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Short Communication

Prevalence and drug susceptibility pattern of *Shigella* and *Salmonella* species in under ten diarrhoeic children admitted to Tirunesh-Beijing hospital

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ABSTRACT

Background: Diarrheal diseases constitute a major burden of disease in the low and middle-income countries of the world with diarrhea being the second leading cause of morbidity. Now a day, food borne disease, caused by *Shigella* and *Salmonella species* is the most common type of bacterial gastroenteritis have gained multiple antimicrobial resistances; the challenge for clinical management. For acute childhood diarrhea that requires antimicrobial therapy correct choice of the drug depends on detailed previous knowledge of local strains. Therefore, the objective of the study was to assess the prevalence and drug susceptibility pattern of diarrheal diseases in under 10 children admitted to Tirunesh-Beijing hospital to provide preliminary information for healthcare providers.
Methods: Selective and differential media for isolation of diarrhea causing bacterial species was used. Further biochemical tests were conducted to confirm the results. The drug sensitivity and resistance pattern of isolated bacteria was assessed by minimum inhibitory concentration (MIC) as well as minimum bactericidal concentration (MBC). Data about patients' sex and age, pathogens isolated and their antimicrobial resistance patterns were recorded.
Results: Out of 22 stool samples collected from diarrhoeic under ten children the majority was males. Two (9.1%) stool samples were found positive for one *Shigella sonnei* and one *Shigilla flexnrei* while no *Salmonella species* was identified.
Conclusions: The isolated *Shigella species* showed high antibiotic resistance to amoxicillin, cotrimoxazole and ampicillin. But it was susceptible to ciprofloxacin. Therefore, frequent assessment of the pattern of resistance and prevalence is needed to keep the community save and use appropriate drug for treatment in the study area.

Keywords: Diarrhea, Antimicrobial susceptibility, *Salmonella*, *Shigella*, Children

INTRODUCTION

Diarrheal diseases constitute a major burden of disease in the world, especially in the low and middle-income countries. Of all medical conditions, diarrhea is the second leading cause of morbidity. About 72.8 million people are exposed to disability due to diarrhea per day. Diarrheal Illnesses are particularly dangerous for young children who are more susceptible to dehydration and nutritional losses during an episode of acute diarrhea. Around 90% of diarrhea-related deaths occur among under-five children living in low-and middle-income countries. Over 1.8 million under-five children die of diarrheal diseases which accounts for 19% of all childhood deaths.[1,2] Of all child deaths from diarrheal diseases 78% occur in the African and South-East Asian regions.

Now a day, food borne disease, caused by S*higella* and salmonella is the most common type of bacterial

gastroenteritis particularly in developing countries like Ethiopia. *Shigella* species belongs to the family Enterobacteriacea. It is small, un-encapsulated, non-motile gram-negative rod with four species of shigella, classified on the basis of biochemical and serological differences: *S. dysenteriae, S. flexneri, S. boydii*, and *S. sonnei*.[3] *Shigella sonnei* is found mostly in industrialized; *S. dysenteriae, S. flexneri*, and *S. boydii*, are more prevalently found in developing countries. Of the estimated 164.7 million *Shigella* diarrheal episodes occurring globally every year, 99% of infections occur in developing countries and the majority of cases and deaths occur amongst children less than five years of age.[4]

Salmonella is generally identified as being a non-lactose fermenting, (NLFs) Gram negative rod shaped organism, ranging 0.7 to 1.5×2 to 5 µm in size.[5] With the exception of *S. pullorum* and *S. gallinarum*, and they are motile with peritrichous flagellate. D-glucose is fermented with the production of acid and usually gas. Other carbohydrates usually fermented are L-arabinose, maltose, D-mannitol, D-mannose, L-rhamnose, D-sorbitol (except ssp VI), trehalose, D-xylose and dulcitol. This diarrheal disease is most likely to occur in children and those who neglect to clean hands thoroughly, including under fingernails after defection.

Many *Shigella species* have obtained multiple antimicrobial resistances; the challenge for clinical management is distinguishing which drugs preserve their activity and clinical efficacy.[6] The Center for Disease Control and Prevention has suggested that sensitivity testing be accomplished to instruct selection of proper antimicrobial therapy for shigellosis and other similar diseases. Because antimicrobial susceptibility patterns of *Shigella*, may differ greatly in different geographical regions and overtime, supervising resistance patterns is necessary to guide selection of appropriate empirical antibiotic treatment.[7] Determination of diarrheal causes is of critical importance to save guard the young children at risk of diarrheal disease. Therefore, in this project assessment of *Salmonella* and *Shigella* infection in under 10 children admitted to Tirunesh-Beijing hospital was conducted to determine their prevalence and drug susceptibility patterns.

