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Prevalence of *Shigella* species and Their Antimicrobial Resistance Patterns in Eastern Nepal

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ABSTRACT

The study was conducted to determine the prevalence of *Shigella* species and their antimicrobial resistance patterns in eastern Nepal. Stool samples submitted to the diagnostic laboratory of B.P. Koirala Institute of Health Sciences, Nepal, during August 2000−July 2004, were cultured for *Shigella* species and were confirmed by biochemical and serological tests. Of 53 *Shigella* species isolated, *Shigella dysenteriae* type 1 was the most predominant isolate (73.7%), followed by *S. flexneri* (23%) and *S. boydii* (4%). The majority (79%) of *Shigella* species were isolated from children aged less than five years. An overall high resistance was observed for trimethoprim-sulphamethoxazole, ampicillin, nalidixic acid, mecillinam, and ciprofloxacin. There was a statistically significant ($p<0.001$) increasing trend in the prevalence of ciprofloxacin resistance in *S. dysenteriae* type 1. The results suggest reconsideration of the empiric use of these antimicrobial agents for shigellosis. A further study is required to evaluate additional antimicrobial agents.

Key words**:** *Shigella*; Dysentery, Bacillary; Drug resistance, Microbial; Drug therapy; Nepal

INTRODUCTION

Shigellosis, an acute diarrhoeal disease, remains a major public-health problem in developing countries. Of total *Shigella* episodes throughout the world annually, the majority of them occur in developing countries (1). The antimicrobial resistance patterns of *Shigella* species vary according to geographic region (2) and in the same place over time, leading to a therapeutic problem (3).

Antimicrobial therapy is recommended for shigellosis (4). However, *Shigella* species has developed antimicrobial resistance since 1940, when resistance of *Shigella* species to sulfonamide was first recognized in Japan (5). Over the past decades, *Shigella* species have become progressively resistant to most widely-used antimicrobials (2,6). The increasing levels of antimicrobial resistance of *Shigella* isolates have complicated the treatment of shigellosis.

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The emergence of resistance to ampicillin and trimethoprim-sulphamethoxazole in 1980 led to the use of nalidixic acid as a first-line drug for *Shigella* species. However, an increasing number of *Shigella* isolates are showing resistance to nalidixic acid and other quinolones leading to a therapeutic problem (2,3). The purpose of the present study was to determine the prevalence of *Shigella* species and their antimicrobial resistance patterns in eastern Nepal for the better management of shigellosis.

MATERIALS AND METHODS

Sample collection

Thirteen hundred ninety-six routinely-collected clinical samples of stools from patients with diarrhoea and dysentery were submitted to the diagnostic laboratory at the Department of Microbiology of B.P. Koirala Institute of Health Sciences, Dharan, Nepal. The Institute, a 700-

bed tertiary-care teaching hospital, provides low-cost medical care to the regional population of eastern Nepal and the population of the border area of the neighbouring country, India. The patients suffering from diarrhoea and dysentery were primarily from the rural area, and the majority of them were economically poor. Stool culture was done at a subsidized rate and free of charge for poor patients on the recommendation of either the treating physician or their concerned village development committee. These samples were referred from the inpatient, outpatient, and emergency departments of the abovementioned teaching hospital for stool culture. The study was conducted during August 2000–July 2004. For most study period, until 2003, the identification of *Shigella* species and their antimicrobial susceptibility monitoring were under the supervision of the Quality Assurance Programme in collaboration with ICDDR,B: Centre for Health and Population Research, Dhaka, Bangladesh, and Antimicrobial Resistance Surveillance of Nepal.

Bacteriological procedure

The stool samples received from patients with diarrhoea and dysentery were inoculated directly on MacConkey agar and deoxycholate citrate agar for the isolation of enteric pathogens. Enrichment was done in selenite F broth and incubated at 37 ^{O}C overnight. After enrichment, sub-culture was done in the above media and further incubated at 37 ^{O}C overnight.

Colony morphology resembling *Shigella* species was further identified by biochemical reaction and confirmed by the slide agglutination test using polyvalent and monovalent antisera (Murex Diagnostics Limited, England).

