Acute gastroenteritis in Nepal with reference to shigellosis and enteropathogenic *Escherichia coli*

Salman KHAN [1], Priti SINGH [2], Ashish ASTHANA [3], Mukhtar ANSARI [4]
[1] Department of Microbiology, [2] Department of Biochemistry, Nepalgunj Medical College, Nepal
[3] Department of Microbiology, Neta ji Subhash Chandra bose subharti Medical College, Swami Vivekanand Subharti University, India
[4] Department of Pharmacology, National Medical College, Nepal

ABSTRACT

Introduction: *Shigellae* play an important role in acute gastroenteritis. We report here the findings of *Shigella* species and relative risk of co-infection with enteropathogenic *Escherichia coli* (EPEC) among patients treated for acute gastroenteritis in the mid and far western region of Nepal. **Materials and Methods:** 507 patients with acute gastroenteritis who presented to the outpatients and inpatients departments of the Nepalgunj Medical College and Teaching Hospital in Banke in Nepal between September 2011 to April 2013 were included in this study. Stool specimens were collected and processed for *Shigella* species and *Escherichia coli* (*E. coli*), following standard bacteriological methods at the Central Laboratory of Microbiology. **Results:** One hundred seventy two isolates were identified as *Shigella* species (n=69, 40.1%) and *E. coli* (n=103, 59.9%). Among the *Shigella* isolated, *S. flexneri*, *S. dysenteriae*, *S. boydii* and *S. sonnei* accounted for 42.0%, 27.5%, 21.7% and 8.7% respectively. The majority was from children (1-10 years old) accounting for 42.0% (n=29), statistically significant ($p<0.05$) compared to the other age groups. Of the E. *coli* isolates, 11 were EPEC, and seven patients also had *Shigella* species isolated in patients aged in the 1–10 years (28.6%), 11–20 years (42.9%) and 21-30 years (28.6%). **Conclusions:** The study revealed the endemicity of Shigellosis with *S. flexneri* being the predominant serogroup. Children were at a higher risk. The continuous analysis and periodic reporting of Shigella species is important in proper therapy of Shigellosis.

Keywords: Epidemiology, dysentery, Shigellosis, gastroenteritis

INTRODUCTION

Shigellosis remains a public-health problem in most developing nations where communities are still ravaged by poverty, poor sanitation, poor personal hygiene, and poor water supplies. [1] Epidemiologic studies estimated between 140 to 160 million cases of Shigellosis occurred worldwide with an estimated 600,000 deaths annually. [1-3] The majority of these cases (99%) occurred in developing countries with 69% occurring in children aged

Correspondence author: Salman KHAN
Department of Microbiology
Nepalgunj medical college, Chisapani
Banke, Nepal
Tel: 009779848354981
E mail: salman186631@gmail.com

less than five years. [3]

Shigella species have been found in most surface-waters, sewage, food, and crops contaminated by human faeces used as fertilisers. [3, 4] Although recovered from these sources, Shigella species are most frequently transmitted via direct person-to-person contact, and 10 to 100 organisms are required to initiate an infection. [5] It is the major cause of dysentery and acute diarrhoeal illness in children and adults. Children are at the highest risk of severe shigellosis. [6] Most are hospitalised immediately after the onset of the disease. Oral rehydration is the principal means of management but antibacterial treatment may be also be required. [7] According to the World Health Organisation (WHO), fluoroquinolones are recommended drugs of choice. [8] However, fluoroquinolone resistance has now been documented in many countries. [9-11] Alternative drug such as the third generation cephalosporins are being used commonly.

There are four major O antigen groups; Group A (*Shigella (S) dysenteriae*), Group B (*S. flexneri*), Group C (*S. boydii*) and (*S. sonnie*). [12] In Eastern Nepal, an active surveillance study (2005) found that *S. dysenteriae* was the major serogroup. [11] However, another study in Western Nepal (2006) on acute gastroenteritis reported that *S. flexneri* was the predominant serogroup. [12] There are also other infections that cause diarrhoeal illness. This study assessed epidemiology of acute gastroenteritis in the mid and far western region of Nepal specifically looking at the types of *Shigella* and the overlap infections.

MATERIALS AND METHODS

Study background and subjects: This was a prospective study conducted on 507 patients of with acute gastroenteritis comes from the mid and far western region of Nepal, attending out-patients and in-patients departments of Nepalgunj Medical college and teaching Hospital, Banke, Nepal, between the periods of September 2011 to April 2013.