METHODS

Study design and collection of stool samples

A cross sectional study was conducted from April to June 2015 at Tirunesh-Beijing hospital (Akaki-Kality sub-city, Addis Ababa), to assess the prevalence and drug susceptibility of *Shigella-Salmonella* species. Parents of under ten diarrhoeic children were request to sign informed consent on behalf of their children to participate in the study. Volunteers who sign the informed consent were requested to provide stool sample of their children. Twenty-two (n=22) stool samples were collected in sterile disposable containers from diarrhoeic under ten children who were admitted to the hospital and whose families agree to sign informed consent. All the samples were sent immediately to Addis Ababa science and Technology University microbiology laboratory within 30 minutes in transport medium that is buffer glycerol saline for isolation and *identification of Shigella-Salmonella species* and drug sensitivity patterns testing. The stool samples were primarily inoculated on Mac Conkey agar, Hektoen plate and *Salmonella-Shigella* agar and incubated at 37^{o}c for 24 hours. Subculture was done in the MacConkey agar, and SS agar. Further incubation were done aerobically at 35-37^{o}c for 18-24 hours.

Morphology characterization

A drop of the isolated colonies was placed on the glass slide and the smear from the isolates was prepared on the microscope slide and crystal violet was added. After 30 second the smears were washed using distilled water and iodine solution dropped on the slide. Then, washed strictly after 30 seconds and alcohol was added. Finally, safranin was added to the smear, washed after 30 seconds using tap water, and observed under microscope.

Biochemical screening tests

Loopful colony from each culture media was transferred to respective nutrient broth to made suspension. Salmonella is oxidase negative, catalase positive, indole and Voges Proskauer negative, methyl red and Simmons citrate positive, H_2S producing and urea negative.[8] The two bacterial isolates were subjected to various tests; triple sugar iron, motility, indole, urease, and citrate tests.

Susceptibility testing of isolates

Antimicrobial susceptibility of *Shigella strains* were assessed by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using Mueller Hinton broth with a concentration of 11.4 g/300 ml and were tested in vitro for susceptibility to four (4) different common antimicrobial agents; Amoxicillin, ciprofloxacin, cotrimoxazole & ampicillin. *E.coli* was used as quality control for susceptibility tests. Using sterile micropipette 4 different drugs with different concentration were transferred to sterile Mueller Hinton broth. Then two isolated colonies were suspended in normal saline (0.85%). The prepared turbidity was matched with a turbidity standard (0.5 McFarland) to have an equivalent suspension. The suspension was inoculated to sterile Mueller Hinton broth mixed with drugs evenly and the broth medium was incubated at 37^{o}c for 16-18 hours. By using a sterile swab the suspension were inoculated on Mueller Hinton agar. Then the result was determined and recorded by observing the growth at different concentration of antimicrobial agent that described as susceptible, intermediate susceptible or resistant as well as the (MIC) and (MBC).

Statistical analysis

Data were recorded accordingly and analyzed. The study findings were explained in tables. Proportions were used to determine prevalence.

RESULTS

In our study, 22 stool samples 6 (27.3%) of patients were female and 16 (72.7%) of them were male as given in Table 1. Isolates were characterized morphologically and biochemically. The morphological examination of S*higella species* were described as a gram negative, rod that exists in the form of staph (cluster) and visualized red rod under the light microscope while our isolates was exhibited indole, urease negative and also non motile but glucose was fermented without gas as shown in Table 2. *Shigella* and *Salmonella* were both non lactose fermenters. Hence, *E.coli* was used as a control to distinguish between both. SS agar is selective medium for salmonella and *Shigella spp*, by inhibiting other contaminants and also pathogenic bacteria. The result was exhibited different characteristics for various tests according to their interpretation as depicted in Tables 3 and 4.

Table 1: Sex and age group of under-ten diarrheic children at Tirunesh-Beijing Hospital, Addis Ababa, Ethiopia.

Variables	Categories	Positive		Negative	
		N	%	N	%
Sex	Male	1	6.25	15	93.7
	Female	1	16.7	5	83.4
Age group (in year)	<1				
	1-5	1	20	4	80
	6-10	1	7.7	12	92.3
Total	<10	2	9.1	20	89.9

Table 2: Characterization of bacteria based on Shape & morphology in gram staining.

Isolates	Gram reaction	Shape	Arrangement
***Shigella* species**	Negative	Rod	Staph

Table 3: Characterization based on biochemical test.

Isolates	Lactose	Indole	Urease	H_2S	Gas in glucose	Citrate	Motility	TSI
Shigella flexneri	-	-	-	-	+/-	-	-	+
Shigella sonnei	+	-	-	-	+/-	+	-	+

+/- ferment glucose without gas, + positive, - negative.

Table 4: Distribution of the pathogens in diarrheal stools according to patients age and sex.

Age	Sex	Shigella spp.	Salmonella spp.	Total
<1 year-old		0	0	0
1-5 year-old	M*	1(4.5%)	0	1(4.5%)
6-10 year-old	F*	1(4.5%)	0	1(4.5%)
Total		2 (9.1%)	0	2 (9.1%)

*M- male, F-female.

Table 5: Interpretation of TSI.

