{(1.96)^2 \times 0.078 \times 0.922}{0.05^2} \quad n = 110.51$$

However, a total of 540 samples (450 from GEPs and 90 from AHIs) were collected, 180 from each senatorial zone.

The stool samples were collected and transported immediately in peptone to the laboratory, Department of Microbiology, Ahmadu Bello University, Zaria.

Culture, Isolation and Characterization of *Shigella* spp

The stool samples were examined physically (macroscopically) for the presence of mucus or blood before were streaked aseptically on MacConkey agar plates and incubated overnight at 37°C.

The suspected colonies of the organism were subjected to Gram staining technique for preliminary identification. Motility of the organism was tested along with H₂S and indole production using sulfide indole motility (Oxoid) medium slants. The slants were stabbed aseptically and incubated overnight at 37°C. Additionally, methyl red (MR) and Voges-Proskauer (VP) tests were carried out using MR-VP broth (Oxoid). The broth was aseptically inoculated with the pure colony of the isolates and incubated for 24 hours at 37°C, after which 1ml of each of the inoculated broth was

aseptically transferred into a sterilized tube. For the VP test, 3 drops of 6% alpha naphthol and 1 drop of 40% potassium hydroxide were added, while for MR test, 5 drops of methyl red was added and the reactions were observed. Furthermore, D-glucose, lactose and sucrose fermentation test were carried out using triple sugar iron (TSI) medium (Oxoid), where the slants were stabbed with the pure colony and were incubated at 37°C for 24 hours after which the reaction was observed. Additionally, sugar fermentation test, was carried out by preparing 1% of each of the D-arabitol, dulcitol, L-rhamnose, raffinose, D-sorbitol, and D-mannitol, D-xylose, and L-arabinose in peptone water, the preparations were inoculated with pure colony and incubated overnight at 37°C after which an indicator (phenol red) was added and the reaction was observed. In addition, decarboxylation of the isolates were tested against lysine and ornithine using lysine and ornithine broth respectively, the broths were aseptically inoculated with pure colony, incubated at 37°C and the reaction was observed after 24 hours (Cowans and Steel, 2004).

Antibiotic Susceptibility Testing

The susceptibility of the isolates obtained to some antibiotics was determined using disc diffusion method. The isolates were suspended in sterilized normal saline and were standardized to 0.5 McFarland scale. Then, 0.1ml of the standardized suspensions were aseptically spread onto Muller-Hinton agar and allowed for complete absorption (Cheesbrough, 2009), after which the plates were seeded with 8 different commonly used antibiotics (discs), namely; augmentin (10µg), ciprofloxacin (30µg), chloramphenicol (10µg), imipenem (10µg), tetracyclin (30µg), gentamicin (30µg), nalidixic acid cefotaxom (30µg) and trimethoprim/sulfamethoxazole (25µg). The plates were inverted and incubated overnight at 37°C, after which were examined for zone of inhibitions, which were compared to CLSI manual (Wayne, 2015), for interpretation. Multiple antibiotic index was calculated as the ratio of the number of antibiotics resisted by an isolate to the total number of antibiotic tested (Cheesebrough, 2009).

RESULTS

Shigella species appeared as non-lactose fermenting, non-mucoid on MacConkey agar plates; Gram negative rods under the microscope. They were non-motile H₂S and indole negative. Similarly, they were both VP and MR negative that could not ferment either sucrose or lactose, but D-glucose (K/A) on the TSI.

Also they were not able to decarboxylate lysine, ornithine or utilize citrate. Additionally, *Shigella* spp did not ferment any of the D-arabitol, dulcitol, L-rhamnose, raffinose, D-sorbitol or D-xylose, except L-arabinose.

The overall prevalence of shigellosis was 0.93%, with 1.1% and 0% among GEPs and AHIs respectively (Odd ratio= 2.2346, 95%CI= 0.1225-40.7708, P value= 0.5873) as presented in Table 1.

