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ORIGINAL ARTICLE

Prevalence and antimicrobial susceptibility pattern of *Salmonella* and *Shigella* in stool among patients presenting with diarrhea in a tertiary care centre in south India

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ABSTRACT

Background and Objectives: Bacterial causes of gastroenteritis include *Salmonella, Shigella* spp, diarrheagenic *Escherichia coli*, *Vibrio cholera* and *Campylobacter* spp. Although infections caused by NTS (Non Typhoidal *Salmonella*) and *Shigella* are usually self-limiting, antibiotic treatment is prefered in severely ill or immunocompromised individuals. The main objective of the study was to find out the prevalence of *Salmonella* and *Shigella* among the stool samples received in Believers Church Medical College hospital and the antimicrobial susceptibility pattern of *Salmonella* spp. and *Shigella* spp.
Materials and Methods: A total of 805 stool samples collected from cases of diarrhea from January 2018 to December 2021 were processed in the laboratory. Standard bacteriological methods were used to isolate, identify, and determine the antimicrobial susceptibility pattern of *Salmonella* and *Shigella* isolates using the disc diffusion method and interpreted according to CLSI.
Results: A total of 100 (12.4%) samples yielded bacterial pathogens. *Salmonella* was isolated from 97 (12%) samples and *Shigella* from 3 (0.4%) samples. *Salmonella enterica* serovar Typhimurium was the predominant serotype, accounting for 53 (54.6%) isolates.
Conclusion: This study showed *Salmonella enterica* serovar Typhimurium as the predominant isolate causing diarrheal illness. The emergence of multidrug resistant phenotypes warrants the continuous monitoring of susceptibility trend of NTS in India.

Keywords: *Salmonella*; *Shigella*; Susceptibility; Resistance; Diarrhea

INTRODUCTION

Acute gastroenteritis is one of the main causes of mortality and morbidity among infants, children, elderly and immunocompromised individuals (1). In India, diarrhea is the cause of hospital admissions in one third of paediatric patients and a cause of death in 17% of all indoor paediatric patients (2). Bacterial causes of gastroenteritis mainly include *Salmonella* spp., *Shigella* spp, Diarrheagenic *E. coli*, *V.cholera* and *Campylobacter* spp. (3). *Salmonella* and *Shigella* are of particular concern and they are Gram-negative rods which commonly inhabit intestinal tracts of humans and many animals. Although most cases of Salmonellosis are self-limiting, some invasive strains can cause bacteremia and systemic infections (4). Salmonellosis usually acquired through ingestion of contaminated food of animal origin and contact

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with infected animals or contaminated environments (5). Food preparation practices, watersupply, waste disposal are some of the factors which determine the prevalence of *Salmonella* in a region (6).

Another important cause of gastroenteritis is *Shigella* which is responsible for approximately 165 million cases annually, of which 98.8% are in developing countries (7). The main mode of transmission of *Shigella* species is by ingestion of contaminated food and water or by direct contact with an infected person and fomites. Majority of cases occur in children less than 5 years of age who contribute 69% of the cases (8). Of particular concern is the emergence of multi drug resistant *Salmonella* and *Shigella*. Terfassa et al. in her study reported that 10% and 33.3% of the isolates of *Salmonella* and *Shigella* respectively were multi drug resistant (9). Another concerning fact was the emergence of XDR (Extensively drug resistant) *Salmonella* in Pakistan (10).

Although infection caused by NTS (Non Typhoidal *Salmonella*) is usually self limiting, antibiotic treatment is preffered in severly ill or immunocompromised individuals. The emerging resistance to ceftriaxone limits the treatment options (11). Intestinal infections with *Shigella* can be managed with rehydration but antibiotics can prevent lethal complications. *Shigella* strains resistant to cephalosporins, fluoroquinolones and azithromycin is of grave concern (12). The main objective of the study was to find out the prevalence of *Salmonella* and *Shigella* in stool among the samples received in the hospital and to find the antimicrobial susceptibility pattern of *Salmonella*, *Shigella* among stool samples.