Result (slant/butt)	Symbol	Interpretation
Red/yellow	K/A	Glucose fermentation only; peptone catabolized
Yellow/yellow	A/A	Glucose and lactose and/or sucrose fermentation
Red/red	K/k	No fermentation; peptone catabolized
Red/no color change	K/NC	No fermentation; peptone used aerobically
Yellow/yellow with bubble	A/A, G	Glucose and lactose and/or sucrose fermentation; gas produced
Red/yellow with bubbles and black precipitate	K/A, G, H_2S	Glucose fermentation only; gas produced; H_2S produced
Red/yellow with black precipitate	K/A, H_2S	Glucose fermentation only; H_2S produced
Yellow/yellow with black precipitate	A/A, H_2S	Glucose and lactose and/or sucrose fermentation; H_2S produced
No change/no change	NC/NC	No fermentation

A=acid production; k=alkaline reaction; G=gas production; H_2S=sulfur reduction.

Table 6: Antibiotics inhibition in minimum inhibitory concentration (MIC).

	Amx			Amp			Cip			Co		
Antibiotics in mg/L	10	8	6	10	8	6	2	1	0.5	5	4	2
Shigella sonnei	+	+	+	+	+	+	-	-	+	+	+	+
Shigella flexneri	+	+	+	+	+	+	-	-	+	+	+	+

Amx=Amoxicillin, Amp=Ampicillin, Cip= Ciprofloxacin, Co=co-trimoxazole; - No growth; + Growth

During the study period *Shigella species* were isolated from two stool samples of 22 (9.09%) from under ten paediatric age group, admitted in paediatric ward of the hospital. The biochemical and morphological test confirmed the serogroup of *S. flexneri* and *S. sonnei* as given in Table 5.

Of 22 sample tested the two positive samples were tested for antimicrobial susceptibility. Four antimicrobial agents were tested to determine minimum inhibitory and minimum bactericidal concentration. Among the isolates, higher rate of drug resistant was observed for ampicillin, amoxicillin, cotrimoxazole that was 50%. Out of four antibiotics drugs *Shigella spp* showed sensitivity for ciprofloxacin and the antimicrobial sensitivity demonstrated that ciprofloxacin was the most effective antimicrobials while the isolate were resistant for ampicillin, amoxicillin, and cotrimoxazole drugs. Ciprofloxacin had exhibited low minimum inhibitory concentration compared to others as in Table 6.

DISCUSSION

The present study provides result of antibiotics resistance pattern of *shigellosis* isolates conducted in Tirunesh-Beijing hospital, Addis Ababa, Ethiopia. Out of 22 stool samples, 2 (9.1%) diarrheagenic bacteria were identified as *Shigella* and no *salmonella strains*. similarly, the study conducted at Harar, Eastern Ethiopia reveals the prevalence of *Shigella* 14.6 isolates from stool samples.[9] Isolation rate of *Shigella species* in our study is comparable with a previous study on handlers.[10] Similar finding was reported in Hawassa, southern Ethiopia for *Shigella* isolates.[11]

In our present study, the prevalence of shigellosis is similar to other studies from other parts of the world. In this studi*es S. flexneri* and *S. sonnei* were found to be common species, which is comparable with studies in developing countries like Pakistan, Kuwait, and India.[12] In endemic regions of the developing countries, shigellosis is predominantly a pediatric disease, with the urban poor being hardest hit. Some studies used similar cultural methods and media, and also sampled all age groups reports dissimilar findings, for instance north of Iran (14.05%), and Tehran (1%).[13]

In the antimicrobial drug susceptibility tests, ciprofloxacin exhibited effective against isolates while there was a resistance for ampicillin, amoxicillin and cotrimoxazole. These indicate that there is a needs to revise specific antibiotic prescription by conducting bacteriological investigation with antibiotic resistance tests. In other studies similar isolates showed high resistance for commonly used antibiotic agents with high resistance to tetracycline (90%), co-trimoxazole (84.6%), ampicillin (78.9%) and chloramphenicol (67.8%) and lower resistance to gentamicin (12.2%), ciprofloxacin (2.2%) and norfloxacin (1.1%).[14] Ciprofloxacin have been shown to be highly effective for treatment of shigellosis.[4] All *Shigella* isolates in our study were sensitive to ciprofloxacin and shown lower resistance to norfloxacin were resistant to amoxicillin (50%), ampicillin (50%).The emergence of antibiotic resistant to this drugs that utilized as an empirical therapy for treatment of shigellosis may be due to excessive and inappropriate use of them in the study area. There is a raise in resistance in various strains of *Shigella* against commonly used antibiotic.[15] Further Analysis of antimicrobial susceptibility profiling, reconsideration of the empiric use of these antimicrobial agents for the treatment of shigellosis is needed in the study area.

In conclusion, the preliminary prevalence of *shigella* species among under-ten children at Tirunesh Beijing hospital was 9.1% *Shigella* species (*Shigella flexneri* and *Shigella sonnei)*. These isolates showed higher resistance to commonly prescribed drugs; Amoxicillin, Co-trimoxazole and Ampicillin while antimicrobial sensitivity to ciprofloxacin was reported as the most effective antimicrobial agent in this study. The immerging resistance to common antimicrobial drugs needs further attention and assessment to obtain the underlying mechanisms of antimicrobial resistance by *Shigella* isolates is necessary to keep the vulnerable children save.

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