The highest prevalence (2.4%) was recorded among patients presented with watery diarrhoea compared to the lowest (0%) among patients presented with formed stool and the differences observed were statistically significant (P= 0.0487). In addition, highest prevalence (23.1%) was recorded among patients presented with bloody stool, compared to the lowest (0.23%) from patients with the non-bloody stool and the differences observed were statistically significant (P = 0.00001). However, prevalence was almost similar among those that experienced constipation (1.9%) and those that did not (1.0%) and the difference observed was statistically insignificant (P = 0.5525) (Table 2).

Furthermore, the highest prevalence (5.3%) was recorded among patients who experienced abdominal pain and the lowest (0.5%) among those who did not experience any of the

localized symptoms, but the differences observed were statistically by chance (P = 0.0618).

For the systemic symptoms, the highest (4.8%) and lowest (0.4%) prevalence were recorded among the patients who presented with fever and those who experienced none respectively, but the observed differences observed were statistically significant (P = 0.0097). There was almost similar prevalence among males (1.3%) and females (1.0%), but the observed difference was statistically insignificant (P = 0.7758). Similarly, the prevalence was higher (1.6%) among children (0-10years) compared the 0% among adults, however, the observed differences were statistically insignificant (P = 0.7652), (Table 3).

All the five isolates of *Shigella* spp were susceptible to augmentin (100%). The second highest activity was shown by nalidixic acid, which was effective against 4 out of the five isolates (80%). However, higher level of resistance (40%) was recorded against ciprofloxacin, imipenem and trimethoprim/sulfamethoxazole (Table 4).

Significant MAR index was recorded against 3 (GG182, YG354 and YG369) out of the 5 isolates (60%), while interestingly isolate YG361 was susceptible to all the 8 antibiotics examined thereby having 0 MAR index (Table 5).

Table 1: Prevalence of *Shigella* species among Gastroenteritis Patients and Apparently Healthy Individuals, in Kano State, Nigeria

Category of patients	No. Examined	No. positive with <i>Shigella</i> sp (%)	Odd's ratio	95% CI	P value
GEP	450	5 (1.1)	2.2346	0.1225- 40.7708	
AHI	90	0 (0.0)			
Total	540	5 (0.93)			

Key: GEP= Gastroenteritis Patients; AHI: Apparently Healthy Individuals (Control)

Table 2: Prevalence of *Shigella* spp in Relation to Stool Characteristics of the Gastroenteritis Patients

Variable	No. tested= 450	No. Positive (%)	x ²	df	P-value
Stool formation					
Formed	134	0 (0.0)	3.8842	2	0.0487*
Semi formed	151	1 (0.7)			
Watery	165	4 (2.4)			
Stool appearance					
Mucoid	19	1 (5.2)	62.959	2	0.00001*
Bloody	13	3 (23.1)			
Normal	418	1 (0.23)			
Constipation					
Yes	52	1 (1.9)	0.3528	1	0.5525
No	398	4 (1.0)			

Key: χ^2 = Chi square; P-value ≥ 0.05 =statistically non-significant, P-value < 0.05 =Significant***Table 3: Prevalence of *Shigella* spp in Relation to Symptoms and Risk Factors among Gastroenteritis Patients**

Variable	No. tested= 450	No. Positive (%)	x2	df	P-value
Localized Symptoms					
Abdominal pain and bloating	52	1 (1.9)	7.3407	3	0.0618
Abdominal pain only	38	2 (5.3)			
Bloating only	139	1(0.7)			
None	221	1(0.5)			
Systemic Symptoms					
Fever and Headache	91	1 (1.1)	9.253	3	0.0097*
Fever only	63	3(4.8)			
Headache only	72	0 (0.0)			
None	224	1(0.4)			
Gender					
Male	153	2 (1.3)	0.0811	1	0.7758
Female	297	3 (1.0)			
Age (years)					
0-10	233	4 (1.6)	0.0892	4	0.7652
11-20	81	1 (1.4)			
21-30	56	0 (0.0)			
31-40	32	0 (0.0)			
>40	48	0 (0.0)			

