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Magnitude of drug resistant shigellosis in Nepalese patients

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ABSTRACT

Background and Objectives: *Shigella* plays an important role as a causative organism of acute gastroenteritis, in children and others. Rapid emergence of antibiotic resistance warrants continuous monitoring of susceptibility pattern of bacterial isolates. We report here our findings about *Shigella* spp. isolates and their drug resistance patterns in Nepalese patients.
Materials and Methods: The study was conducted on 507 Nepalese patients with acute gastroenteritis attending outpatient and inpatient departments of Nepalgunj Medical college and teaching Hospital, Banke, Nepal from September 2011 to April 2013. Stool specimens were processed for isolation and identification of *Shigella* species following the standard microbiological methods while the disc diffusion test was used to determine antimicrobial resistance patterns of the recovered isolates at the central Laboratory of Microbiology.
Results: Sixty nine isolates were identified as *Shigella species*. *S. flexneri, S. dysenteriae*, *S. boydii* and *S. sonnei* accounted, respectively, for 42.03%, 27.54%, 21.74% and 8.70% of the total number of *Shigella* isolates. Resistance to nalidixic acid (95.65%), ampicillin (85.51%), co-trimoxazole (82.61%) and ciprofloxacin (47.83%) was observed. Among 69 isolates, 29 (42.03%) were from children aged 1-10 years and this group was statistically significant ($P < 0.05$), compared to the other age groups.
Conclusions: The study revealed endemicity of shigellosis with *S. flexneri* as the predominant serogroup in Nepalese patients. Children were at a higher risk of severe shigellosis. Nalidixic acid, ampicillin, co-trimoxazole and ciprofloxacin should not be used empirically as the first line drugs in treatment of shigellosis. Continuous local monitoring of resistance patterns is necessary for the appropriate selection of empirical antimicrobial therapy.

Keywords: gastroenteritis, *Shigella*, Antimicrobial resistance, Nepal.

INTRODUCTION

Shigellosis still remains a public-health problem in most developing countries where communities are ravaged by poverty, war, poor sanitation, personal hygiene, and water supplies (1). Epidemiological reports show that about 140 million people suffer from shigellosis with estimated 600,000 deaths per year worldwide (2, 3). They are four serogroups, Serogroup *A*: *S. dysenteriae* (12 serotypes), Serogroup *B*: *S. flexneri* (6 serotypes), Serogroup *C*: *S. boydii* (18 serotypes), Serogroup *D*: *S. sonnei* (1 serotype)(4). *Shigella spp*. is a major cause of dysentery/diarrhea in children and others. Many of them are hospitalized immediately after the onset of the disease. Though, oral rehydration is the principal tool of management, but, because of the enteroinvasiveness antibacterial treatment may be necessary (5). The emergence of antimicrobial resistance within members of the *Enterobacteriaceae* family is posing serious problems in the treatment of outbreaks of infections. Since its first report in studies

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conducted in the 1950s, multiple-drug resistance transmitted by plasmids among *Shigella* species has been reported from many countries (6-8). Moreover, an increase in resistance against many different drugs has been observed in the last two decades. In India, over 70% of *Shigella* isolates were resistant to two or more drugs including ampicillin and co-trimoxazole during 2002 to 2007 (9). Reports from Indonesia (10), Bangladesh (11), Malaysia (12), and Nepal (13) show increasing prevalence of *Shigella* isolates with multiple resistance to ampicillin, trimethoprim-sulphamethoxazole, tetracycline, and nalidixic acid. Similar resistance profiles have been also reported from Africa (14), Central America (15), Europe (16), and South America (17). Besides the temporal changes in the susceptibility patterns of *Shigella* species, it is well known that they may differ between geographical areas. Such differences are never stable and may change rapidly, especially in places where antibiotics are excessively used (particularly in developing countries) (18). This warrants for continuous monitoring of antibiotic susceptibility of this organism. This study was carried out to determine the antimicrobial resistance patterns of *Shigella* species in Nepalese patients.

MATERIALS AND METHODS

Study background and subjects. This was a prospective study conducted on 507 Nepalese patients with acute gastroenteritis, attending outpatients and inpatients departments of Nepalgunj Medical College and teaching Hospital, Banke, Nepal, between September 2011 and April 2013.