Sample collection and processing: Stool sample from affected patients were collected and sent (in plastic containers) to the central Laboratory of Microbiology of Nepalgunj Medical College for examination. The specimens were inoculated on Hektoen Enteric Agar plates, *Salmonella-Shigella* agar, deoxycholate citrate agar, and MacConkey agar (Himedia Lab. Pt Ltd.) and incubated at 37^{o}C for 24 hours. Among those that isolated organisms, the colourless (non-fermentation) colonies were chosen for identification of enteropathogenic bacteria such as *Shigella* spp. Enrichment was done in selenite F broth and incubated at 37^{o}C for 24 hours. After enrichment, sub-culture was done in the above media and further incubated at 37^{o}C for 24 hours.

Tests for distinguishing *Shigella* spp. from other enteric pathogens included: gram negative rods, non-motile, non-lactose fermenter, oxidase, lysine decarboxylase, simmon's citrate, voges prousqure, urease were negative, mannitol and sucrose fermentation, ONPG test, ornithine decarboxylase test and indole were variable positive and triple sugar iron agar were alkaline/acid with no production of hydrogen sulfur. Finally the identified *Shigella* spp. was confirmed by using the slide agglutination test using polyvalent and monovalent anti-sera (Denka Seiken, Japan). *E. coli* were also further tested for enteropatho-

genicity by slide and tube agglutination tests using *E. coli* polyvalent antisera A, B, and C (Biotec Laboratories, UK).

Statistical analysis: Data obtained were analysed using the Statistical Package for Social Sciences (SPSS) software (version 16, SPSS, Inc, Chicago, IL, USA). Comparison of data in respect of sex, and age-groups were performed by Chi-square. P values of < 0.05 were considered to be statistically significant.

Table 1: Distribution of *Shigella* species by gender.

Shigella species	Distribution of positive cases		
	Male	Female	Total (%)
S. flexneri	16	13	29 (42.0)
S. dysenteriae	10	9	19 (27.5)
S. boydii	8	7	15 (21.7)
S. sonnei	4	2	6 (8.7)
Total (%)	38 (55.1)	31 (44.9)	69 (100)

RESULTS

Of the 507 stool samples screened, 53.9% was from male and 46.1% from female patients. Overall, 103 (20.3%) *E. coli* strains and 69 (13.6%) *Shigella* strains were identified.

S. flexneri was the predominant isolate accounted for 42.0% (n=29) followed by *S. dysenteriae* in 27.5% (n=19). This is shown. Among those positive for *Shigella*, 55.1% (n=38) were from male and 44.9% (n=31) from female (Table 1). The age range of patients was between 1 to more than 60 years. The majority was in the 1-10 years group (n=29, 42.0%), statistically significant (p<0.05), compared to the other age groups (Table 2). However, there was no significant difference in the overall number of isolates recovered based on gender (p>0.05).

For *E. coli* 62.1% (n=64) were from male and 37.9% (n=39) from female (Table 3).

Of the 11 EPEC strains isolated, seven were isolated with *Shigella* species from patients in the 1–10 (28.6%, n=2), 11–20 (42.9%, n=3), and 21–30 (28.6%, n=2) age groups (Table 4).

DISCUSSION

Shigellosis remains a significant cause of morbidity and mortality, especially in developing countries. [13] In this study, the predominant serotype was *S. flexneri* followed by *S. dysentriae*. Differences in the distribution of *Shigella* serogroups and serotypes have been reported from time to time, not only in Nepal but other countries. [6, 14] In western Nepal *S. flexneri*

Table 2: Distribution of *Shigella* species by age groups.

Shigella species	Age groups (years) distribution n (%)							
	1-10	11-20	21-30	31-40	41-50	51-60	>60	**Total**
S. flexneri	14	5	6	2	1	-	1	29 (42.0)
S. dysenteriae	9	4	5	-	1	-	-	19 (27.5)
S. boydii	5	2	4	3	-	-	1	15 (21.7)
S. sonnei	1	1	1	1	1	1	-	6 (8.7)

Table 3: Distribution of *Shigella* species by gender.

