ORIGINAL RESEARCH ARTICLE

Antibiotic Resistance Pattern of *Shigella* spp. Among Gastroenteritis Patients at Tertiary Care Hospital in Pokhara, Nepal

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Abstract

Shigellosis, a disease caused by *Shigella* species. It is a major public health problem in developing nations like Nepal, where communities having poverty; poor sanitation, personal hygiene, and water supplies. The main aim of our study is to isolate and identify *Shigella* spp. from gastroenteritis patients and to find out its drug resistance pattern.

A cross-sectional study was carried out based on routinely attending outpatients and inpatients. A total of 225 stool samples collected from gastroenteritis patients were processed from 20 April to 24 September 2014 in Western Regional Hospital, Pokhara, Nepal. Standard microbiological procedures were followed for the isolation of *Shigella* spp. After that slide agglutination kit method was used for identification of *Shigella* spp. Finally, Kirby-Bauer disc diffusion method was done for an antimicrobial resistance test.

Of the total 225 gastroenteritis patients, 133 were detected as bacterial positive cases. Among positive cases, *Shigella* spp. was identified in 10.5%. Age wise, an infection rate of *Shigella* in patients <15-years old was found higher i.e. 7.3% than in patients ≥ 15 years old i.e. 4.5% with the (p = 0.432) at 95% CI. The infection rate of *S. dysenteriae, S. flexneri,* and *S. sonnei* was detected in 28.6%, 57.1%, and 14.3% respectively. For the antimicrobial test, eight types of antibiotics were used. The most resistance pattern of isolated *Shigella* spp. was found in nalidixic acid, and co-trimoxazole 92.8% followed by ampicillin 64.3% and ciprofloxacin 42.8% etc.

Our study reported that endemicity of Shigellosis with *S. flexneri* is the predominant group in gastroenteritis patients. This finding suggests that co-trimoxazole, nalidixic acid, ciprofloxacin and ampicillin should not be used experimentally as first-line drugs for shigellosis treatment.

Keywords: *Shigella*, Antibiotic Resistance Pattern, Shigellosis, Gastroenteritis, Tertiary Care Hospital, Nepal

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Introduction

Shigellosis is caused by members of the bacterial genus *Shigella*. It is a severe and occasionally life-threatening gastrointestinal infection. Worldwide, *Shigella* spp. is the most common cause for acute and bloody diarrhea (dysentery). They are also responsible for a significant proportion of the burden of morbidity and mortality associated with diarrheal disease [1, 2]. The incidence of shigellosis in developing countries is nearly 20 times more than in developed countries [3]. Annually, it is estimated that there are 125 million infections and 14,000 deaths due to shigellosis in Asia. [4].

The first emerging antimicrobial studies conducted in the 1950s, reported multiple drug resistance transmitted by plasmids among Shigella spp. from many countries [5, 6]. As a result of the considerable global burden, low infectious dose, clinical severity, and frequent reports of emerging antimicrobial resistance against first-line and, more recently, second-line therapies [7-9]. Over 70% of *Shigella* isolates were resistant to two or more drugs including ampicillin and co-trimoxazole during 2002 to 2007 in India [10]. Reports from Indonesia, Bangladesh, Malaysia and Nepal, showed an increasing prevalence of *Shigella* isolates that are resistant to multiple drugs like trimethoprim-sulphamethoxazole, ampicillin, nalidixic acid and tetracycline [11-16].

Shigellosis is associated with significant morbidity and mortality among the pre-school children. It is caused by any one of the four species of *Shigella,* namely Serotype A - *S. dysenteria,* Serotype B - *S. flexneri,* Serotype C - *S.*

sonnei, and Serotype A - *S. boydii,* and the classification is based upon the serological antigen [17]. The frequencies of *S. flexneri*, *S. sonnei*, *S. boydii*, and *S. dysenteriae* are 16.0%, 7.0%, 2.0%, and 1.0% in developed countries and 60.0%, 15.0%, 6.0%, and 6.0% in developing countries, respectively [18]. In Nepal, *Shigella* spp. was detected in 13.6% of stool samples examined at Nepalgunj Medical College and Teaching Hospital, Banke, between September 2011 and April 2013 [19]. The current study was carried out in stool specimens collected from gastroenteritis patients at a Tertiary Care Hospital in Pokhara, Nepal. The main aim of this study is to isolate and identify *Shigella* spp. from gastroenteritis patients and find out its antimicrobial resistant pattern by modified Kirby-Bauer disk diffusion method.

