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# Isolation of *Shigella* species and their resistance patterns to a panel of fifteen antibiotics in mid and far western region of Nepal

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## PEER REVIEW

## Peer reviewer

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## Comments

The work is very good in which the authors evaluated the distribution of *Shigella* species and their antibiotic resistance pattern that were found to be different between isolates. Overall the work done can add additive support to treat the shigellosis.  
Details on Page 33

## ABSTRACT

**Objective:** To determine the antimicrobial resistance patterns of *Shigella* species to the most commonly used antibiotics in mid and far western part of Nepal.

**Methods:** Stool samples were collected from 458 patients who came from mid and far western region of Nepal, attending OPD & IPD Departments of Nepalgunj Medical College, Nepal, between the periods of September 2011 to March 2013. Standard microbiological procedures were used for isolation and identification of *Shigella* species while the disc diffusion test was used to determine the antimicrobial resistance patterns of the recovered isolates.

**Results:** A total of 65 isolates were identified as *Shigella* species. *Shigella flexneri*, *Shigella dysenteriae*, *Shigella boydii* and *Shigella sonnei* were accounted respectively for 43.07%, 27.69%, 21.53% and 7.69% of the total number of *Shigella* isolated. Resistances to nalidixic acid (95.38%), ampicillin (84.62%), co-trimoxazole (81.54%) and ciprofloxacin (46.15%) were observed. Greater number of isolates (38.46%) was recovered from those aged 1–10 years. This was statistically significant ( $P < 0.05$ ), compared to the other age groups.

**Conclusions:** The study revealed the endemicity of shigellosis with *Shigella flexneri* as the predominant serogroup. Children were at a higher risk of severe shigellosis. The results also suggest that nalidixic acid, ampicillin, co-trimoxazole and ciprofloxacin should not be used empirically as the first line drugs in the treatment of shigellosis. Periodic analysis of resistance patterns is necessary for the appropriate selection of empirical antimicrobial therapy.

## KEYWORDS

*Shigella*, Dysentery/diarrhea, Antimicrobial resistance, Mid & far western region of Nepal

## 1. Introduction

Shigellosis remains a public-health problem in most developing countries where communities are ravaged by poverty, war, poor sanitation, personal hygiene, and water supplies[1]. Epidemiologic reports show that about 140 million people suffer from shigellosis with estimated 600,000 deaths per year worldwide[2,3]. There are four major

O antigen groups, viz. group A as *Shigella dysenteriae* (*S. dysenteriae*), group B as *Shigella flexneri* (*S. flexneri*), group C as *Shigella boydii* (*S. boydii*) and *Shigella sonnei*[4]. It 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 means of management, as the enteroinvasiveness antibacterial treatment may be necessary[5]. The

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emergence of antimicrobial resistance to members of the Enterobacteriaceae family is posing serious problems in the treatment of outbreaks of infections. Since its first report in studies 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 one study, a significant decrease was observed in the susceptibility of the species to ampicillin and cotrimoxazole from 1988–89 to 1991–92[9]. In another report, it was shown that co-trimoxazole resistance of *Shigella* increased from 3% to 40% within ten years[10]. Another study showed that the percentage of resistant *Shigella* strains in Madrid (Spain) increased from 39.6% to 97.9% for ampicillin, from 34.4% to 96.9% for co-trimoxazole, from 6.3% to 18.0% for tetracycline, and from 1.6% to 15.1% for chloramphenicol[11]. In Ethiopia, strains of *Shigella* that were resistant to many commonly used drugs have been reported in different parts of the country by several studies[12–14]. Belay *et al.* have reported a strain that was resistant to eight drugs out of the nine antimicrobials they used[14]. In India, Over 70% of *Shigella* isolates were resistant to two or more drugs including ampicillin and co-trimoxazole during 2002 to 2007[15]. Reports from Indonesia, Bangladesh, Malaysia, and Nepal showed increasing frequency of *Shigella* with multiple resistance to ampicillin, trimethoprim-sulphamethoxazole, tetracycline, and nalidixic acid[16–19]. Similar resistance profiles were reported from Africa[20], Central America[21], Europe[22–24], and South America[25,26]. Besides the temporal changes in the antibiogram of *Shigella* species, it is well known that antibiotic susceptibility patterns in *Shigella* may differ between geographical areas. Such differences are never stable and may change rapidly, especially in places where antibiotics are used excessively (particularly in developing countries)[27]. This warrants for frequent observation on the change in the pattern of antibiogram for this organism. To our best knowledge, no report exists regarding the antibiotic resistance pattern of *Shigella* species in mid and far western region of Nepal. This study was thus carried out to determine the antimicrobial resistance patterns of *Shigella* species to the most commonly used antibiotics in mid and far western part of Nepal.

