

Prevalence and Antibiotic Resistance Patterns of *Salmonella* and *Shigella* Species among under Five Age Children at Jimma University Medical Center and Serbo Health Center, Southwest Ethiopia

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Abstract

Background: Worldwide, an estimated 2,00,000 deaths occur due to diarrhea caused by *Salmonella* where 80% of deaths occur among under-five children. *Shigella* species is the leading pathogen among the top six attributable pathogens causing childhood diarrhea. The study was undertaken to determine the prevalence of *Salmonella* and *Shigella* species among under-five children and their antibiotic resistance patterns.

Methods: A cross-sectional study design was employed to collect data. The stool samples were inoculated on macconkey agar, xylose lysine dextrose agar and incubated aerobically at 37°C for 18 hrs to 24 hrs. All positive stool cultures were identified and characterized based on morphology, cultural characters and biochemical tests. The antibiotic susceptibility testing was done on Mueller Hinton agar.

Results: From 348 stool samples screened for *Salmonella* and *Shigella* species. The overall prevalence of *Salmonella* and *Shigella* species was 5.2% and 6.0% respectively. About 76.2% of *Shigella* species and 66.7% of *Salmonella* species isolated from a stool sample were multidrug-resistant. *Shigella* and *Salmonella* species showed the highest frequency of drug resistance for ampicillin (100%, 88.9%).

Conclusion: Prevalence of *Salmonella* and *Shigella* species still higher and higher level of drug resistance observed. Fluoroquinolones and ceftriaxone are still the treatment of options for *Salmonella* and *Shigella* species.

Keywords: Antibiotic resistance; Diarrhea; Ethiopia; *Salmonella*; *Shigella*

groups. In the world, an estimated 21 million cases of diarrhea were due to *Salmonella* species, resulting in 2,00,000 deaths each year where 80% of deaths occur among under-five children. *Shigella* species are one of the leading causes of childhood diarrhea with case-fatality rates of up to 28% among under-five children. About 113 million cases of diarrhea due to *Shigella* occurred each year among under-5-year olds in developing countries. In 2016, the Global Enteric Multicenter Study (GEMS) reported that *Shigella* is a pathogen among the top six attributable pathogens causing childhood diarrhea [1].

Diarrhea due to *Salmonella* and *Shigella* species and its complications remain a major cause of morbidity and mortality among under-five children, especially in developing countries including Ethiopia. In Ethiopia, diarrhea caused by *Salmonella* species and *Shigella* species is common among under-five children.

On the other hand, the emergence of multi-drug resistance pathogen is a global challenge, particularly in developing countries where increased misuse of antimicrobial agents occurs. Limited studies have been conducted on prevalence and antibiotic resistance of *Salmonella* and *Shigella* species in the study area. Therefore, this study aimed to determine the prevalence of *Salmonella* and *Shigella* species among under-five children and to test antibiotic resistance of the isolates in the study area [2,3].

Materials and Methods

Study area and study period

The study was conducted in Jimma university medical center and Serbo health center from February 17/2017 to June 30/2017.

Study design

Cross-sectional study design was used.

Introduction

Globally, diarrheal diseases accounted for 8% of all deaths in children under five years of age. *Salmonella* and *Shigella* species are microorganisms that have the potential of causing disease in the intestinal tract. Children below five years of age are more susceptible to diarrheal illnesses compared with other age

Study population and eligibility criteria

Under-five children who had diarrhea presented to the pediatric outpatient department during the study period were the study population. Children aged between 0-59 months with diarrhea and not had been inpatient for longer than 24 hours were included. Under-five children with diarrhea who started treatment at a time of data collection were excluded.

Sample size determination and sampling technique

The sample size for this study was calculated using a single population proportion formula ($n = Z^2pq/d^2$), where: P=was taken from the prevalence of *Shigella* species from a study conducted in Ambo hospital 29%. Considering 95% confidence interval the total sample size was n (including 10% non-response rate) \approx 348. The participants were recruited using a convenient sampling technique [4].