Key: χ^2 = Chi square; P-value ≥ 0.05 =statistically non-significant, P-value < 0.05 =Significant***Table 4: Antibacterial Sensitivity profile of *Shigella* spp. isolated from stool samples of Gastroenteritis Patients**

Antibiotics (Conc.)	Sensitivity Pattern (n=5)		
	Susceptible (%)	Intermediate (%)	Resistant (%)
Augmentin (10 μ g)	5 (100)	0 (0)	0 (0)
Chloramphenicol (10 μ g)	2 (40)	2 (40)	1 (20)
Ciprofloxacin (30 μ g)	2 (40)	1 (20)	2 (40)
Imipenem (10 μ g)	2 (40)	1 (20)	2 (40)
Gentamicin (30 μ g)	3 (60)	1 (20)	1 (20)
Cefotaxim (30 μ g)	1 (20)	3 (60)	1 (20)
Nalidixic Acid (30 μ g)	4 (80)	1 (20)	0 (0)
Trimethoprim/ Sulfamethoxazole (25 μ g)	2 (40)	1(20)	2 (40)

Table 5: Multiple Antibiotic Resistance (MAR) Indices of *Shigella* spp isolated from Gastroenteritis Patients (n=8)

Isolate Code	No. of Antibiotic Resisted	Resistance Pattern	MAR Index
GG182	3	CH, CPX, IMP	0.375
BG298	1	CTX	0.125
YG354	3	CPX, IMP, SXT	0.375
YG361	0	-	0
YG369	2	CN, SXT	0.25

AU=Augmentin, CH= Chloramphenicol, CPX= Ciprofloxacin, IMP= Imipenem, CN= Gentamicin, CTX= Cefotaxime, NA=Nalidixic Acid, SXT= Trimethoprim/sulfamethoxazole

DISCUSSION

From the current findings, the odd ratio showed that *Shigella* spp was 2.3 times more likely to occur as enteric pathogen than normal flora. This implicates *Shigella* as an absolute pathogen, which is in agreement with recent findings which reported that *Shigella* infection is always associated with gastroenteritis (Roya *et al.*, 2017). The current prevalence (1.1%) is much lower than 8% reported from the north-eastern states of Nigeria (Ngoshe *et al.*, 2017) and 7.8% reported from Kano (Abdullahi, 2017). The difference may be due to the variation in the population studied and the sample analyzed.

From the current findings, shigellosis is more likely to be presented as watery stool accompanied with blood and may alternate with constipation. However, constipation may not be a clear cut symptom of the disease, as the difference observed within the category was statistically insignificant ($p=0.5528$). Similarly, another finding reported *Shigella* as the major cause of dysentery with symptoms typically as diarrhoea usually presented with blood and mucus (Hussen *et al.*, 2019). The destructive effect of shiga toxin on the intestine, together with the ability of the pathogen to invade and kill macrophages may result to inflammatory reaction manifested as bloody diarrhoea with or without mucus (Ferarri *et al.*, 2019). The result is in agreement with the previous findings, where *Shigella* was reported as strongly associated with bloody diarrhoea in Kano (Kotloff *et al.*, 2018).

Although some findings have limited *Shigella* infection to colonic epithelium (Willey *et al.*, 2008), however, the highest prevalence (4.5%) of the infection among febrile patients with significant statistical difference ($p=0.0097$) in the current study, further implicate the pathogen in causing post-intestinal infections, which is in agreement with a finding previously reported in Kano (Abdullahi *et al.*, 2010).