## 2. Materials and methods

### 2.1. Study background and subjects

This was a prospective study conducted on 458 patients of diarrhea/dysentery who come from mid and far western region of Nepal, attending out-patients and in-patients of the Departments of Nepalgunj Medical college and teaching Hospital, Banke, Nepal, between the periods of September 2011 to March 2013.

### 2.2. Sample collection and processing

Stool specimens were collected and processed following the standard microbiological methods at the central Laboratory of Microbiology[28]. The specimens were inoculated on plates of Hektoen enteric agar, *Salmonella-Shigella* agar and deoxycholate citrate agar (Himedia Lab.

Pvt Ltd.). The plates were incubated at 37 °C for 24 h. The *Shigella* isolates were speciated biochemically as outlined by Cowan and confirmed by the slide agglutination test using polyvalent and monovalent antisera (Denka Seiken, Japan) [29].

### 2.3. Antibiotic susceptibility testing

Antimicrobial sensitivity testing was determined by the Kirby–Bauer disc diffusion method on Mueller Hinton agar using the antimicrobial agents including amikacin, ampicillin, amoxycylav, cefotaxime, ceftazidime, ceftriaxone, chloramphenicol, ciprofloxacin, cotrimoxazole, doxycycline, gentamicin, imipenem, nalidixic acid, norfloxacin, and ofloxacin[30]. The plates were incubated at 37 °C for 24 h, and the diameters of zone of inhibition were compared with recorded diameters of the reference isolate (*Escherichia coli* ATCC 25922) in order to determine susceptibility or resistance.

### 2.4. Statistical analysis

Data obtained were analyzed using the SPSS software for windows version 18. Comparison of data in respect of *Shigella*, sex, and age-groups were performed by *Chi-square*.  $P < 0.05$  was considered to be statistically significant.

## 3. Results

Of the 458 diarrheal/dysenteric stool samples screened, *Shigella* strains were identified in 65 (14.19%) samples, 36 (55.38%) in male and 29 (44.62%) in female (Table 1). *Shigella* spp. were isolated from patients with ages ranging between 1 to >60 years. Of these, 38.46% ( $n=25$ ) were from children from 1–10 years age groups, which was statistically significant ( $P < 0.05$ ) compared to the other age groups. However, there was no significant difference in the overall number of isolates recovered in the study based on sex ( $P > 0.05$ ). *S. flexneri* strains were identified in 28 (43.07%) *Shigella* positive cultures, while *S. dysenteriae* accounted for 18 (27.69%), *S. boydii* in 14 (21.53%) and *S. sonnei* in 5 (7.69%) of the total number of isolates (Table 2). *S. flexneri* has been the predominant isolate during the period of the study.

**Table 1**

Sex distribution of all positive cases in different species of *Shigella*.

| <i>Shigella</i> species | Sex distribution of positive cases |            |              |
|-------------------------|------------------------------------|------------|--------------|
|                         | Male                               | Female     | Total No (%) |
| <i>S. flexneri</i>      | 16                                 | 12         | 28 (43.07)   |
| <i>S. dysenteriae</i>   | 9                                  | 9          | 18 (27.69)   |
| <i>S. boydii</i>        | 8                                  | 6          | 14 (21.53)   |
| <i>S. sonnei</i>        | 3                                  | 2          | 5 (7.69)     |
| Total No. (%)           | 36 (55.38)                         | 29 (44.62) | 65 (100)     |

The resistance pattern of *Shigella* spp. isolated between September 2011 to March 2013 is shown in Table 3. Over 80% of *Shigella* isolates were resistant to two or more drugs including ampicillin, nalidixic acid and co-trimoxazole. Resistance rate of nalidixic acid was 95.38%, ampicillin 84.62%, co-trimoxazole 81.54%, ciprofloxacin 46.15%, gentamicin, 24.62%, norfloxacin 24.62%, ceftriaxone 24.62%, ofloxacin 21.54%, ceftazidime 20%, amikacin 16.92%,