Sample collection and processing. Stool specimens were collected and processed following the standard microbiological methods (19) at the central Laboratory of Microbiology of Nepalgunj medical college and teaching hospital, Banke, Nepal. The specimens were inoculated on plates of Hektoen Enteric Agar, Salmonella-Shigella agar and deoxycholate citrate agar (Himedia Lab, Pvt Ltd, India). The plates were incubated at 37°C for 24 hours. The *Shigella* spp. isolates were speciated biochemically as outlined by Cowan (20) and confirmed by the slide agglutination test using polyvalent and monovalent antisera (Denka Seiken, Japan).

Antibiotic susceptibility testing. Antimicrobial sensitivity testing was determined by the Kirby-Bauer disc diffusion method (21) on Mueller Hinton agar (Himedia Lab, Pvt Ltd.) using the following antimicrobial agents: ampicillin (10 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), cotrimoxazole (25 µg), gentamicin (10 µg), imipenem (10 µg), nalidixic acid (30 µg) and ofloxacin (5 µg) (Himedia Lab, Pvt Ltd.). The plates were incubated at 37°C for 24 h, and the diameters of zone of inhibition were compared with those of the reference isolate (*Escherichia coli* ATCC 25922). Ethical approval for the study was taken from institutional research ethical committee.

Statistical analysis. Data obtained were analyzed using the SPSS (v. 18) Chicago, USA. Association of gender and age-groups with prevalence of *Shigella* spp. was assessed using chi-square test. P values <0.05 were considered to be statistically significant.

RESULTS

A total of 507 diarrheal/dysenteric stool samples were screened, *Shigella* spp. was identified in 69 (13.61%) samples. The prevalence of *S. flexneri* was identified in 29 isolates (42.03%), while *S. dysenteriae* in 19 (27.54%), *S. boydii* in 15 (21.74%) and *S. sonnei* in 6 (8.70%) of the total number of isolates (Table 1). *S. flexneri* has been the predominant isolate during the period of the study. Among 69 positive sample, 38 (55.07%) were male (Table 2). *Shigella* spp. were isolated from patients aged between 1 and > 60 years. A high prevalence (42.03%) was identified in subjects aged 1-10 years and this age group was statistically significant ($P < 0.05$) compared to the other age groups. However, there was no significant difference in the overall prevalence of isolates according to sex.

The resistance pattern of *Shigella* spp. isolated between September 2011 to April 2013 is as shown in Table 3. Over 80% of *Shigella* isolates were resistant to 2 or more drugs including ampicillin, nalidixic acid and co-trimoxazole. All the isolates recovered from the age group 51 - 60 and > 60 were resistant to these drugs. The maximum resistant isolates were observed in 1 -10 age group. Resistance rate to nalidixic acid was 95.64%, ampicillin 85.51%, co-trimoxazole 82.61%, ciprofloxacin 47.83%, gentamicin 24.64%, ceftriaxone 24.64%, ofloxacin 21.74%, ceftazidime 18.84%, cefotaxime 15.94%. No resistance was observed to imipenem during the study period.

Table 1. Distribution of *Shigella spp*. recovered from different age groups.

Shigella species	Age groups (years) distribution							
	0-10 No (%)	**11-20 No (%)**	**21-30 No (%)**	**31-40 No (%)**	**41-50 No (%)**	**51-60 No (%)**	**> 60 No (%)**	**Total No (%)**
Shigella flexneri	14	5	6	2	1	-	1	29 (42.03)
Shigella dysenteriae	9	4	5	-	1	-	-	19 (27.54)
Shigella boydii	5	2	4	3	-	-	1	15 (21.74)
Shigella sonnei	1	1	1	1	1	1	-	6 (8.70)
Total No (%)	29 (42.03)	12 (17.39)	16 (23.19)	6 (8.70)	3 (4.35)	1 (1.45)	2 (2.90)	69 (13.61)

Considering resistance patterns in the different serogroups, *S. boydii* showed 100% resistance to nalidixic acid and co-trimoxazole; *S. sonnei* was 100% resistant to ampicillin, 83.33% to co-trimoxazole and nalidixic acid; *S. flexneri* showed 96.55% resistance to nalidixic acid and ampicillin and *S. dysenteriae* showed most frequently resistance to nalidixic acid (94.74%) and co-trimoxazole (84.21%).

DISCUSSION

Shigellosis still accounts for a significant proportion of morbidity and mortality, especially in developing countries (22). The majority of the *Shigella* strain were isolated from children aged 1-10 years, according with previous studies (9, 22, 23). The changing patterns in the distribution of *Shigella* serogroups and serotypes have been reported from time to time (24-26). The shift in the prevalence of serogroups and the changing patterns in antimicrobial susceptibilities among *Shigella* isolates pose a major difficulty in the determination of an appropriate drug for the treatment of shigellosis (24, 25). In the present study, *S. flexneri* has been the predominant serogroup among *Shigella* species in Nepalese patients, as recent studies have showed in different countries (22) and in western Nepal (13). Different epidemiological patterns have been reported by other studies (23, 28, 29). This could be attributed to geographic, socio-demographic, climatic and environmental differences.

