Research Article

Prevalence and Antimicrobial Susceptibility Testing of Salmonella and Shigella Species among Children with Diarrhea in Khartoum and Al Jazeera States, Sudan

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Abstract:

Background*:** It has been estimated that in low and middle-income countries (LMICs) each child suffers up to 15 to 19 episodes of diarrhea per year. Diarrhea kills more young children than Malaria, AIDS, and Measles combined a large proportion of which were attributed to *Salmonella and Shigella* spices. In developing countries, *Salmonella* and *Shigella* species remain major contributors to acute enteric infection in children. ***Objectives: To detect the prevalence of *Salmonella* and *Shigella* species and antibiotic susceptibility testing among children with diarrhea in Khartoum and Al Jazeera states, Sudan (2022). ***Methods***: A descriptive, prospective cross-sectional study was carried out from July to October 2022 among 120 children with diarrhea aged 3-14 years in Khartoum and Al Jazeera states. Information about patient demographics and clinical status was obtained from each patient using a written questionnaire. Stool specimens from diarrheic children were collected and processed for wet mount examination and isolation of *Salmonella* and *Shigella* using conventional microbiology procedures. Antibiotic susceptibility patterns were determined by using the Kirby-Bauer disc diffusion method through culturing the isolates on Mueller- Hinton agar. The collected data and laboratory results were analyzed using SPSS version 26. ***Results***: In this study; only 2/120 (1.7%) of the specimens showed growth of *Shigella sonnei* and *Shigella species* both were isolated from patients from Al Jazeera state (Al Sameer village), both *Shigella* isolates were sensitive to ciprofloxacin, gentamicin, cefotaxime, amikacin and only resistant to amoxicillin. *G. lamblia* and *E. histolytica* were detected in wet mount examination in stool specimens with a prevalence of (8.3%) and (4.2%) respectively. In our study, there was no association between positive stool culture and type and duration of diarrhea, symptoms with diarrhea, hospitalization, household income, source of drinking water, and hand washing. ***Conclusion***: In this study, only *Shigella* was detected 2/120 (1.7%) among children aged 3-14 years suggesting the dominance of other causes of diarrhea including intestinal parasites. A further study targeting other causes of diarrhea should be conducted to establish the major causes of diarrhea in children in the study areas.

Keywords: Salmonella, Shigella, Antibiotic resistance, Children diarrhea, Khartoum, Al Jazeera, Sudan.

Introduction:

Diarrhea was defined as the excessive and frequent evacuation of watery faces, usually indicating gastrointestinal distress or disorder during 3-7 days it is a frequent illness in developing countries and contributes to the deaths of 4.6 to 6 million children annually in Asia, Africa, and South America. It has been estimated that in the very poor countries of these regions each child suffers up to 15 to 19 episodes of diarrhea per year **[1].** Diarrhea kills more young children than Malaria, AIDS, and Measles combined **[2].** Infections caused by enteric pathogens are common public health problems in many parts of the world, and it is a major cause of morbidity and mortality among children under 5 years old in Sub-Saharan Africa **[3,4].** In developing countries, *Salmonella* and *Shigella* species remain major contributors to acute enteric infection in children **[5].** WHO diarrhea guidelines estimated that children less than five years of age have 3.3 diarrheal episodes per year, and more than one-third of the deaths in this age group is associated with diarrhea **[6].** Therefore, every year there are approximately 1.5 billion diarrheic episodes and 4 million deaths in children less than five years of age (most from 6 months to 12 years) **[7].** Africa and South Asia are still home to more than 80% of child death. Among 15 high-burden countries that have three-quarters of all deaths from diarrhea, Ethiopia was ranked **[8].** Diarrhea is mainly caused by viruses, bacteria, and parasites, the latter characterized by slow onset of action compared with the infection caused by viruses and bacteria **[9].** Under normal conditions, the gastrointestinal tract has a great capacity to absorb fluids and electrolytes, however, enteric pathogens disturb this balance by different mechanisms, and these results in fluids and electrolytes loss and may lead to death **[10].** *Salmonella* and *Shigella* are Gram-negative rods that commonly inhibit the intestinal tracts of humans and many animals, they are transferred from person to person usually by asymptomatic carriers and via contaminated food and water. Infection of *Salmonella* and *Shigella* can be asymptomatic which can be treated with rehydration solutions unless the infection is by invasive strains **[11,12].** *Salmonella* species are a leading cause of acute gastroenteritis in different countries, especially in developing countries where substandard hygienic conditions and unsafe water supplies prevail **[13].** Infectious diarrhea, especially those due to enteric bacterial pathogens, remains a significant public health problem worldwide. While it primarily contributes to morbidity in developed countries, it accounts for significant mortality among children in low and middle-income countries (LMICs), despite declining diarrhea-related death in the last 20 years **[14,15].** *Salmonella* causes Salmonellosis which can be characterized by diarrhea, fever, vomiting, and abdominal cramps after 12-72 hours of infection. *Salmonella enterica* serotype Typhi is a common serotype of *Salmonella* and can cause typhoid fever- is a systemic disease with diarrhea and it is a