**Table 2**Distribution of *Shigella* species recovered from different age groups.

| <i>Shigella</i> species | Age groups distribution |               |               |               |               |               |             | Total No. (%) |
|-------------------------|-------------------------|---------------|---------------|---------------|---------------|---------------|-------------|---------------|
|                         | 1–10 No. (%)            | 11–20 No. (%) | 21–30 No. (%) | 31–40 No. (%) | 41–50 No. (%) | 51–60 No. (%) | >60 No. (%) |               |
| <i>S. flexneri</i>      | 13                      | 5             | 6             | 2             | 1             | –             | 1           | 28 (43.07)    |
| <i>S. dysenteriae</i>   | 8                       | 4             | 5             | –             | 1             | –             | –           | 18 (27.69)    |
| <i>S. boydii</i>        | 4                       | 2             | 4             | 3             | –             | –             | 1           | 14 (21.53)    |
| <i>S. sonnei</i>        | –                       | 1             | 1             | 1             | 1             | 1             | –           | 5 (7.69)      |
| Total No. (%)           | 25 (38.46)              | 12 (18.46)    | 16 (24.61)    | 6 (9.23)      | 3 (4.61)      | 1 (1.53)      | 2 (3.07)    | 65 (14.19)    |

**Table 3**

The resistance patterns of the recovered bacteria isolates from diarrhoeal/dysenteric patients in mid and far western region of Nepal expressed in percentage to a panel of fifteen antibiotics.

| Concentration of antimicrobial agent (mcg) | <i>Shigella</i> species    |                               |                          |                          | Total % (No.) (n=65) |
|--|----------------------------|-------------------------------|--------------------------|--------------------------|----------------------|
|  | <i>S. flexneri</i> % (No.) | <i>S. dysenteriae</i> % (No.) | <i>S. boydii</i> % (No.) | <i>S. sonnei</i> % (No.) |                      |
| Amikacin (30)                              | –                          | 16.66 (3)                     | 28.57 (4)                | 80 (4)                   | 16.92 (11)           |
| Ampicillin (10)                            | 92.85 (26)                 | 72.22 (13)                    | 78.57 (11)               | 100 (5)                  | 84.62 (55)           |
| Amoxycylav (30)                            | 14.29 (4)                  | –                             | 35.71 (5)                | –                        | 13.85 (9)            |
| Cefotaxime (30)                            | 14.29 (4)                  | –                             | 28.57 (4)                | 40 (2)                   | 15.38 (10)           |
| Ceftazidime (30)                           | 46.43 (13)                 | –                             | –                        | –                        | 20 (13)              |
| Ceftriaxone (30)                           | 35.71 (10)                 | 33.33 (6)                     | –                        | –                        | 24.62 (16)           |
| Chloramphenicol (30)                       | –                          | 16.66 (3)                     | 35.71 (5)                | 40 (2)                   | 15.38 (10)           |
| Ciprofloxacin (5)                          | 60.71 (17)                 | 66.66 (12)                    | –                        | 20 (1)                   | 46.15 (30)           |
| Co–trimoxazole (25)                        | 67.86 (19)                 | 88.89 (16)                    | 100 (14)                 | 80 (4)                   | 81.54 (53)           |
| Doxycycline (30)                           | 7.14 (2)                   | –                             | 57.14 (8)                | –                        | 15.38 (10)           |
| Gentamicin (10)                            | 14.29 (4)                  | 38.89 (7)                     | 35.71 (5)                | –                        | 24.62 (16)           |
| Imipenem (10)                              | –                          | –                             | –                        | –                        | –                    |
| Nalidixic acid (30)                        | 96.43 (27)                 | 94.44 (17)                    | 100 (14)                 | 80 (4)                   | 95.38 (62)           |
| Norfloxacin (10)                           | 14.29 (4)                  | –                             | 57.14 (8)                | 80 (4)                   | 24.62 (16)           |
| Ofloxacin (5)                              | 39.29 (11)                 | 16.66 (3)                     | –                        | –                        | 21.54 (14)           |

cefotaxime 15.38%, chloramphenicol 15.38%, doxycycline 15.38%, and amoxycylav 13.85%. No resistance was observed to imipenem during the study period. Considering resistance patterns in different serogroups, *S. boydii* showed 100% resistance to nalidixic acid and co–trimoxazole. *S. sonnei* were 100% resistance to ampicillin, 80% to amikacin, co–trimoxazole, norfloxacin and nalidixic acid. *S. flexneri* showed the highest resistance to nalidixic acid (96.43%) and ampicillin (92.85%) and *S. dysenteriae* showed the highest resistance to nalidixic acid (94.44%), and co–trimoxazole (88.89%).