The current findings implicated neither gender nor age group as a risk factor, although the prevalence was higher in males than in females, and in children 0-10years of age than the other age groups, which is in agreement with the previous study, where the prevalence of shigellosis was reported higher (64.3%) in

males than in females (35.7%) (Abdullahi, 2017), also higher in children 1-2years compared to other age groups (Abdullahi *et al.*, 2010).

From our study augmentin is the drug of choice for the antibiotic therapy of *Shigella* infection, followed by nalidixic acid and gentamicin. The study, however proscribe using ciprofloxacin, imipenem and trimethoprim/sulfamethoxazole for the antibiotic therapy in the study area, as the efficiency of each of the 3 antibiotics was below 50%. The result is in agreement with the previous work, where augmentin was reported as the most effective (75%) against *Shigella* isolates from north eastern Nigeria (Jomezadeh *et al.*, 2014). The efficacy of augmentin may be due to the presence of clavulanate known to act against the β -lactamase produced by bacteria to help them resist the traditional antibiotics (Damrikarnlert *et al.*, 2000), while that of gentamicin may be due to its route of administration (intravenous injection) and nalidixic acid is not commonly found over the counter.

Three (60%) out of the 5 isolates of *Shigella* spp had MAR indices above 0.2, suggesting their isolation from individuals where antibiotics were inappropriately used (Paul *et al.*, 1997). This percentage occurrence is less than what was recorded in the previous study in Ile-Ife, where significant MAR indices was reported in 80% of the *Shigella* spp, isolated from freshly sold vegetable (*Amaranthus viridis*) (Olaniyi *et al.*, 2019). Perhaps, the variation is due to differences in the source of isolation.

CONCLUSION

Shigella spp were implicated as enteric pathogen with the prevalence of 1.1% among gastroenteritis patients studied. The patients typically presented with watery stool stained with blood and fever. Augmentin was the drug of choice. The possibility of inappropriate use of antibiotics was observed in 60% of the patients. The study identifies the need for adherence to hygiene practices to control the spread of the pathogen and the importance of laboratory diagnosis prior to treatment for successful control of the infection. Also, there is need for more enlightenment directed against inappropriate use of antibiotics.

REFERENCE

Abdullahi, M. (2017). Shigellosis and Socio-Demography of hospitalized Patients in Kano, North-West, Nigeria. *International Journal of*

Pharmaceutical Science Invention, 6 (3):31-37

Abdullahi, M., Olonitola S.O., and Inabo, I.H. (2010). Isolation of bacteria associated with diarrhoea among children attending some hospitals in Kano