MATERIALS AND METHODS

A retrospective cross sectional study was conducted in a tertiary care hospital in Kerala, India from January 2018 to December 2021. The study (IEC/09/84/2019) was approved by the Institutional Ethical Committee(ECR/1098/Inst/KL/2018).

Inclusion criteria- Stool samples of all patients presenting with diarrhea.

Specimen collection and bacterial identification. A total of 805 freshly collected stool samples were processed in the clinical microbiology laboratory during the study period. Only one sample per patient was included in the study. Samples were collected in sterile containers and transported to the laboratory within 6 hrs of collection. Stool specimens were inoculated onto MacConkeys agar (MA) (Himedia,New Delhi, India) and Xylose-Lysine-Deoxycholate (XLD)) (Himedia, New Delhi, India) and incubated at 37°C for 18-24 hrs. Specimens were also inoculated into Selenite-F enrichment broth (Himedia, New Delhi, India) and subcultured on XLD after 8-12 hours to improve bacterial recovery. Alkaline peptone water broth was used in suspected cases of cholera.

Salmonella and *Shigella* were both suspected by their characteristic presence on MacConkeys agar showing non lactose fermenting colonies. On XLD, *Salmonella* produced red colonies with a black centre, while *Shigella* produced red colonies without a black centre. All the suspected colonies grown on MA and XLD were analyzed by routine biochemical and microbiological tests. Indole, Triple Sugar Iron (TSI), Citrate, Urease and Mannitol Motility were the biochemichal tests used for identification. Isolates of *Shigella* were non motile, K/A(Alkali/Acid) with no gas and no H_2S on TSI and negative on Citrate and Urease tests. Isolates of *Salmonella* were motile, K/A(Alkali/Acid) with gas and H_2S on TSI , positive Citrate test and negative Urease test. Specific polyvalent antisera (Denka Seikan Co. Ltd, Japan) were used for the serotype identification of *Salmonella* and *Shigella* (13). All clinical and demographic details were obtained from medical records.

Antimicrobial susceptiblity testing. Antimicrobial susceptibility testing was done by using disk diffusion method, according to guidelines of Clinical Laboratory Standards Institute (CLSI 2018-2021) (14). All the antibiotic discs were obtained from HIMEDIA (Himedia, New Delhi, India). The antibiotic discs used and their concentration were: ampicillin (AMP, 10-µg), nalidixic acid (NA, 30-µg), ceftriaxone (CRO, 30-µg), cotrimoxazole (SXT, 25-µg), and ciprofloxacin (CIP,5-µg). A standard inoculum which was adjusted to 0.5 McFarland was swabbed on to Muller-Hinton agar (Himedia, New Delhi, India). After dispensing the discs on the plate they were incubated at 35-37°C for 18-24hrs. The reference strain *Escherichia coli* ATCC 25922 was used as control for each batch of drugs.

Statistical analysis. The data was entered in MS Excel. JASP V.18 was used to analyze the data. Proportions and percentages were calculated and ex-

pressed to arrive at meaningful conclusions.

RESULTS

Among the 805 samples processed in the microbiology laboratory, 100 (12.4%) samples yielded bacterial pathogens. *Salmonella* was isolated from 97 (12%) samples and *Shigella* from 3 (0.4%) samples. Among the 97 isolates of *Salmonella*, *Salmonella enterica* serovar Typhi was not present. *Salmonella enterica* serovar Typhimurium was the predominant serotype, accounting for 53 (54.6%) isolates. Remaining isolates could not be serotyped. Among the three cases of *Shigella*, there were two cases of *Shigella flexneri* and one case of *Shigella sonnei*. Identification of *Salmonella* and *Shigella* were confirmed by colony morphology, biochemical reactions and agglutination by antisera. Socio demographic data, clinical features and laboratory findings among the positive cases are shown in Table 1. The yearly isolation rate of Non typhoidal *Salmonella* from 2018 to 2021 was 7.2%, 6.7%, 35.7% and 16.6% respectively.

Antimicrobial susceptibility pattern of *Salmonella* and *Shigella* isolated during the study is shown in Table 2. Resistance distribution of pathogens isolated during the study is shown in Table 3.