Drug-susceptibility test

Antibiotic susceptibility patterns were determined by the Kirby-Bauer disc-diffusion method performed on Muller-Hinton agar plates following the guidelines of the National Committee for Clinical Laboratory Standards (7). Commercially-manufactured disc (Oxoid, Basingstoke, Hampshire, England) of antimicrobial agents and their concentration in μg/mL were as follows: ampicillin–10, nalidixic acid–30, ciprofloxacin–5, mecillinam–25, and trimethoprim-sulphamethoxazole–25. A control strain of *Escherichia coli* (ATCC-225922) was included in each test.

Statistics

The SPSS (version 10.0) was used for the analysis of data. The chi-square test was applied to calculate trends for the most prevalent species.

RESULTS

Of the 1,396 routine stool specimens, 53 (4%) yielded *Shigella* species. Forty-two (79%) *Shigella* species were isolated from children aged less than five years (Table 1). *S. dysenteriae* type 1 was the most prevalent isolate (n=39, 73.6%), followed by *S. flexneri* (n=12, 23%) and *S. boydii* (n=2, 4%).

Table 1. Distribution of *Shigella* isolates (n=53) by age-group, eastern Nepal, 2000-2004

Age-group (years)	*Shigella* species	
	No.	%
≤4	42	79.2
5-10	3	5.7
11-25	4	7.5
>25	4	7.5

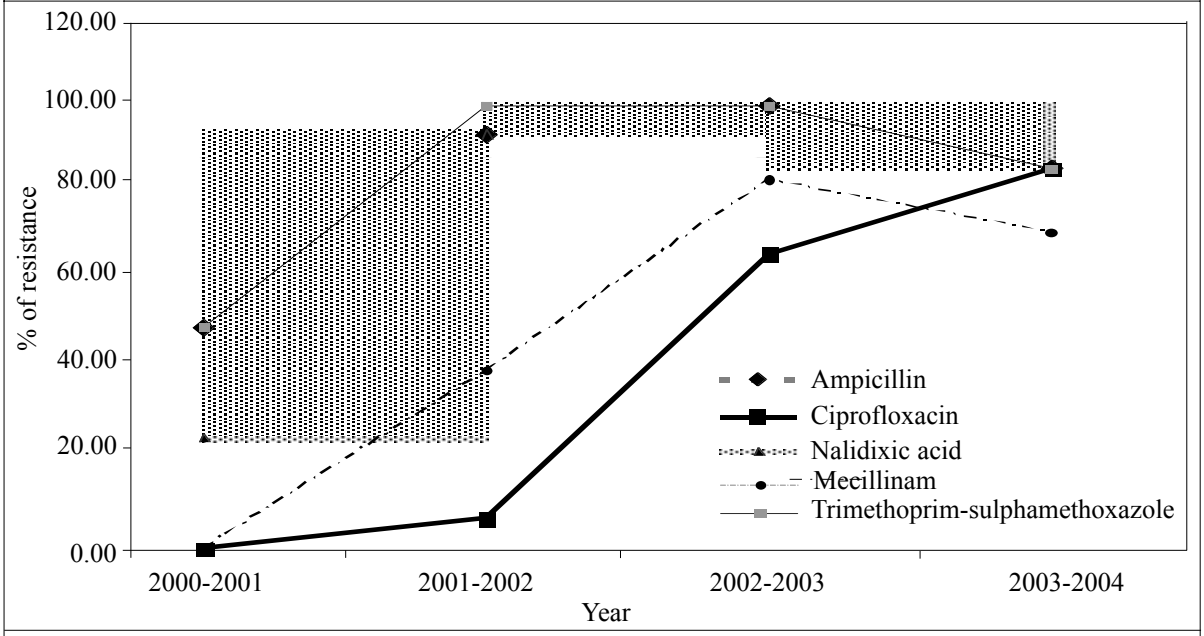
Among *Shigella* species, an overall resistance of 88.7%, 86.8%, 69.8%, 49.0%, and 28.3% was observed for trimethoprim-sulphamethoxazole, ampicillin, nalidixic acid, mecillinam, and ciprofloxacin respectively. The overall susceptibility patterns among the *Shigella* isolates are shown in Table 2. The general trends of antimicrobial resistance in *S. dysenteriae* type 1, the most common isolate, are shown in the figure. There was a statistically significant ($p<0.001$) increasing trend in the prevalence of ciprofloxacin resistance among *S. dysente riae* type 1 from 2000 to 2004 (Fig.).