Materials and Methods

Study design and setting

A cross-sectional study was conducted in 225 stool samples collected during 20 April to 14 September 2014. Clinical specimens of gastroenteritis patients were examined which is referred by a physician. The patient's symptoms like diarrhea, abdominal pain, vomiting, reddish & watery stool; age, gender etc. were also recorded. Both outpatient and in patients with acute gastroenteritis but not undertaking any antibiotics were taken as subject from Western Regional Hospital.

Sample collection

One gram of fresh stool sample was collected in a clean and sterile screw-capped plastic container. The collected samples were labeled properly with the patient's name, age, sex, address, and date. These samples were processed immediately following Standard Operating Procedures (SOPs) of microbiology in the laboratory of Western Regional Hospital, Pokhara, Nepal. If there was a delay in processing freshly passed stool sample within 2 hours, the specimens were kept in a refrigerator at 2-8^{o}C.

Macroscopic examination

Microscopic physical examination of stool sample was done for the color, consistency (formed, semi-formed, unformed), presence of blood, mucus or pus.

Isolation and identification of *Shigella* on Culture method:

0.1 ml of stool sample were inoculated in MacConkey (MA), Xylose Lysine Deoxycholate (XLD) and Salmonella-Shigella (SS) agar (Himedia Lab. Pvt. Ltd. India) and incubated at 37^{o}C for 24 hours. The suspected colonies were grouped as Gram Positive and Gram Negative by Gram's staining reaction. Biochemically, *Shigella* are found Indole negative, Citrate negative, Urea negative and Hydrogen Sulphide negative in TSI agar's slant and non-motile [14].

Identification of *Shigella* spp. in slide agglutination test

Shigella spp. was further tested by type-specific antisera of group A, B, C and D *Shigella* antisera in a serological kit (Denka Seiken Co. LTD. 3-4-2, Nihonbashi Kayabacho, Chuo-ku, Tokyo, Japan). Agglutination appeared on (Group A) monovalent, (Group B) polyvalent, (group C) polyvalent and (Group D) polyvalent in Kit, were identified for *S. dysenteriae*, *S. flexneri, S. boydii* and *S. sonnei* respectively.

Antibiotic susceptibility test

Antibiotic susceptibility tests of different clinical isolates against various antibiotics were performed by modified Kirby-Bauer disk diffusion method in Mueller Hinton Agar (Hi-Media Laboratories, India) according to Clinical Laboratory Standards Institute (CLSI) guidelines [20]. The antibiotics used for analysis were ampicillin, azithromycin, ciprofloxacin, co-trimoxazole, ceftriaxone, gentamycin, and nalidixic acid. The diameters of inhibition of zone for *Shigella* were compared with strains *Escherichia coli* ATCC 25922 (National Committee for Clinical Laboratory Sciences, Document M100-56: Sixth informational Supplement).

Statistical analysis

Pearson's Chi-Square test was used to determine the significant association of dependent variable by using Win-pepi Software (Copyright J. H. Abrmson, 23 Aug

2016 Version 11.65) with the p-value > 0.05 is considered as significant at 95% CI.

Results

Of the total 225 gastroenteritis patients, 133 were as detected bacterial positive cases (Gram-positive = 54 and Gram-negative = 79). Among bacterial positive cases, *Shigella* spp. was detected in 10.5% (14/133). The infection rate of *S. flexneri, S. dysenteriae,* and *S. sonnei* were detected in 57.1% (8/14), 28.6% (4/14) and 14.3% (2/14) respectively (**Figure 1**).