major cause of morbidity and mortality worldwide in under the age of five children **[16].** Shigellosis is caused by *Shigella* species, and it is a worldwide problem although more prevalent in developing countries **[17].** It was estimated worldwide about 1.8 million cases of children died from diarrheal illness, a large proportion of which were attributed to *Shigella* and *Salmonella spp* **[18].** *Shigella* also known as bacillary dysentery or Marlow syndrome is caused by *Shigella* species and it can rarely occur in animals other than humans **[19].** Accurate estimates of the burden of diarrheal diseases caused by *Salmonella* species and other food-borne pathogens remain essential in setting effective public health goals and allocation of resources to reduce the disease burden **[20].** Therefore, consideration of the significance of fecal carriage of *Salmonella* by food handlers is important to public health. Typhoid fever caused by *Salmonella enterica* serotype Typhi (*S. typhi)* is a major cause of morbidity and mortality among children and adults in developing countries it is estimated that 21.6 million illnesses and 216,500 deaths worldwide are due to *S. typhi* infection annually **[21].** In addition, *Shigella dysentery* species release Shiga toxin, an AB exotoxin similar to EHEC that diarrhea **[22].** For most patients, the illness is a self-limited one; but the disease can cause severe fluid and electrolytes loss, which require prompt treatment. The management of acute diarrhea is based on the replacement of fluids. However, antibiotics might be required for the management of the same cases and may reduce the duration of the disease, but their prescription is restricted due to the emergence of resistance or due to lack of availability in some countries **[23].**

Materials and methods:

Study design:

A descriptive, prospective cross-sectional study.

Study area:

The study was conducted at selected hospitals and medical centers in Khartoum and Jazeera states. In Khartoum state, the participants were selected from: Mohammed Alamin Hammed children teaching hospital- Omdurman, Noon children hospital- Omdurman, Alrazi medical center- Omdurman, Aljamoiya medical center, Aljamoiya is a village located in the southern countryside of Omdurman city, about 5 kilometers from the White Nile, and about 10 kilometers from Omdurman city center, the majority of the population were farmers. The second area from where our participants were selected was Alsameer village -Jazeera state, which is located 15 kilometers east of Khartoum city.

Study duration:

This study was carried out from July to October 2022.

Study population:

Children with diarrheal disease.

Sampling type:

Non- probability, convenience sampling technique.

Sample size:

One hundred and twenty child patients with diarrhea were enrolled in our study. The

sample size was determined using the Raosoft sample size software calculator at a confidence interval (CI) of 95%, and a margin of error of 8.92%.

Data collection tools:

Each participant was taught to collect stool specimens in a clean dry container. A written questionnaire was used to collect socio-demographic data (age, gender, household income, domestic animals in the house, source of drinking water), medical information data; hospitalization, type of diarrhea, duration of diarrhea, symptoms of diarrhea: recurrent infection (malaria, enteric fever, others).

Isolation of bacteria:

A loop full of stool samples was inoculated in selenite F broth mixed well then incubated aerobically at 37 0C overnight incubation in the incubator. after overnight incubation, subcultured on MAC, XLD, and DCA using a sterile wire loop then the inoculated plates were incubated in the incubator overnight at 37 ^{0}C.

Preparation of the bacterial suspension:

Using a sterile wire loop, 3–5 well-isolated discrete colonies of similar appearance to the test organism were picked up and emulsified in 3-4 ml of sterile normal saline in a sterile test tube. In good light, the turbidity of the suspension was compared to the turbidity of 0.08-0.1 optic density in colorimeter these equal to 0.5 McFarland standard.

Ethical consideration:

The study clearance was obtained from the ethical committee of Alfajr College of Sciences and Technology, and the Medical Laboratory Sciences Program. Departmental permission was obtained from different hospitals. Verbal consent was taken from each participant before the collection of specimens.

Data analysis:

Statistical analysis of the data was performed using Statistical Package for the Social Sciences (SPSS) software, version 26.