Screening and identification of bacteria

Stool sample collection, inoculation and incubation: About 2 ml-5 ml fresh diarrheic stool sample was collected in a clean tight-fitting container and then transported to Jimma university microbiology laboratory within 2 hours for isolation and identification. The stool samples were inoculated on MacConkey agar (MAC) and Xylose Lysine Dextrose agar (XLD) and the samples were also plated onto selenite F broth for the enrichment of *Salmonella* species and incubated at 37°C for 18 hrs to 24 hrs. Growth conditions were controlled by parallel incubation of control strain *i.e.*, *Shigella flexneri* ATCC 12022 and *Salmonella typhimurium* ATCC 14028 obtained from Ethiopian public health institutes [5].

Isolation and identification: After overnight incubation, culture plates were examined for *Salmonella* and/or *Shigella*-like colonies. *i.e.*, colorless and lactose non-fermenting colonies on MAC and/or clear to light pink colonies with distinct black centers and clear to white/pale-red colonies on XLD. All positive stool cultures were identified and characterized on the basis of morphology, cultural characteristics, biochemical tests using standard procedures. The reaction pattern was then used in the identification of *Salmonella* species and *Shigella* species (Figure 1) source [6].

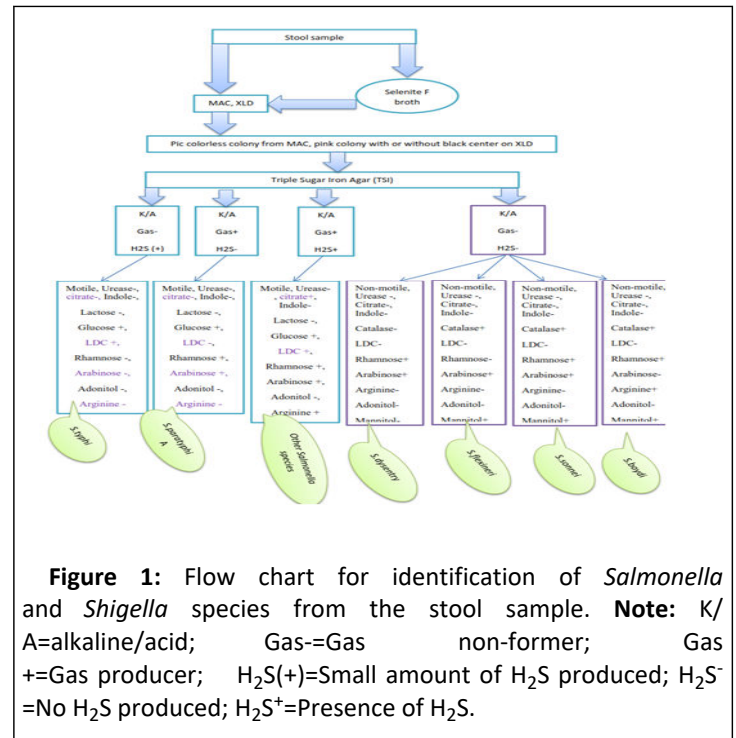


Figure 1: Flow chart for identification of *Salmonella* and *Shigella* species from the stool sample. **Note:** K/A=alkaline/acid; Gas=Gas non-former; Gas +=Gas producer; H₂S(+)=Small amount of H₂S produced; H₂S⁻=No H₂S produced; H₂S⁺=Presence of H₂S.

Antibiotic sensitivity test

The antibiotic resistance patterns of *Salmonella* and *Shigella* species were determined by the modified Kirby-Bauer method on Mueller Hinton Agar (MHA). Following overnight incubation, at 37° C, clear zones of inhibitions were measured in mm using a straight-line ruler and the results were recorded as Sensitive (S) or Resistance (R) based on EUCAST guidelines. For the susceptibility testing the following ten antimicrobial drugs and concentrations were used: Chloramphenicol (30 µg), ampicillin (10 µg), ciprofloxacin (5 µg), cefotaxime (5 µg), ceftazidime (10 µg), cefuroxime 30 µg, ceftriaxone (30 µg), norfloxacin (10 µg) and trimethoprim-sulfamethoxazole (1.25/23.75) µg and amoxicillin-clavulanic acid (20/10) µg. Multidrug resistance was defined as resistance to \geq 3 of the antimicrobial agents tested [7].