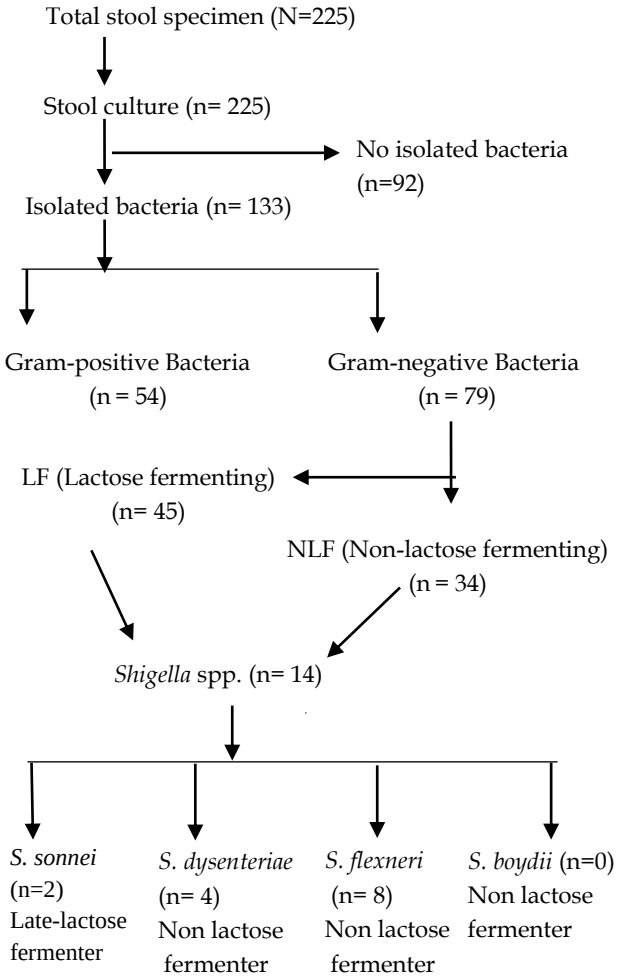

Figure 1. Flow chart for the isolation and identification of *Shigelle* isolates.

Sex wise distribution of all positive cases in different species of *Shigella* in gastroenteritis patients:

Among bacterial positive cases, male patients were 129 and female patients were 96 in the ratio of 1.3:1. In male patients, infection rate of *S. flexneri* was the highest i.e. 42.8% (6/14), followed by *S. dysenteriae* 7.1% (1/14) and *S. sonnei* 7.1% (1/14). Whereas in female patients, the highest rate of infection was observed for *S. dysenteriae*, i.e. 21.1% (3/14), followed by *S. flexneri* 14.3% (2/14) and *S. sonnei* 7.1% (1/14) were depicted in **figure 2.**

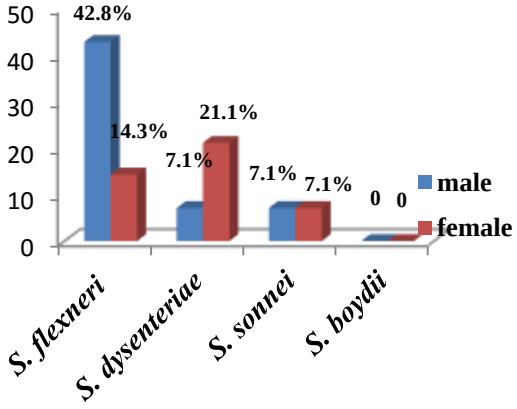

Figure 2: Sex wise distribution of all positive cases in different species of *Shigella* in gastroenteritis patients.

Age-wise distribution of gastroenteritis patients with *Shigella* and its infection:

Infection rate for *Shigella was* 7.3% (10/137) and 4.5% (4/88) in patient <15 years old and >15 years old respectively, confirming that infection rate for *Shigella* spp. is higher in children patients than in patients elder than 15 years old with the p-value (p = 0.432) at 95% CI (**Table 2**).