Results:

This cross-sectional study was carried out on children aged 3-14 years with diarrhea attending different hospitals in Khartoum and Al Jazeera states to determine the prevalence and antimicrobial susceptibility pattern of *Salmonella* and *Shigella* isolated from children. Out of the 120 child participants; 75 (62.5%) were from Khartoum state, and 45 (37.5%) were from Al Jazeera state. Out of them; 71/120 (59.2%) were males and 49/120 (40.8%) were females. The age-mean of the study participants was 6.78 ± 2.7, half of them (50.8%) their age in the range of 3 - 6 years, more than half of the participants (65.8%) had low household income, (96.7%) were having no domestic animals. Tap water was the most common drinking water source among children (98.3%) and (88.3%) had good hand behavior **(Table1).** The loose stool was the most common type of diarrhea among children (71.7%), and (86.7%) had diarrhea for 1-3 days. Fever was the most common symptom with diarrhea among children (35.8%), and (80.8%) of them were outpatients and more than two third of the participants (87.5%) responded with no recurrent infections

(Table1). According to the microscopic examination of stool specimens; (43.3%) of the specimens had pus cell count of > 10/HPF, (76.7%) had RBCs count of 1-5/HPF, and (87.5%) of the specimens revealed no parasite, but *G. lamblia* was detected in (8.3%), followed by *E. histolytica* which was detected in (4.2%) of the specimens **(Table2).** According to stool culture; only 2/120 (1.7%) of the specimens showed growth of *Shigella sonnei* and *Shigella species* both were isolated from patients from Al Jazeera state. While (98.3%) of the specimens were negative for *Salmonella- Shigella* isolates. The antimicrobial susceptibility testing of Shigella isolates showed the two shigella isolates were sensitive to amikacin, ciprofloxacin, gentamycin, and cefotaxime and resistant to amoxicillin only. **(Table3).** In this study, there was no significant association between stool culture result and type of diarrhea (*P. value* 0.600) **(Table 4).** In our study also there was no correlation between hospitalization, duration of diarrhea symptoms with diarrhea, recurrent infections, pus cells, RBCs, intestinal parasites, and the result of stool culture **(Table 5).**

Table-1: Socio-demographic status of study population

Variables	*Categories*	*Frequency*	*Percent (%)*
Age group /Years	3-6	61	50.8
	7-10	42	35
	11-14	17	14.2
	Total	**120**	**100**
Householdincome	Low	79	65.8
	Medium	39	32.5
	High	2	1.7
	Total	**120**	**100**
Domestic animals	Yes	4	3.3
	No	116	96.7
	Total	**120**	**100**
Hospitalization	In patient	23	19.2
	Out patient	97	80.8
	Total	**120**	**100**
Type of diarrhea	Blood and mucous	8	6.7
	watery	26	21.7
	Loose stool	86	71.7
	Total	**120**	**100**
Duration of diarrhea/ Days	1-3	104	86.7
	4-6	13	10.8
	>6	3	2.5
	Total	**120**	**100**
Symptoms with diarrhea	Fever	43	35.8
	Fever and abdominal pain	17	14.2
	Fever and vomiting	7	5.8
	Abdominal pain	26	21.7
	Nausea	4	3.3
	Vomiting	7	5.8
	Abdominal pain and vomiting	16	13.3
	Total	**120**	**100**
Drinking- water source	Tap water	118	98.3
	River	2	1.7
	Total	**120**	**100**
Recurrent infections	Malaria	14	11.7
	Enteric fever	1	0.8
	No	105	87.5
	Total	**120**	**100**
Hand washing	Yes	106	88.3
	No	14	11.7
	Total	**120**	**100**

Table-2: Distribution of stool analysis and culture results

Variables	*Categories*	*Frequency*	*Percent (%)*
Pus cells/HPF	Nil	31	25.8
	1-5	19	15.8
	6-10	18	15.0
	>10	52	43.3
	Total	**120**	**100.0**
RBCs/HPF	Nil	28	23.3
	1-5	92	76.7
	Total	**120**	**100**
Intestinal parasite	No	105	87.5
	E. histolytica	5	4.2
	G. lamblia	10	8.3
	Total	**120**	**100**
Stool culture	Negative	118	98.3
	Positive	2	1.7
	Total	**120**	**100**

Table-3: Antibiotics pattern among isolated *Shigella* species

Isolate /Antibiotic	*Amikacin*	*Ciprofloxacin*	*Gentamycin*	*Cefotaxime*	*Amoxicillin*
Shigella sonnei	S	S	S	S	R
Shigella spp	S	S	S	S	R

Table-4: Association between type of diarrhea and stool culture result.

Type of diarrhea	*Negative*	*Positive*	*Total*	*P. value*
Blood and mucous	8(100.0%)	0 (0.0%)	8(100.0%)	0.600
watery	25(96.2%)	1(3.8%)	26(100.0%)	
Loose stool	85(98.8%)	1(1.2%)	86(100.0%)	
Total	118(98.3%)	2(1.7%)	120 (100.0%)	

Table-5: Correlation between clinical characteristics, stool analysis and stool culture result.