There were only two MDR pathogens (*Salmonella*-1, *Shigella*-1) isolated during the study. Both isolates were resistant to ampicillin, ceftriaxone and ciprofloxacin. As shown in Table 1, 88% of the positive patients were treated with antibiotics. Among the antibiotics used to treat patients ceftriaxone was the most frequent intravenous antibiotic,used in 50% of the patients. Cefexime was the most frequent oral antibiotic and it was used in 39% of the patients. Ciproflxacin was used both IV and orally and it was used in 21% of the patients. Other less commonly used drugs include cefotaxime, cotrimoxazole, piperacillin-tazobactam, meropenem and ampicillin. Among the 100 positive cases, 2 patients expired, while others improved on treatment. Both patients who expired had *Salmonella* isolated from stool. Death of two patients were due to multi organ dysfunction. It was unrelated to salmonella in stool.

DISCUSSION

Our study reinforces the importance of Non typhoidal *Salmonella* as an enteric pathogen. Overall

Table 1. Demographic, Clinical features and laboratory findings among the positive cases

Variable	*Salmonella*, n (%)	*Shigella*, n (%)
Age		
<1 year	7 (7.2)	0
1-5 years	10 (10.3)	1 (33.3)
16-30 years	7 (7.2)	2 (66.7)
31-45 years	6 (6.2)	0
46-60 years	22 (22.7)	0
61-80 years	41 (42.3)	0
>80 years	4 (4.1)	0
Gender		
Male	59 (60.8)	1 (33.3)
Female	38 (39.2)	2 (66.7)
Duration of Diarrhoea		
1-5 days	91 (93.8)	2 (66.7)
6-10 days	3 (3.1)	1 (33.3)
11-15 days	0	0
≥ 16 days	3 (3.1)	0
Prior Respiratory Infection	7 (7)	0
Symptoms		
Fever (T ≥ 38°C)	61 (62.9)	2 (66.7)
Abdominal Pain	31 (32)	0
Vomiting	50 (51.5)	2 (66.7)
Nausea	5 (5.2)	0
Headache	7 (7.2)	0
Malaise	6 (6.2)	0
Stool Colour		
Yellow	25 (25.8)	2 (66.7)
Brown	60 (61.9)	1 (33.3)
Green	11 (11.3)	0
Red	1 (1)	0
Stool Consistency		
Watery	44 (45.4)	1 (33.3)
Bloody	13 (13.4)	0
Mucoid	11 (11.3)	0
Mixed (Blood + Mucus)	29 (29.9)	2 (66.7)
Laboratory Findings		
Anemia (Hb< 110 g/L)	10 (10.3)	
Leukocytosis (>12 × 10^9/L)	24 (24.7)	1 (33.3)
Thrombocytosis (>400 × 10^9/L)	5 (5.2)	1 (33.3)
CRP (≥1 mg/L)	73 (75.3)	1 (33.3)
Stool WBC ≥ (++)	76 (78.4)	2 (66.7)
Stool RBC ≥ (++)	50 (51.5)	2 (66.7)
Stool occult blood (+)	8 (8.2)	1 (66.7)
Antibiotics		0
Oral only	4 (4.1)	0
IV only(Intravenous)	25 (25.8)	0
IV + Oral	56 (57.7)	3 (100)
None	12 (12.4)	0

Table 2. Sensitivity distribution (%) of *Salmonella* and *Shigella*

Bacterial isolate	AMP n (%)	CIP n (%)	NA n (%)	CRO n (%)	ST n (%)
Salmonella n=97	82 (84.5)	75 (77.3)	75 (77.3)	96 (99)	92 (94.8)
Shigella n=3	0	0	0	2 (66.7)	3 (100)

AMP = Ampicillin, CIP = Ciprofloxacin, NA = Nalidixic acid
CRO = Ceftriaxone, ST = Cotrimoxazole.