Data analysis

The collected data were checked for completeness, then it was coded, entered and cleaned using epi-data version 3.02. The analysis of data were done using SPSS version 20. Logistic regression was performed to evaluate whether variables were significantly associated with the outcomes of interest at 95% confidence limits or a 5% level of significance [8].

Results

Socio-demographic information

In this study a total of 348 stool samples were collected from under-five children with diarrhea. The mean age of children

enrolled in the study was 15 months with standard deviation of ± 12 months. About 188 (54%) of them were female. About 47 (38.8%) of urban resident have domestic animals in their home (Table 1) [9].

Table 1: Socio demographic status of under-five children attending Jimma university medical center and Serbo health center, southwest Ethiopia.

Socio-demographic information		Number (%)
Age	0-6 months	116 (33.3%)
	7-36 months	214 (61.5%)
	37-59 months	18 (5.2%)
Total		348 (100%)
Gender	Male	160 (46.0%)
	Female	188 (54.0%)
Total		348 (100%)
Presence of domestic animals	Yes	135 (38.8%)
	No	213 (61.2%)
Total		348 (100%)
Types of domestic animal	Cattle	56 (41.5%)
	Sheep and goat	9 (6.7%)
	Dog	46 (43.1%)
	Cat	24 (17.8%)
Total		135 (100%)

Environmental factors

The main sources of drinking water for the participants were tap water 274 (78.7%) while 70 (20.1%) sources of water were stream water particularly for 67% of rural residents. Only about

55 (15.8%) of the participant's parents, treated drinking water in their home. The practice of hand washing before feeding was significantly decreased infection with *Salmonella* and *Shigella* species ($P=0.001$) (Table 2).

Table 2: Environmental factors associated to diarrhea in under-five children in Jimma university medical center and Serbo health center, southwest of Ethiopia.

Environmental factors		Number (%)
Water source	Tap water	274 (78.7%)
	Stream water	70 (20.1%)
	Well water	4 (1.1%)
Total		348 (100%)
Private latrine	Yes	271 (77.9%)

	No	77 (22.1%)
Total		348 (100%)
Water treatment	Yes	55 (15.8%)
	No	293 (84.2%)
Total		348 (100%)
Hand washing before feeding	Yes	315 (90.5%)
	No	33 (9.5%)
Total		348 (100%)
Means of hand washing	Ash and water	11 (3.2%)
	Water only	217 (62.4%)
	Soap and water	120 (34.5%)
Total		348 (100%)

Clinical data

About 201 (57.8%) and 187 (53.7%) of the patients had signs and symptoms of fever and vomiting respectively. Out of 348 stool samples collected about 194 (55.7%) of stool was watery diarrhea, while 31 (8.9%), 29 (8.3%) was bloody and mucoid diarrhea respectively [10].

Prevalence of *Salmonella* and *Shigella* species

From a total of 348 samples examined, *Salmonella* and *Shigella* species were isolated in 39 samples. The overall prevalence of *Salmonella* species was 18 (5.2%). Frequently

isolated *Salmonella* species was *S. typhi* 44.5% (8/18). The overall prevalence of *Shigella* species was 21 (6.0%) and presumptive identification of *Shigella* species showed *S. flexneri* was the most frequent species which accounted for 57.1% (12/21). Majority of *Shigella* species 61.9% (13/21) were isolated from mucoid diarrhea followed by bloody diarrhea from which 19.0% (4/21) *Shigella* species isolated, while least numbers of *Shigella* species were isolated from watery diarrhea and loose stool which was 9.52% (2/21) each (Table 3).

Table 3: Characteristics of under-five children and organism isolated from the stool sample.