Age groups	Male	Female	Total
1-10	8 (7.8)	5 (4.9)	13 (12.6)
11-20	16 (15.5)	6 (5.8)	22 (21.4)
21-30	14 (13.6)	9 (8.7)	23 (22.3)
31-40	13 (12.6)	11 (10.7)	24 (23.3)
41-50	9 (8.7)	7 (6.8)	16 (15.5)
51-60	4 (2.9)	0 (0)	4 (2.9)
>60	0 (0)	1 (0.9)	1 (0.9)
Total	64 62.1)	39 (37.9)	103 (20.3)

was reported to be the predominant serogroup. [12] *S. flexneri* has also been reported to be the predominant serogroup in Nigeria, India, and Bangladesh. [7, 14-18] *S. sonnei* was the most frequently-isolated serogroup in the West Indies and Iran. [19, 20] In northern Pakistan, *S. dysenteriae* was the predominant serogroup. [21] This was the second most common serogroup in our study. 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. [14]

All the four *Shigella* serogroups coexist in different proportions in many countries. However, in most developing countries, *S. flexneri* is the predominant serogroup isolated from patients with infectious diarrhoea and usually account for 50-90% of all Shigella isolates. [7, 12, 15-18, 22, 23]

The majority of the *Shigella* species were isolated from children aged 1-10 years, followed the 21-30 and 11-20 age groups. The incidence became less in the older age groups. Therefore, in mid and western region of Nepal based on our study, children and youth are the most commonly affected groups. Children are most susceptible to shigellosis primarily because of poor resistance, lack of previous exposure, poor personal hygiene, and higher exposure to contaminated environment due to play-related activities. [21, 22] This is similar to other studies from the Indian Subcontinent. Srinivasa *et al*. (India) reported that 41.8% affected were children between 0 to 14 years. [23] Another study from India by Sonawala *et al*. reported that 80% of *Shigella* was isolated from children aged below five years with the remaining 20% isolated from those age 5-20 years old. [24] A study from eastern Nepal by Bhattacharya *et al*. reported that 79% were isolated from children aged less than five years old. [6]

We also examined the incidence of co-infection with EPEC. We found a proportion of patients with EPEC also had *Shigella* isolated

Table 4: Co-infections of *Shigellae* with enteropathogenic E. coli in different age groups.

Age	EPEC		Shigellae	
Groups	(+ve) Shigella	(-ve) Shigella	(+ve) EPEC	(-ve) EPEC
1-10	2	11	2	27
11-20	3	19	3	9
21-30	2	21	2	14
31-40	0	24	0	6
41-50	0	16	0	3
51-60	0	4	0	1
>60	0	1	0	2
Total	7	96	7	62

in the stool. The most common groups, similar to the demographic of shigellosis to be affected were the children, youths and young adults. EPEC has been reported as the major cause of acute diarrhoea in children aged more than two in Latin America. [25] Gomez *et al.* reported that death were more common among children with EPEC and *Shigella* co-infections. [26]

There were some limitations with this study. We did not perform any antibiotic sensitivity test and we did not assess for other bacteria, viruses, parasites and fungi which are also responsible for diarrheal illness or dysentery in the mid and western region of Nepal. We also did not assess the outcomes of the patients.

In conclusion, the findings of this study show that *Shigella* remains an important cause of acute gastroenteritis. *S. flexneri* was the predominant Shigella serogroup and that infection with additional organism occurs. Continued surveillance should be carried to assess for any change and to guide management.

REFERENCES

1: World Health Organization. Vaccine research and development: new strategies for accelerating Shigella vaccine development. Wkly Epidemiol Rec. 1997; 72:73-80.

2: World Health Organization. Diarrhoeal disease due to Shigella disease. In: Vaccines, immunization and biologicals. Geneva: World Health Organization. 1998:1-5.

3: Geldreich EE, Bordner RH. Faecal contamination of fruits and vegetables during cultivation and processing for marketing: a review. J Milk Food Technol.1971; 34:184-95.

4: Taylor BC, Nakamura M. Survival of Shigellae in food. J Hyg (Lond). 1964; 62:303-11.

5: World Health Organization. Programme for Control of Diarrhoeal Diseases. Guidelines for the control of epidemics due to *S. dysenteriae* 1. Geneva: World Health Organization. 1988:1-15.

6: Bhattacharya S, Basudha Khanal, Narayan R, et al. Prevalence of Shigella species and Their Antimicrobial Resistance Patterns in Eastern Nepal. J health popul nutr. 2005; 23:339-42.

