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Bacterial Etiology of Acute Diarrhea in Children Under Five Years of Age

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ABSTRACT

Background: Diarrheal diseases are major problem of developing countries. Though precise data on childhood mortality associated with diarrheal diseases in Nepal is not available, it has been estimated that approximately 25% of child death are associated with diarrheal disease, particularly acute diarrhea. The purpose of this study was to assess the incidence of bacterial pathogens causing acute diarrhea in children under 5 years of age.

Methods: A total of 525 children with acute diarrhea in a children's hospital of Kathmandu, Nepal were enrolled between April 2011 to September 2011. Feacal specimens for culture were inoculated to the several media. The organisms were identified by different biochemical tests and serotyping. Their antibiotic sensitivity tests were performed by Kirby-Bauer's disc diffusion method as recommended by CLSI.

Results: Out of total 525 enrolled cases bacterial infection was found to be 46 (8.8%). Bacterial infection was found to be of highest, 36 (78.3%) in the age group between 6-24 months. Among the total enrolled cases the prevalence of Shigella species was 24 (4.6%) followed by *Escherichia coli* 12 (2.3%) and Salmonella species 10 (1.9%). Chloramphenicol and Tetracycline showed efficacy in 9 (90.0%) isolates of Salmonella species, Gentamycin showed efficacy in 22 (91.7%) isolates of Shigella species and Chloramphenicol showed 100% efficacy against *Escherichia coli* whereas 7 (70.0%) isolates of Salmonella species were resistant to ampicillin in vitro. MDR was highest 7 (70.0%) in Salmonella species.

Conclusions: The bacterial pathogens were found to be a significant cause of acute diarrhea. The most common causative organism for acute diarrhea were Shigella spp. Awareness of improving hygiene and infectious diseases may reduce the burden of infection.

Keywords: acute diarrhea; bacteria; pathogen.

INTRODUCTION

Diarrhea is defined by World Health Organization (WHO) as having 3 or more loose or liquid stools per day or as having more stools than is normal for that person.[1] Acute diarrhea, defined as an increased frequency of defecation (three or more times per day or at least 200 g of stool per day) lasting less than 14 days, may be accompanied by nausea, vomiting, abdominal cramping, clinically significant systemic symptoms, or malnutrition.[2] In 2009 diarrhea was estimated to have caused 1.5 million deaths in children under the age of 5 years.[3]

Though precise data on childhood mortality associated with diarrheal diseases in Nepal is not available, it has been estimated that approximately 25% of child death are associated with diarrheal diseases, particularly acute diarrhea.[4]

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In developing countries 50-60% cases are of bacterial (Enteropathogenic *Esherichia coli* 25%, *Campylobacter jejuni* 10-18%, *Salmonella* spp. and *Shigella* spp. 5% each), 35% of viral (15-25% rotavirus) origin, and in many the cause is unidentified or mixed.[5-9] Among the bacteriological pathogens *Escherechia coli* play an important role.[10]

METHODS

This study was a cross-sectional study conducted at Tribhuvan University Teaching Hospital, Department of Microbiology-Public Health Research Laboratory. Ethical approval was taken from the Institutional Review Board (IRB), Institute of Medicine, Tribhuvan University Teaching Hospital, Kathmandu, Nepal. Written informed consent was obtained from the children's parents or guardian before enrollment. A total of 525 stool samples were collected from the children under 5 years of age visiting Kanti Children's Hospital, Kathmandu, Nepal with acute diarrhea in the periods between April 2011 to September 2011. From each participating children, clinical data were obtained and stool sample was collected in a sterile container.

Faecal specimens for culture were inoculated to several media for maximal yield, including solid agar and broth. Specimens received for detection of the most frequently isolated Enterobacteriaceae and *Salmonella* spp. and *Shigella* spp. were plated to a supportive medium, a slightly selective and differential medium. The specimen were inoculated to a MacConkey agar (Himedia) for the selection of *Escherichia coli* and Salmonella-Shigella agar (Himedia) for the selection of *Shigella* spp. and *Salmonella* spp. and thiosulfate citrate bile salt sucrose (TCBS) agar (Himedia) for the selection of *Vibrio* spp. Salmonell-Shegella broth (Himedia), Selenite F broth (Himedia) were used as the enrichment media for *Salmonella* spp. and *Shigella* spp. Alkaline peptone water was used as the enrichment medium for *Vibrio* spp. The aseptic condition was maintained through the experiment. Purity plate and quality control was maintained during the experiment. All culture plates and the enrichment media were incubated at 37 ^{0}C for overnight. All samples were tested for *Escherichia coli*, *Shegella spp.*, *Salmonella spp.* and *Vibrio cholerea* by using gram's stain, colony morphology, biochemical tests and agglutination with specific antisera.