Table 3. Resistance distribution of *Salmonella* and *Shigella*

Bacterial isolates	R1, n (%)	R2, n (%)	R3, n (%)	R4, n (%)	S, n (%)
Salmonella n=97	11#(11.3)	14*(14.4)	1 (1)	0	71 (73.2)
Shigella n=3	0	2**(66.7)	1 (33.3)	0	0

R1= Resistance for one drug only, R2= Resistance for two drugs belonging to different groups, R3=Resistance for three drugs belonging to different groups (MDR) , R4= Resistance to all drugs, S=Pan sensitive, *= Resistant to ampicillin and ciprofloxacin, **= Resistant to Ampicllin and cotrimoxazole, #= 9/11 were resistant to ciprofloxacin alone and remaining two were resistant to cotrimoxazole alone.

prevalence of Non typhoidal *Salmonella* and *Shigella* in our study were 12% and 0.4% respectively. Two studies conducted in Ethiopia reported a lower prevalence in NTS of 7.3% and 2.5% respectively (15, 16). Prevalence of *Shigella* in India varies from 1-15% and a 12 year study in Karnataka reported a prevalence of 4.1% (17). The very low *Shigella* prevalence in our study is probably due to the fact that most infections are mild and self limiting, not needing medical attention or laboratory evaluation. Overall prevalence of enteric pathogens in our study was 12.4%, probably due to the fact that only two enteric pathogens were isolated in our study. This finding is low when compared to the prevalence in Tanzania (42.7%), Ethiopia (22.3%), Mozambique (27.2%) (16, 18, 19). A study conducted in North india showed an overall prevalence in enteric pathogens of 23.2% and a prevalence in NTS of 2.37% (20).

In our study although all the isolates could not be serotyped, *Salmonella* enterica serovar Typhimurium was still the most prevalent serotype (54.6%). This finding is concordant with the study conducted by Ren et al. which showed that *Salmonella enterica* serovar Typhimurium was the most prevalent NTS with a reported prevalence of 82.4% (21). This finding is discordant with the study conducted by Ran et al. which showed that *Salmonella enterica* serovar Enteritidis was the most common serotype, with a prevalence of 31% (22). The difference in serotype prevalence is probably due to changing epidemiological trend, the regional variation, and the specific patient population (21). *Salmonella enterica* serovar Weltevreden is gaining importance as a significant pathogen causing gastroenteritis globally. In India it has emerged as a significant food borne pathogen which has caused outbreaks in Pune, Kolkata and Mangalore (23). This isolate has also been increasingly isolated from vegetables,meat and sea foods (23). However, despite being a dominant pathogen in many parts of India, this isolate was not found in our study.

In our study majority of cases (42.3%) occurred in the 41-60 year age group. Study conducted by Leung et al. also showed higher percentage of cases in the elderly age group when compared to the younger group (24). This is discordant with a study conducted in China which showed higher prevalence in the younger age group (25). The common symptoms in our study were diarrhea, fever, vomiting and abdominal pain. Most patients reported watery diarrhea which was a similar finding to the study conducted by Mulatu et al. (16). More than 90% of the positive cases in our study had duration of diarrhea less than 6 days. Laboratory study revealed that the majority (73%) of patients had elevated CRP which was a similar finding to the study conduced by Ren et al. Contrary to the findings of Ren et al., our study showed that anaemia, leucocytosis and thrombocytosis was present in less than 25% of the positive cases. Stool examination in our study revealed that majority of patients had increased RBC and WBC. The study conducted by Ren et al. showed similar results with respect to WBC in stool but showed discordant results on RBC in stool (21). Occult blood in stool was found in only 8% of the positive cases.

In our study the isolation rate of NTS increased substantially in the third year to 35.7% which although concerning probably hints at improved surveillance in the hospital. Jacob et al. in his study also reported

a marked yearly increase in the isolation rate of NTS (26). In our study isolation rate showed an increase from June to November (59%), which are considered the rainy season in Kerala, hinting at an increased contamination of food and water. This association of NTS infection with rainfall was reported by Tack et al. (27). Eventhough gastroenteritis caused by *Salmonella* and *Shigella* can be self limiting, antibiotics were used in 88% of the positive patients. This is likely due to most of our patients being referred from surrounding hospitals where initial supportive care failed.