7: Iwalokun BA, Gbenle GO, Smith SI, Ogunledun A, Akinsinde KA, Omonigbehin EA. Epidemiology of Shigellosis in Lagos, Nigeria: trends in antimicrobial resistance. J Health Popul Nutr. 2001; 19:183-90.

8: Guidelines for the Control of Shigellosis, including epidemics due to Shigella dysenteriae type 1. Geneva: WHO; 2005.

9: Naheed A, Kalluri P, Talukder KA, Faruque AS, et al. Fluoroquinolone and resistant Shigella dysenteriae type 1 in northeastern Bangladesh. Lancet Infect Dis. 2004; 4:607–8.

10: Hirose K,Terajima J,Izumiya H, Tamura K,et al. Antimicrobial susceptibility of Shigella sonnei isolates in Japan and molecular analysis of S.sonnei isolates with reduced susceptibility to fluoroquinolones. Antimicrob Agents Chemother. 2005; 49:1203–5.

11: Roy S, Bhattacharya D, Thanasekaran K, et al. Emergenceof fluoroquinolone resistance in Shigella isolated from Andaman & Nicobar Islands, India. Indian J Med Res. 2010; 131:720–2.

12: Wilson G, Easow JM, Mukhopadhyay C, Shivananda PG. Isolation and antimicrobial susceptibility of Shigella from patients with acute gastroenteritis in Western Nepal. Indian J Med Res. 2006; 123:145-50

13: Kotloff KL, Winickoff JP, Ivanoff B, et al. Global burden of Shigella infections: implications for vaccine development and implementation of control strategies. Bull World Health Organ. 1999; 77:651-66.

14: Shrestha CD, Malla S, Maharjan L. Multi Drug Resistant Shigella Species in Nepal, a Retrospective Study Conducted at National Public Health Laboratory (NPHL), 1999 to 2002. J Nepal Health Research Council. 2003; 4: 75-8.

15: Niyogi SK, Mitra U, Dutta P. Changing patterns

of serotypes and antimicrobial susceptibilities of Shigella species isolated from children in Calcutta, India. Jpn J Infect Dis. 2001; 54:121-2.

16: Jesudason MV. Shigella isolation in Vellore, South India (1997-2001). Indian J Med Res. 2002; 115:11-3.

17: Zaman K, Yunus M, Baqui AH, Hossain KMB. Surveillance of shigellosis in rural Bangladesh: a 10 years review. J Pak Med Assoc. 1991; 41:75-8.

18: Bhattacharya D, Sugunan AP, Bhattacharjee H et al. Antimicrobial resistance in Shigella - rapid increase & widening of spectrum in Andaman Islands, India. Indian J Med Res. 2012; 135:365-70.

19: Orrett FA. Prevalence of Shigella serogroups and their antimicrobial resistance patterns in Southern Trinidad. J Health Popul Nutr. 2008; 26:456-62.

20: Gharibi O, Zangene S, Mohammadi N et al. Increasing antimicrobial resistance among shigella isolates in the Bushehr, Iran. Pakistan J Bio Sci. 2012; 15:156-9.

21: Ahmed K, Shakoori FR, Shakoori AR. Aetiology of shigellosis in northern Pakistan. J Health Popul Nutr. 2003; 21:32-9.

22: Hossain MA, Albert MJ, Hasan KZ. Epidemiology of shigellosis in Teknaf, a coastal area of Bangladesh: a 10-year survey. Epidemiol Infect. 1990; 105:41-9.

23: Srinivasa H, Baijayanti M, Raksha Y. Magnitude of drug resistant shigellosis: A report from Bangalore. Indian J Med Microbiol. 2009; 27:358-60.

24: Sonawala M, Saraswathi K, Deodhar LP. Serogroup prevalence of Shigellae in Bombay. J Postgrad Med. 1995; 41:104-6.

25: Notario R, Borda N, Gambande T, Sutich E. Species and serovars of enteropathogenic agents associated with acute diarrheal disease in Rosario, Argentina. Rev Inst Med Trop Sao Paulo. 1996; 38:5 -7.

26: Gomes TAT, Rassi V, MacDonald KL, et al. Enteropathogens associated with acute diarrheal disease in urban infants in São Paulo, Brazil. J Infect Dis. 1991; 164:331-7.