The antibiotic sensitivity tests of the pathogens isolated from the clinical specimen against different antibiotics were done using Mueller Hinton agar (MHA) (Himedia India) by the standard disk diffusion technique of modified Kirby-Bauer method as recommended by CLSI. For disk susceptibility testing, Ampicillin (10 µg), Norfloxacin (10 µg), Nalidixic acid (30 µg), Cotrimoxazole (1.25/23.75 µg), Gentamycin (10 µg), Amikacin(30 µg), Ciprofloxacin (5 µg), Ofloxacin (5 µg), Cefotaxime (30 µg), and Ceftazidime (30 µg), were used. In this study if the isolates were resistant to at least two or more than two groups of antimicrobial agents, they were regarded as MDR.[11]

Differences in proportions were assessed by Chi-square test. P values <0.05 were considered statistically significant.

RESULTS

Out of total 525 enrolled cases 323 (61.5%) were from IPD and 202 (38.5%) were from OPD. Boys had higher diarrheal cases (64.2%) than girls (35.8%). The higher rate of diarrhea was in the age group of less than 2 years among which the rate was highest 367 (69.9%) in the age group of 6-24 months, 101 (19.2%) in the age group of less than 6 months and the least rate of 14 (2.7%) was found in the age group of 49-60 months (Table 1). The rate of diarrhea in less than 2 years of age was found to be statistically significant (P<0.01).

Table 1. Age and gender wise distribution of diarrheal cases

Age groups in months	Male n (%)	Female n (%)	Total n (%)
Less than 6	60 (17.8%)	41 (21.8%)	101 (19.2%)
6 -24	238 (70.6%)	129 (68.6%)	367 (69.9%)
25-36	18 (5.3%)	9 (4.8%)	27 (5.2%)
37-48	10 (3.0%)	6 (3.2%)	16 (3.0%)
49-60	11 (3.3%)	3 (1.6%)	14 (2.7%)
Total	337 (100%)	188 (100%)	525 (100%)

Out of total enrolled cases bacterial infection was found to be 46 (8.8%). Bacterial infection was found to be of highest, 36 (78.3%) in the age group between 6-24 months (Figure 1), the lower age group of infants under 6 months was 7 (15.2%). The least infection (2.2%) was found in higher age group between 49-60 months. There were no detectable cases of bacterial infection among the age group of 37-48 months in this study. Occurrence of bacterial pathogens in children below 2 years of age was statistically significant than in those above 2 years of age (P<0.01). Bacterial pathogen infected cases were 22 (47.8%) among male while it was 24 (52.2%) among female.

Among the total enrolled cases the *Escherichia coli* was 12 (2.3%), *Shigella* species was 24 (4.6%) and *Salmonella* species was 10 (1.9%) as shown in figure 2. The rate of *Shigella* spp. was found to be statistically significant (P<0.01).