In our study ceftriaxone, cefexime and ciprofloxacin were the most commonly used antibiotics. Ampicillin and ciprofloxacin susceptibility among *Salmonella* was 84.5% and 77.3% respectively. Jacob et al. also reported ampicillin and ciprofloxacin susceptibility of 75% and 82% respectively which is similar to our findings (26). There was only one multi drug resistant *Salmonella* isolated in our study but the emergence of ceftriaxone resistant NTS is of grave concern and needs further attention. The study conducted in India by Pragasam et al. showed a 5% prevalence of ceftriaxone resistance in Non typhoidal *Salmonella*, which is extremely concerning (28). Study conducted in Nepal by Bastola et al. showed that ciprofloxacin resistance in *Salmonella* was only 6.9% (29). Study conducted in Korea by Kim et al. showed that the resistance rates to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole were 32.6%, 12.1%, and 8.4%, respectively. The resistance rates to cefotaxime and ciprofloxacin were 8.1% and 3.0%, respectively and in this study 5.4% of the isolates were multi drug resistant (30). Our Patient who had MDR *Salmonella* in stool was a 38 year old female who was successfully managed without antibiotics.

There were only 3 isolates of *Shigella* (*S. flexneri*-2, *S. sonnei*-1) in our study. Only one among them was multi drug resistant. This was an isolate of *Shigella sonnei* isolate which was resistant to ampicillin, ciprofloxacin and ceftriaxone. Eventhough the number of isolates of *Shigella* in our study was small, all three were resistant to ampicillin and ciprofloxacin. High resistance to ciprofloxacin and ampicillin among *Shigella* has been reported by others (29). Study conducted by Bastola et al. in Nepal showed that ciprofloxacin resistance in shigella was 25% (29). Ceftriaxone resistance among *Shigella* is very concerning as it severly limits the treatment options. A recent study conducted in North India showed that nearly 20% of *S. flexneri* were resistant to third generation cephalosporin by disc diffusion and 33.7% had MIC ≥1 μg/mL (31). Another study conducted in India showed that 91.6% of the *Shigella* isolates were multi drug resistant and presence of ESBL (Extended spectrum betalactamase) was detected in five isolates (32). Our patient who had MDR *Shigella* in stool was a 21 year old female, who was successfully managed with cotrimoxazole and other supportive measures. Global emergence of resistance in *Shigella* is a matter of concern and calls for systematic monitoring and periodic updates of countrywide and local antibiogram.

CONCLUSION

The impact of NTS in causing diarrheal illness in Kerala is not well defined. The geographical distribution of NTS, their susceptibility profiles or the prevalent serotypes in the state are not well documented. This may also be due to the fact that the most common manifestation is a mild self-limiting gastroenteritis, and hence very few of the affected individuals seek medical care. Prevalence of Non typhoidal *Salmonella* and *Shigella* in our study were 12% and 0.4% respectively. This study showed *Salmonella enterica* serovar Typhimurium (54.6%) as the predominant isolate causing diarrheal illness. There were only two MDR pathogens (*Salmonella*-1, *Shigella*-1) isolated during the study. Emergence of ceftriaxone resistant NTS is extremely concerning and needs urgent attention. Ceftriaxone resistant *Shigella sonnei* was also isolated in the study. With appropriate antibiotic therapy and other supportive care, the majority (98%) of our patients improved. Physicians should be aware of the evolving antimicrobial resistance rates among *Salmonella* and *Shigella*. The emergence of multidrug resistant phenotypes warrants the continuous monitoring of susceptibility trend of NTS in India. Increasing resistance to fluoroquinolones and third generation cephalosporins may restrict future treatment options. Also monitoring the susceptibility of carbapenems and colistin in NTS may be considered, as these may be the last resort options in the future. Therefore, there is an urgent need to control the spread of drug resistant *Salmonella* to improve the treatment strategy for salmonellosis. This study had a few limitations. All the 100 isolates could not

be serotyped due to lack of sufficient antisera. Molecular characterization of *Salmonella* could not be done due to financial limitations.

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