Antibiotic resistance wise distribution of gastroenteritis patients with *Shigella* and its species infection:

In antibiotic resistance test, the most resistance pattern of isolated *Shigella* were found in nalidixic acid and co-trimoxazole 92.8% (13/14) followed by ampicillin 64.3% (9/14) and ciprofloxacin 42.8% (6/14), detail description of *Shigella* spp. were shown in **Table 3.**

Discussion

Worldwide, acute gastrointestinal infections including diarrhea are among the leading causes of morbidity and mortality among children, particularly in underdeveloped countries [21]. Poor access to safe water, inadequate sanitary conditions, lower literacy rate, and unavailability of healthcare facilities in the remote area are the major factors

Table 2: Age with isolated *Shigella* spp.; *S. flexnari, S. dysenteriae,* and *S. sonnei* in patients of gastroenteritis

Characteristics		Total stool sample (n=225)	*Shigella* (n=14)	P value	*S. flexnari* (n=8)	*S. dysenteriae* (n=4)	*S. sonnei* (n=2)	P-value
			No. (%)		No. (%)	No. (%)	No. (%)	
Age (year)	<15	137	10 (7.3)	>0.05	5 (3.6)	3 (2.1)	2 (1.4)	>0.05
	≥15	88	4 (4.5)		3 (3.4)	1(1.3)	0 (0.0)	

Table 3 Antibiotic Resistance pattern of *Shigella* and its species

Antibiotics	*S. flexnari* (n=8)	*S. dysenteriae* (n=4)	*S. sonnei* (n=2)	Total (n=14)
	No. (%)	No. (%)	No. (%)	No. %
Ampicillin (10µm)	4 (50.0)	3 (75.0)	2 (100.0)	9 (64.3)
Azithromycin (10µm)	1 (12.5)	0 (0.0)	0 (0.0)	1 (7.1)
Ciprofloxacin (5µm)	2 (25.0)	3 (75.0)	1 (50.0)	6 (42.8)
Ceftriaxone (30µm)	2 (25.0)	2 (50.0)	0 (0.0)	4 (28.6)
Co-trimoxazole (25µm)	8 (100.0)	3 (75.0)	2(100.0)	13 (92.8)
Gentamycin (10µm)	2 (25.0)	0 (0.0)	0 (0.0)	2 (14.3)
Nalidixic acid (30µm)	7 (87.5)	4 (100.0)	2 (100.0)	13 (92.8)

predisposing diarrheal illness among developing countries [22]. *Shigella* spp. was detected in 10.5%, among which, infection rate of *S. flexneri, S. dysenteriae,* and *S. sonnei* was detected in 57.1%, 28.6%, and 14.3%, respectively in gastroenteritis patients. *S. boydii* could not identify, this might be due to small sample size or error in the kit method in diarrheal patients, or might be used by kit method rather than molecular methods. In the previous study, it was reported that *S. flexneri* (43.2%) is the predominant of the four species followed by *S. dysenteriae* (41.5%), *S. boydii* (7.6%) and *S. sonnei* (7.6%) in Nepal [19]. Similarity, the prevalence of *S. flexneri* is identified in 42.0% of isolates, while *S. dysenteriae* in 27.5%, *S. boydii* in 21.7% and *S. sonnei* in 8.7% in Eastern Nepal [18]. In western Nepal, *S. flexneri, S. dysenteriae, S. boydii,* and *S. sonnei* were accounted respectively for 43.1%, 27.7%, 21.5%, and 7.7% of the total number of *Shigella* isolated [14].

Infection rate for *Shigella* spp. was higher (7.3%) in gastroenteritis patients of <15 years old than in patients ≥15 years old (4.5%) which is not statistically significant (p=0.432). This finding is consistent with the study done by Khan et al and Shakya et al found *Shigella* 42.0% and 38.4% and 30.1% respectively [14, 18, 32]. The reported high prevalence of *Shigella* spp. from children aged 1-10 years, compared to the other age groups. They also reported high prevalence of *Shigella* spp. from male patient compared to the female [14, 32].

Shigellosis or severe bacillary dysentery is a disease of public health importance because it is associated with increased mortality and morbidity especially among the children of developing countries [23]. The seasonal tendency of shigellosis was summer-monsoon [24]. Our study was conducted in summer-monsoon (April to September); when school going children may get dysentery and diarrhea due to unsafe water like rainfall, flood and drinking contaminated water. On the other hand the no. of male cases with the pathogen exposer were high may be due to they go out from home more frequently for playing, eating purpose that compares to female.