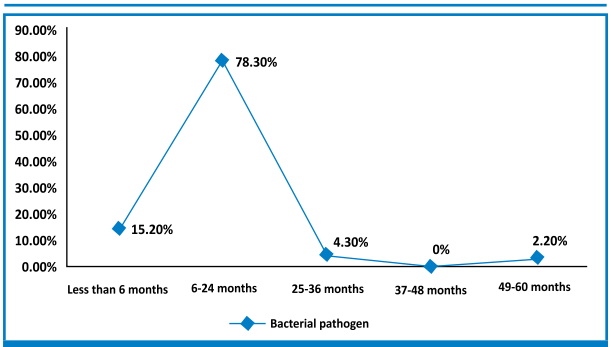

Figure 1. Distribution of bacterial positive cases in different age groups

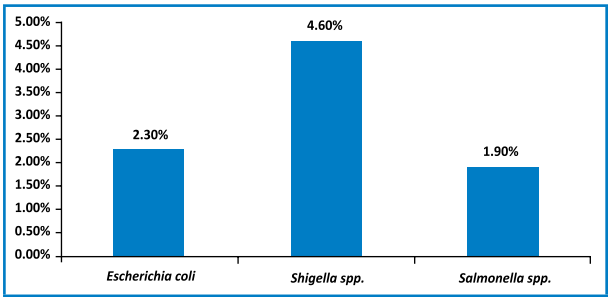

Figure 2. Distribution of different bacteria found in stool samples

Table 2. Distribution of bacterial pathogen (n= 46)

Type of organisms	n (%)
Escherichia coli	**12 (26.1)**
Enteropathogenic *Escherichia coli* (EPEC)	8
Enterotoxigenic *Escherichia coli* (ETEC)	2
Enteroheamorrhagic *Escherichia coli*(EHEC)	2
Enteroinvasive*Escherichia coli* (EIEC)	0
Shigella spp.	**24 (52.2)**
Shigella boydii	12
Shigella sonnei	6
Shigella flexnerri	5
Shigella dysenteriae	1
Salmonella spp.	**10 (21.7)**
Salmonella Typhi	2
Salmonella Paratyphi A	1
Other *Salmonella* spp.	7

Out of total bacterial enteropathogens positive cases, *Shigella* species were found to be highest constituting 24 (52.2%) followed by *Escherichia coli* constituting 12 (26.1%) and *Salmonella* species 10 (21.7%) as depicted in table 2.

Among the *Escherichia coli*, chloramphenicol showed 12 (100%) efficacy while fluoroquinolones, gentamycin and 3rd generation cephalosporin showed better efficacy whereas 5 (41.7%) isolates were resistant to ampicillin followed by 4 (33.3%) resistant to nalidixic acid, cotrimoxazole and amikacin each in vitro (Table 3).

Table 3. Antimicrobial sensitivity pattern of Escherichia coli (n=12)

Antibiotics	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Ampicillin	7 (58.3)	0 (00)	5 (41.7)
Nalidixic acid	8 (66.7)	0 (00)	4 (33.3)
Norfloxacin	11 (91.7)	0 (00)	1 (8.3)
Chloram-phenicol	12 (100)	0 (00)	0 (00)
Cotrimoxazole	8 (66.7)	0 (00)	4 (33.3)
Gentamycin	11 (91.7)	0 (00)	1 (8.3)
Ciprofloxacin	11 (91.7)	0 (00)	1 (8.3)
Tetracycline	9 (75.0)	0 (00)	3 (25.0)
Ofloxacin	11 (91.7)	0 (00)	1 (8.3)
Amikacin	8 (66.7)	0 (00)	4 (33.3)
Ceftazidime	11 (91.7)	0 (00)	1 (8.3)
Cefotaxime	11 (91.7)	0 (00)	1 (8.3)

Among the total *Shigella species*, 22 (91.7%) isolates were susceptible to gentamycin followed by 21 (87.5%) isolates susceptible to fluoroquinolones and amikacin whereas 13 (54.2%) isolates were resistant to nalidixic acid followed by 12 (50.0%) isolates resistant to ampicillin and cotrimoxazole each in vitro (Table 4).

Table 4. Antimicrobial sensitivity pattern of *Shigella species* (n=24)

Antibiotics	Sensitive n (%)	Intermediate resistant n (%)	Resistant n (%)
Ampicillin	12 (50.0)	0 (0.0)	12 (50.0)
Nalidixic acid	11 (45.8)	0 (0.0)	13 (54.2)
Norfloxacin	21 (87.5)	1 (4.2)	2 (8.3)
Chloram-phenicol	19 (79.2)	0 (0.0)	5 (20.8)
Cotrimoxazole	12 (50.0)	0 (0.0)	12 (50.0)
Gentamycin	22 (91.7)	0 (0.0)	2 (8.3)
Ciprofloxacin	21 (87.5)	1 (4.2)	2 (8.3)
Tetracyclin	14 (58.3)	0 (0.0)	10 (41.7)
Ofloxacin	21 (87.5)	1 (4.2)	2 (8.3)
Amikacin	21 (87.5)	0 (0.0)	3 (12.5)
Ceftazidime	18 (75.0)	0 (0.0)	6 (25.0)
Cefotaxime	16 (66.7)	0 (0.0)	8 (33.3)

Among the *Salmonella* species, chloramphenicol and tetracycline showed efficacy in 9 (90.0%) isolates in vitro followed by amikacin, cotrimoxazole in 8 (80.0%) isolates and fluoroquinolones in 7 (70.0%) isolates whereas 7 (70.0%) isolates were resistant to ampicillin followed by 6 (60.0%) isolates resistant to nalidixic acid in vitro (Table 5).