Demographic factors		Salmonella species isolated			Shigella species isolated No (%)
		S. typhi No (%)	S. paratyphi A No (%)	Other Salmonella spp. No (%)	
Age	0-6 months	0 (0%)	0 (0%)	0 (0%)	8 (38.1%)
	6-36 months	8 (44.4%)	4 (22.2%)	6 (33.3%)	6 (28.6%)
	37-59 months	0 (0%)	0 (0%)	0 (0%)	7 (33.3)
Total		8 (44.4%)	4 (22.2%)	6 (33.3%)	21 (100%)
Gender	Male	6 (33.3%)	3 (16.7%)	1 (5.5%)	3 (14.3%)
	Female	2 (11.1%)	1 (5.5%)	5 (27.8%)	18 (85.7%)
Total		8 (44.4%)	4 (22.2%)	6 (33.3%)	21 (100%)

Clinical sign and symptoms	Fever	2 (11.1%)	1 (5.5%)	5 (27.8%)	7 (33.3%)
	Abdominal cramps	8 (44.4%)	4 (22.2%)	6 (33.3%)	21 (100%)
	Vomiting	8 (44.4%)	4 (22.2%)	6 (33.3%)	14 (66.7%)
Total		18 (75%)	9 (75%)	17 (94%)	42 (66.6%)
Stool consistency	Loose	6 (33.3%)	3 (16.7%)	1 (5.5%)	2 (9.5%)
	Bloody diarrhea	2 (11.1%)	0 (0%)	3 (16.7%)	4 (19.1%)
	Mucoid diarrhea	0 (0%)	1 (5.5%)	0 (0%)	13 (61.9%)
	watery diarrhea	0 (0%)	0 (0%)	2 (11.1%)	2 (9.5%)
Total		8 (44.4%)	4 (22.2%)	6 (33.3%)	21 (100%)

Detection of *Salmonella* and *Shigella* species was higher in those who washed hands with water alone than those who washed with ash and soap. A higher prevalence of *Salmonella* and *Shigella* species was also seen in under-five children who had the previous history of contact with patients with diarrhea [11].

isolates were resistant to chloramphenicol. Resistance to ciprofloxacin and ceftriaxone was observed in 17% (3/18) and 11% (2/18) respectively. All *Shigella* isolates showed 100% (21/21) resistance to ampicillin and 86% (18/21) isolates were resistant to cefuroxime while 14% (3/21) and 5% (1/21) of the isolates showed resistance to norfloxacin and ciprofloxacin respectively (Table 4) [12].

An imicrobial resistance pattern

Antimicrobial susceptibility tests showed that 16 (88%) of the *Salmonella* isolates were resistant to ampicillin and 13 (73%)

Table 4: Antibiotic resistance of *Salmonella* and *Shigella* isolates. Note: AMP=Ampicillin; CHLOR=Chloramphenicol; CIPRO=Ciprofloxacin; NOR=Norfloxacin; CEFO=Cefotaxime; CEFU=Cefuroxime; CEFTA=Ceftazidime; CEFTRA=Ceftriaxone; SXT=Trimethoprim sulfamethoxazole; AM-CLAV=Amoxicillin clavulanic acid.

Antibiotics	Resistance of <i>Salmonella</i> species			Resistance of <i>Shigella</i> species
	<i>S. typhi</i>	<i>S. paratyphi A</i>	Other <i>Salmonella</i> spp.	
AMP. 10 µg	7 (38.9%)	4 (22.2%)	5 (27.8%)	21 (100%)
CHLOR. 30 µg	5 (27.8%)	3 (16.6%)	5 (27.8%)	12 (57%)
CIPRO. 5 µg	1 (5.6%)	1 (5.6%)	2 (11.1%)	1 (4.8%)
NOR. 10 µg	2 (11.1%)	2 (11.1%)	3 (16.6%)	3 (14.3%)
CEFU. 30 µg	5 (27.8%)	3 (16.6%)	5 (27.8%)	18 (85.7%)
CEFO. 5 µg	2 (11.1%)	0 (0%)	0 (0%)	3 (14.3%)
CEFTA. 10 µg	5 (27.8%)	1 (5.6%)	3 (16.6%)	13 (61.9%)
CEFTRA. 30 µg	1 (5.6%)	1 (5.6%)	0 (0%)	0 (0%)
SXT 1.25/23.75 µg	3 (16.6%)	2 (11.1%)	2 (11.1%)	11 (52.4%)
AM-CLAV20-10 µg	6 (33.3%)	3 (16.6%)	3 (16.6%)	20 (95.2%)