- metropolis, Kano state, Nigeria. *BayeroJournal of Pure and Applied Sciences*,3 (1): 10 - 15.
- Aranda, K. R. S., Fagundes-Neto, U. and Scaletsky, I. C. A. (2004). Evaluation of Multiplex PCRs for Diagnosis of Infection with Diarrheagenic *Escherichia coli* and *Shigella* spp. *American Society for Microbiology*, 42 (12): 5849-5853.
- Baker, S., and The, H.C. (2018). Recent insights into *Shigella*: a major contributor to the global diarrhoeal disease burden. *Current Opinion on Infectious Diseases*, 31:449-454.
- Cheesebrough M. (2009). District Laboratory Practice in Tropical Countries. Cambridge University Press, 2nd ed., 96-97.
- Center for Disease Control and Prevention (CDC) (2019). Gastroenteritis. http://www.health.nt.gov.au/library/scripts/objectifyMedia.aspx?file=pdf/48/21.pdf&siteID=1&str_title=Gastroenteritis.pdf. Accessed 16th September, 2019.
- Cowan, S.T. and Steel, K.J. (2004). Manual for identification of medical bacteria. 3rd Edition, 122-141. Cambridge University press London.
- Damrikarnlert, L., Jauregui, A.C. and Kzadri, M. (2000). Efficacy and Safety of Augmentin twice Daily Versus Three times Daily in the Treatment of Otitis Media. *Journal of Chemotherapy*, 12(1)79-87.
- Ferrarri, M.L., Malarde, V., Grassart, A., Salavessa, L., Nigro, G., Descorps-Declere, S., Rohde, J.R., Schnupf, P., Masson, V., Arras, G., Loew, D., Sansonetti, P.J. and Sauvonnet, N. (2019). *Shigella* promotes major alteration of gut epithelial physiology and tissue invasion by shutting off host intracellular transport. *National Academy of Sciences of the United States of America*, 116 (27):13582-13591
- Jomezadeh, N., Babamoradi, S., Kalantar, E. and Javaharizadeh H. (2014). Isolation and Antibiotic Susceptibility of *Shigella* species from Stool Samples among Hospitalized Children in Abadan, Iran. *Gastroenterology and Hepatology from Bed to Bench*, 7(4):218-231
- Kotloff, K.L., Riddle, M.S., Platts-Mills, J.A., Pavlinac, P. and Zaidi, A.K. (2018). Shigellosis. *The Lancet*, 391(10122)801-812
- Hussen, S., Mulatu, G. and Kassa, Z.Y. (2019). Prevalence of *Shigella* species and its drug resistance pattern in Ethiopia: a systematic review and meta-analysis. *Annual of Clinical Microbiology and Antimicrobials*, 8:1-22.
- Helmy, O.M., Ragab, Y.M. and Hussein M.M. (2013). Multiplex polymerase chain reaction (PCR) for the detection of diarrheagenic *Escherichia coli* and *Shigella* directly from stool. *African Journal of Microbiology*, 7(34):4368-4372.
- Hyma, K.E., Lacher, D.W., Nelson, A.M., Bumbaugh, A.C., Janda, J.M. and Strockbine, N.A. (2005). Evolutionary genetics of a new pathogenic *Escherichia* species: *Escherichia albertii* and related *Shigella boydii* strains. *Journal of Bacteriology*, 187 (2):619-628.
- Murray, P.R., Baron, E.J., Jorgensen, J.H., Landry, M.L. and Pfaller, M. A. (2007): Manual of Clinical Microbiology. *American Society for Microbiology*, 9 (1):670-687.
- Ngoshe, I.Y., Denu, B.A., Bello, H.S., Akawu, C.B., Gashau, W. (2017). Prevalence and antimicrobial susceptibility of *Shigella* species isolates from diarrheal stool of patients in a tertiary health facility in northeastern Nigeria. *Sub-Saharan African Journal of Medicine*, 4:96-101.
- Olaniyi, F.T., Akanro, I. R., Oguh, C. E., Fashina, C. D., Ahmed, I. and Ezech, C. C. (2019). Characterization and Antimicrobial Resistance Profile of Pathogenic Bacteria Isolated from Freshly Sold *Amaranthus viridis* in Ile-Ife, Southwest Nigeria. *Journal of Advances in Microbiology*, 18(1):1-8
- Paul, S., Bezbaruah, R.L. and Ghosh, A.C. (1997). Multiple antibiotic resistance (MAR) index and its reversion in *Pseudomonas aeruginosa*. *Letter of Applied Microbiology*, 24(3):169-71.
- Roya, N., Ahmad, S., Marjan, D. Khaghani, S. and Mina, M. (2017). A study of Prevalence of *Shigella* species and Antimicrobial Resistance Patterns in Paediatric Medical Center. *Iranian Journal of Microbiology*, 9 (5):277-283
- Wayne, P.A. (2015). Clinical and Laboratory Standards Institute. Performance standards for antimicrobial

- susceptibility testing; Twenty-first informational supplement; M100-S21.
- Willey, J.M., Sherwood, L.M. and Woolverton, C.J. (2008). Microbiology. *McGraw-Hill Companies, Inc.*, 1221 Avenue of the Americas, New York, NY 10020,7:41
- Zubair, K.O. (2012). Sample size determination. *Technology, Health and Medicine*, 12: 103-122.