For antibiotic resistance pattern, eight types of antibiotics were used. Nalidixic acid and co-trimoxazole (92.8%) was found to be the most resistant antibiotics in isolated *Shigella spp*. followed by ampicillin (64.3%) and ciprofloxacin (42.8%). Among isolated *Shigella Spp.*, almost all of the *S. flexneri* is most resistant to co-trimoxazole (100.0%) while *S. dysenteriae* is resistant to nalidixic acid (100.0%). In addition, most of the *S. sonnei* was found 100.0 % resistant in ampicillin, nalidixic acid, and co-trimoxazole. In a study, resistance rate of

Shigella spp. for nalidixic acid was 95.6%, ampicillin 85.5%, co-trimoxazole 82.6%, gentamicin 24.6%, ofloxacin 21.7% etc. [19]. 33% of the total *Shigella* studied was found multi drug resistant (MDR) i.e. they showed resistance to 3 or more antibiotics. However, none of the *Shigella* spp. was resistant to azithromycin and ceftriaxone. Ciprofloxacin resistance was seen only among *Shigella dysenteriae* strains [14]. *Shigella* spp. resistant to nalidixic acid (95.4%); ampicillin (84.6%) co-trimoxazole (81.5%) and ciprofloxacin (46.2%) were detected in Nepal [16]. Over the past decades, it has been reported that a significant number of *Shigella* spp. isolates resistant to normally prescribed drugs [25]. In the early 1990s, many isolates of *Shigella* spp. were susceptible to norfloxacin, nalidixic acid, gentamicin and furazolidone [26, 27]. However, in the late 1990s, most *Shigella* isolates showed an increased resistance to these antibiotics [28, 29] but most were susceptible to ciprofloxacin [30]. Nowadays, alternative drugs such as the third generation cephalosporins are being commonly used. Although, the present study shows that *Shigella* strains are rapidly acquiring resistance to these substituted drugs as well. The emergence of plasmid-borne resistance to cephalosporins is another reason which further reduces the therapeutic option for the treatment of shigellosis [19]. *Shigella* spp. is highly necessary to start a prompt and rational antibiotic regimen to minimize the clinical effects of severe dysentery and its complications [31].

We could not able to perform confirmatory tests like other biochemical tests, molecular level test like PCR, gel electrophoresis due to lack of resources and additional clinical patients issues like clinical complication and informed consents. Moreover, the study was limited to the single hospital so the results do not represent a broad population.

Conclusions

Our findings also revealed that endemicity of Shigellosis with *S. flexneri* is the predominant strain in gastroenteritis patients. To prevent school going children from Shigellosis they should keep away from untreated water. As shown by antibiotic resistant pattern, co-trimoxazole, nalidixic acid, ciprofloxacin,and ampicillin should not use experimentally as first-line drugs for the treatment of shigellosis. Frequent analysis of resistance pattern and periodic reporting of *Shigella* spp. is necessary for Shigellosis therapy. In addition, continuous cultural surveillance of multidrug-resistant test of *Shigella* spp. is necessary to know changing the time-to-time resistant pattern of its serogroup. Isolation and sensitivity testing for *Shigella* spp. should be done regularly. Monitoring of emergence of resistance is highly recommended.

Ethical approval and consent to participant

All *Shigella* strains were routinely collected in the microbiology laboratory. No patient-related data were collected. Ethical approval was therefore not required. The study was laboratory-based basic science study.

Written informed consent was taken from all participating patients or from guardian on the behalf of their children.

Consent for publication

Not applicable

Availability of data and materials

All supplementary files, data generated and analyzed during this study will be made available as per request to co-author.

Source of support:

None

Conflict of interest:

None declared

Author's contributions

KT and AK designed the study. KT collected sample at Western Regional Hospital. KT and AK performed an investigation and recorded the laboratory findings with the validation. JBK supervised and provide methodology for the study. RRG and BB administered the project, reviewed literature, and written original manuscript; curated data to perform statistical

data analysis and data interpretation. RRG helped in review and revision of the draft by compiling, formatting, editing and writing the final version of the article. Thus, all authors made a substantial contribution to the study. All of them read and approved the final manuscript.

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