Table 2. Overall distribution of drug-susceptibility patterns among *Shigella* species, eastern Nepal, 2000-2004

Antimicrobial agent	*Shigella dysenteriae* (n=39)			*Shigella flexneri* (n=12)			*Shigella boydii* (n=2)		
	Resistant	Intermediate	Sensitive	Resistant	Intermediate	Sensitive	Resistant	Intermediate	Sensitive
Ampicilin	32 (82.1)	2 (5.1)	5 (12.8)	12 (100.0)	0 (0)	0(0)	2 (100.0)	0 (0)	0 (0)
Nalidixic acid	33 (84.6)	0 (0)	6 (15.4)	4 (33.3)	0 (0)	8 (66.7)	0 (0)	0 (0)	2 (100.0)
Ciprofloxacin	13 (33.3)	4 (10.3)	22 (56.4)	2 (16.7)	0 (0)	10 (83.3)	0 (0)	0 (0)	2 (100.0)
Mecillinam	21 (54)	0 (0)	18 (46.2)	4 (33.3)	0 (0)	8 (66.7)	1 (50.0)	0 (0)	1 (50.0)
Trimethoprim-sulphamethoxazole	35 (89.7)	0 (0)	4 (10.3)	11 (91.7)	0 (0)	1 (8.3)	1 (50.0)	0 (0)	1 (50.0)

Figures in parentheses indicate percentages

Fig. Antibiotic resistance patterns of *Shigella dysenteriae* type 1, eastern Nepal, 2000-2004



Moderately-susceptible *S. dysenteriae* type 1 (n=4 for ciprofloxacin and n=2 for ampicillin) were considered resistant to plot this figure

DISCUSSION

Shigellosis still accounts for a significant proportion of morbidity and mortality, especially in developing countries (1). In the study, the majority of the *Shigella* species were isolated from children aged less than five years, which is similar to other studies (1,8). The changing patterns in the distribution of *Shigella* serogroups and serotypes have been reported from time to time (3,8-10).

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 (3,9).

In the present study, *S. dysenteriae* type 1 was the most prevalent strain among *Shigella* species, which was similar to some earlier studies (11,12) but dissimilar to more recent studies (1,9,10,13). This could be attributed to geographic variation and to changing patterns of serogroup, and serotypes of *Shigella* species from time to time. Although our sample size may not reflect the genuine spectrum of *Shigella* species in this country, this survey is still significant, because, to our knowledge, this study is the first to define the prevalence of *Shigella* species and their antimicrobial resistance patterns in eastern Nepal.

Over the past decades, a significant number of *Shigella* isolates, resistant to commonly-prescribed antimicrobials, have been reported (6). In early 1990s, many isolates were susceptible to nalidixic acid, norfloxacin, furazolidone, and gentamicin (8,9,11,12). In the late 1990s, most isolates, especially *S. dysenteriae* type 1, showed an increased resistance to these antimicrobials (2,3), but most were susceptible to ciprofloxacin (13,14).

In the present study, the overall increased resistance was observed for trimethoprim-sulphamethoxazole, ampicillin, nalidixic acid, and mecillinam in *Shigella* species, which was more or less similar to some studies conducted during the late 1990s. In addition, these isolates, resistant to ciprofloxacin, showed a trend towards an increased incidence of resistance, especially in *S. dysenteriae* type 1, during the study period. The high level of ciprofloxacin resistance observed in our study was similar to recently-published reports from India (15,16) and Bangladesh (17,18), having a potential for a large-scale epidemic. In the present context, it is worth noting the prediction of these studies about the likely spread of this new clone of *S. dysenteriae* type 1 in a wider geographic area (15-18).

In conclusion, the present study demonstrates that *S. dysenteriae* type 1 is the predominant species. There is a significant increase in resistance to several commonly-used antimicrobial agents. The rapid increase in ciprofloxacin resistance, especially in *S. dysenteriae* type 1, is a major cause of concern. The results suggest reconsideration of the empiric use of these antimicrobial

agents for the treatment of shigellosis. A further study is required with additional drugs, such as tetracycline, norfloxacin, ofloxacin, ceftriaxone, and azithromycin. These drugs may help formulate the empirical therapy of shigellosis. Our findings stress the need for distributing reliable information about antimicrobial resistance patterns and for ongoing drug resistance surveillance.

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