Study variable	*R*	*P. value*
Hospitalization	0.063	0.432
Duration of diarrhea	0.048	0.601
Symptoms with diarrhea	0.144	0.116
Recurrent infections	0.043	0.537
Stool pus cells	0.129	0.160
Stool RBC's	0.179	0.051
Stool parasites	0.072	0.436

Discussion:

This study was conducted to detect the prevalence of *Salmonella* and *Shigella* species. Associated with childhood gastroenteritis and to evaluate the antimicrobial susceptibility of *Salmonella* and *Shigella* isolates to commonly used antibiotics. This study was one of the fewest reports on the prevalence and antimicrobial susceptibility pattern of *Salmonella* and *Shigella* gastroenteritis among children aged between 3-14 years. In our study the overall prevalence of *Salmonella* and *Shigella* was (1.7%) among children aged between 3–14 years, this result agreed with the result of a study carried out by Terfassa (2018) in Nekemte Ethiopia where the prevalence was (2.1%), and by Mulatu G *et al* (2014) in Hawassa town, south Ethiopia the prevalence was (2.5%) (40) **[24,25].** Conversely, a very high prevalence was found by Mache A (2002), in Jimma South West Ethiopia, the prevalence was (20.1%) **[26].** All *Shigella* isolates in this study displayed resistance to amoxicillin, this agreed with the result, by Reda AA *et al* (2011) in Eastern Ethiopia Harar where (100%) resistance to amoxicillin was found, same as by Beyene *et al* (2014) in South West Ethiopia Jimma **[27,28].** Ciprofloxacin showed good antimicrobial activity against the isolates, this is also comparable with the result reported by Molla *et al*. (2006) from the central part of Ethiopia **[29].** Watery diarrhea and loose stool were the predominant types of diarrheas of *Shigella* isolate in this study; this difference may result from differences in the species involved: *Shigella dysentery* and *Shigella sonnei* cause bloody and watery diarrhea respectively **[30].** In this study, we found children from rural areas were more susceptible to diarrhea due to *Shigella* infection than children from urban areas, a result in conformity with the study of Vargas *et al*. (2004) in Tanzania. Absence of a latrine at home, source of drinking water, and failure to wash hands before meals were found significantly associated with shigellosis; which is similar to reports from elsewhere **[31].** In this study most the children complained of diarrhea the age 3-6 years (61%) children at this age are naturally taking contaminated soils, food, and water into the mouth and may acquire disease microbes including pathogenic *Salmonella* and *Shigella* spp. From the environment easily. Children from rural areas were more susceptible to diarrhea due to *Salmonella* and *Shigella* infection than children from urban areas, a result in conformity with the study of Vargas *et al* (2004) in Tanzania, this was a result of the fecal-oral transmission of these bacteria from the parents to their children during feeding and /or handling **[31].** In this study Blood and mucous, watery, loose stool were the predominant type of diarrhea in the cases of intestinal parasites *Giardia lamblia* and *Entamoeba histolytica*. Our study did not find any association between diarrhea duration and the positive culture *of Salmonella* and *Shigella* spp. another study agreed, that mild symptoms are self-limited, but antibiotics are prescribed in extreme dysentery cases to shorten the duration of diarrhea Williams and Berkley *et al*. (2018) **[32].** In our study there was no association between domestic animals and the positive culture of *Salmonella* and *Shigella* spp. another study was greed, which revealed that unhygienic living circumstances and close relations between humans and animals may substantially contribute to the occurrence of *Salmonellosis* and *Shigellosis* Birhaneselassie *et al*. (2013) in Southern Ethiopia **[33].** In our study we did not find any association between the source of drinking water and the positive culture of *Salmonella* and *Shigella* spp. another study agreed with this result. It was indicated that the consumption of contaminated food and/ or water is responsible for diarrhea diseases caused by *Salmonella* and *Shigella* isolates Madigan *et al* (2012) in San Francisco: Personal Education **[34].**

Conclusion:

In this study, the prevalence of *Salmonella and Shigella* was detected only in 2/120 (1.7%) of children aged 3-14 years with diarrhea in studied areas in Khartoum and Al Jazeera states, suggesting the dominance of other causes of diarrhea including intestinal parasites. *Shigella* but not *Salmonella* was detected in this study, which revealed sensitivity to ciprofloxacin, gentamycin, amikacin, and cefuroxime, in contrast, both isolated *Shigella* species showed (100%) resistance to amoxicillin. In this study, the association between the studied risk factor and infection with *Shigella* was not found.

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Conflict of Interest:

The author has affirmed that there are no conflicting interests.

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