Multidrug resistance was considered when the isolate was resistant to three and more classes of drugs. Out of 21 isolates of *Shigella* species, 16 (76.2%) were multidrug-resistant. From a

total of 18 isolates of *Salmonella* species only one isolate was found to be susceptible to all drugs while 12 (66.7%) of *Salmonella* isolates were multidrug-resistant.

Discussion

In this study *Salmonella* and *Shigella* species were isolated in 11.2% of stool sample taken from under-five children with diarrhea. This result is lower than a study conducted in Sudan where *Salmonella* and *Shigella* species were isolated in about 47.37% of diarrheas. This can be due to geographic and time differences.

In the current study, the prevalence of *Salmonella* species among under-five children was about 5.2% which is slightly higher than a study conducted among under-five children in Vietnam (4%) and in Kenya 3.5%. These variations might be due to method differences where this study used biochemical tests alone while they include PCR.

The prevalence of *Salmonella* species in this study was higher than the prevalence reported in Hawassa (2.5%) and Addis Ababa (3.95%). On the other hand, the prevalence of *Salmonella* species was comparable with the prevalence reported in Jimma in 2014 in children less than 15 years of age where the prevalence of *Salmonella* was 6.2%.

On the other hand the prevalence of *Shigella* species of the present study is comparable with the prevalence reported in Hawassa Adare hospital and Millennium health center (7%).

Antimicrobial susceptibility tests of this study showed that about 88% of *Salmonella* isolates were resistant to ampicillin and 72.2% to chloramphenicol which showed a higher rate of resistance than study in Gonder. But it is comparable to study in Addis Ababa where 95.7% of isolated *Salmonella* species were resistant for ampicillin. In line with this study, in Addis Ababa, low resistance to ciprofloxacin (4.3%) and ceftriaxone (4.3%) were reported.

Antimicrobial susceptibility tests of this study showed that 76.2% of *Shigella* species were multidrug-resistant which is lower than the study done in Addis Ababa where more than 87% of *Shigella* species were multidrug-resistant. This difference might be due to the fact that in this study multidrug resistance was considered when the isolate was resistant to three and more drugs unlike in Addis Ababa which considered resistance to more than one drug [13].

Limitations of the study

We have recognized limitations; one of which was the study design (cross-sectional) which cannot rule out cause and effect relationship of associated factors which indeed require control groups. Presumptive identification of the isolated strain was made with series of biochemical reaction but has not been serotyped.

Conclusion

In the present study, *Salmonella* and *Shigella* species were isolated in 11.2% of under-five children with diarrhea. In the present study, about 76.2% of *Shigella* species and 66.7% of *Salmonella* species were multidrug resistant. In this study, 66.7% of *Salmonella* isolates were Multidrug-Resistant (MDR) which is comparable with study conducted in Nepal, where 70.0% of

Salmonella species were MDR and study done in Addis Ababa where more than 70.0% of *Salmonella* species were MDR.

Ethical Consideration

Institutional ethical clearance was obtained from Jimma university health research ethics review committee. During data collection, each participant's parent/legal guardians were informed about the aim of the study and written consent was obtained before the start of data collection.

Recommendation

This study indicated, ampicillin is no longer effective for the treatment of diarrhea that might be caused by *Salmonella* and *Shigella* species at least in the study area and its surroundings.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Declaration

I declare that there is no conflict of interest.

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