#### 4. Discussion

Shigellosis still accounts for a significant proportion of morbidity and mortality, especially in developing countries[31]. In this study, the majority of the *Shigella* species were isolated from children aged 1–10 years, which is similar to other studies[15,31–33]. The changing patterns in the distribution of *Shigella* serogroups and serotypes have been reported from time to time[33–36]. 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[34,35]. In the present study, *S. flexneri* has been the predominant isolates among *Shigella* species, which was more similar to recent studies[15,37], but dissimilar to other studies[32,38–40]. This could be attributed to geographic variation and to

changing patterns of serogroup, and serotypes of *Shigella* species from time to time. To the best of our knowledge, this study is the first to define the isolation of *Shigella* species and their resistance patterns to a panel of 15 antibiotics in mid and far western region of Nepal. Over the past decades, a significant number of *Shigella* isolates resistant to commonly–prescribed antimicrobials have been reported[41]. In early 1990s, many isolates were susceptible to nalidixic acid, norfloxacin, furazolidone, and gentamicin[33,35,39,40]. In the late 1990s, most isolates showed an increased resistance to these antimicrobials[42,34], but most were susceptible to ciprofloxacin[19,43,44]. In the present study, the overall increased resistance was observed for ampicillin, nalidixic acid and co–trimoxazole in *Shigella* species, which was more or less similar to some recently studies conducted in India, Iran, Ethiopia, and Nigeria[15,27,37,45,46]. 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[47], emergence of fluoroquinolone resistance among *Shigella* spp. has been documented in many countries[48–50]. At present, alternate drugs like the third generation cephalosporins are being used commonly. The present study shows that *Shigella* strains are rapidly acquiring resistance (ceftriaxone 24.62%, ceftazidime 20%, and cefotaxime 15.38%) to these drugs as well. The emergence of plasmid borne resistance to these cephalosporins further reduces the choice of drugs 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, plus the potential for plasmid and mediated quinolone resistance, will be sure to create significant therapeutic problems in the future. Widespread selective pressure and efficient dissemination channels for multi and drug resistant organisms are major factors that might have contributed to the rapid emergence and spread of resistant organisms.

The emergence of resistance to several new drugs such as flouroquinolones and the third generation cephalosporins in *Shigellae* is a cause of great concern not only at local level but also at regional level. The culture of antimicrobial abuse needs to be stopped and continuous surveillance of multidrug resistant strains is very important to know the changing antibiotic susceptibility pattern as well as the cyclical change of the serogroup from time to time as the resistance pattern also changes with the change in the serogroup. Analysis and periodic reporting is important in proper therapy of shigellosis.

### Conflict of interest statement

We declare that we have no conflict of interest.

### Comments

#### Background

Shigellosis is known to be an important cause of acute invasive diarrhea in children and others. Rapid emergence of antibiotic resistance warrants continuous monitoring of sensitivity pattern of bacterial isolates. This study reported the findings of *Shigella* species and their resistance patterns in mid and far western part of Nepal.

#### Research frontiers

The emergence of resistance to several new drugs such as flouroquinolones and the third generation cephalosporins in *Shigellae* is a cause of great concern not only at local level but also at regional level. The culture of antimicrobial abuse needs to be stopped, and continuous surveillance of multidrug resistant strains is very important to know the changing antibiotic susceptibility pattern as well as the cyclical change of the serogroup from time to time as the resistance pattern also changes with the change in the serogroup. Analysis and periodic reporting is important in proper therapy of shigellosis.

#### Related reports

Resistant to ciprofloxacin showed a trend towards an increased incidence of resistance, especially in *S. dysenteriae* and *S. flexneri*, WHO, 2005.

#### Innovations & breakthroughs

Endemicity of shigellosis with *S. flexneri* is as the predominant serogroup.

#### Applications

Greater number of isolates (38.46%) was recovered from those aged 1–10 years. This was statistically significant,

compared to the other age groups.

#### Peer review

The work is very good in which the authors evaluated the distribution of *Shigella* species and their antibiotic resistance pattern that were found to be different between isolates. Overall the work done can add additive support to treat the shigellosis. The manuscript can be published.

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