Table 2. Distribution of *Shigella spp*. by gender.

***Shigella* species**	**Gender**		
	Male	**Female**	**Total No (%)**
Shigella flexneri	16	13	29(42.03)
Shigella dysenteriae	10	9	19(27.54)
Shigella boydii	8	7	15(21.74)
Shigella sonnei	4	2	6(8.70)
Total No (%)	38 (55.07)	31 (44.93)	69 (100%)

Over the past decades, a significant number of *Shigella* isolates resistant to commonly-prescribed antimicrobials have been reported (30). In early 1990s, many isolates were susceptible to nalidixic acid, norfloxacin, furazolidone, and gentamicin (25, 29). In the late 1990s, most isolates, showed an increased resistance to these antimicrobials (24, 31) but most were susceptible to ciprofloxacin (13, 32, 33). In the present study, an overall high rate of resistance was observed to ampicillin, nalidixic acid and co-trimoxazole. Of interest, all the isolates recovered from the age group 51-60 and >60 were resistant to these drugs. The maximum resistant isolates were observed in 1-10 age group, which was more or less similar to some studies conducted in India, Iran, Ethiopia and Nigeria (9,27,34-36). In addition, these isolates, resistant to ciprofloxacin, showed a trend towards an increased incidence of resistance, especially in *S. dysenteriae* and *S. flexneri* during the study period. Although fluoroquinolones are recommended as the drugs of choice for shigellosis by World Health Organization (37), emergence of fluoroquinolone resistance among *Shigella* spp. has now been documented in many countries (38-40). At present, alternative drugs such as the third generation cephalosporins are being commonly used. However, the present study shows that *Shigella* strains are rapidly acquiring resistance to these drugs as well. The emergence of plasmid borne resistance to cephalosporins further reduces the therapeutic option for the treatment of shigellosis. The genetic transfer of drug resistance genes may not be of immediate concern for the treating clinicians, but will pose a potential problem in the future. Their presence, along with the potential for plasmid mediated quinolone resistance, will be surely create significant therapeutic problems in the future. Widespread selective pressure

Table 3. Resistance of the *Shigella spp*. isolates to a panel of ten antibiotics.

Antimicrobial agent (Concentration)	Bacterial species				Total N = 69
	Shigella flexneri No (%)	*Shigella dysenteriae* No (%)	*Shigella boydii* No (%)	*Shigella sonnei* No (%)	No (%)
Ampicillin	28 (96.55)	14 (73.68)	11 (73.33)	6 (100)	59 (85.51)
Cefotaxime	5 (17.24)	-	4 (26.67)	2 (33.33)	11 (15.94)
Ceftazidime	13 (44.83)	-	-	-	13 (18.84)
Ceftriaxone	10 (34.48)	7 (36.84)	-	-	17 (24.64)
Ciprofloxacin	18 (62.07)	13 (68.42)	-	2 (33.33)	33 (47.83)
Co-trimoxazole	21 (72.41)	16 (84.21)	15 (100)	5 (83.33)	57 (82.61)
Imipenem	-	-	-	-	-
Nalidixic acid	28 (96.55)	18 (94.74)	15 (100)	5 (83.33)	66 (95.65)
Gentamicin	5 (17.24)	7 (36.84)	5 (33.33)	-	17 (24.64)
Ofloxacin	11 (37.93)	4 (21.05)	-	-	15 (21.74)

and efficient dissemination routes for multi drug resistant organisms are major factors contributing to the rapid emergence and spread of drug resistant organisms.

CONCLUSION

The emergence of resistance to many drugs, such as flouroquinolones and third generation cephalosoprins, in *Shigella* is a cause of great concern not only at local and regional level, but also in a national and international scale. The culture of antimicrobial abuse needs to be soon stopped. Continuous surveillance of multidrug resistant strains is very important to know the changing antibiotic susceptibility patterns as well as the cyclical changes of the serogroups from time to time because the resistance patterns are related to the serogroup. A network of laboratories for real time monitoring of antibiotic resistance of *Shigella* and timely dissemination of such information to the clinicians for modification of treatment strategy are urgently necessary.

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