Table 5. Antimicrobial sensitivity pattern of *Salmonella species* (n=10)

Antibiotics	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Ampicillin	3 (30.0)	0 (00)	7 (70.0)
Nalidixic acid	4 (40.0)	0 (00)	6 (60.0)
Norfloxacin	7 (70.0)	0 (00)	3 (30.0)
Chloram-phenicol	9 (90.0)	0 (00)	1 (10.0)
Cotrimoxazole	8 (80.0)	0 (00)	2 (20.0)
Gentamycin	6 (60.0)	0 (00)	4 (40.0)
Ciprofloxacin	7 (70.0)	0 (00)	3 (30.0)
Tetracycline	9 (90.0)	0 (00)	1 (10.0)
Ofloxacin	7 (70.0)	0 (00)	3 (30.0)
Amikacin	8 (80.0)	0 (00)	2 (20.0)
Ceftazidime	6 (60.0)	0 (00)	4 (40.0)
Cefotaxime	6 (60.0)	0 (00)	4 (40.0)

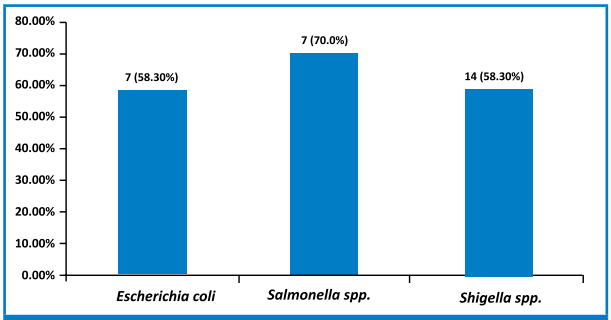

Figure 3. Distribution of MDR bacterial isolates

MDR was highest 7 (70.0%) in *Salmonella* species followed by 14 (58.3%) in *Shigella* species and 7 (58.3%) in *Escherichia coli* (Figure 3).

DISCUSSION

In this study out of 525 cases enrolled 337 were male and 188 were female with male to female ratio being 1.79:1. Children below 5 years of age were enrolled in this study. The maximum number of samples were from the age group of less than 2 years in which the age group of 6- 24 months constitutes maximum number 367 (69.9%) followed by the age group of less than 6 months 101 (19.2%). Among the total cases enrolled, the bacterial pathogen was found in 46 (8.8%) of cases.

The rate of diarrhea was higher in male 337 (64.2%) than female 188 (35.8%) in this study. The higher positivity rate among boys was in agreement with the numerous studies.[12-15] The most of the diarrheal cases were found in the age group less than 2 years of age among which the higher rate (69.9%) was found in the age group of 6-24 months than the age group of less than 6 months (19.2%). The prevalence of diarrhea in age less than 2 years was found to be statistically significant (P<0.01).

In present study 323 (61.5%) of cases were from IPD and 202 (38.5%) were from OPD. Bacterial infected cases showed non-significant difference between male (47.8%) and female (52.2%) (P>0.05).

In our study bacterial pathogens were found in 46 (8.8%) of cases. Similar results were also found by Sherchand et al. in Nepal[13] and Patel et al. in Oman.[16] The prevalence of bacterial infection in cases of diarrhea among children in other countries varies between 5.3% and 54%.[16]

It appeared that infants below 6 months of age were initially protected to some extent by maternal antibodies against severe diarrhea, and they seem to have acquired adequate immunity between 12 and 16 months of age. The greater risk of infants and young children in the period between 6 to 12 months with declined levels of maternal antibodies have been documented.[13]

In this study too the age below 2 years were found to be the most infected by bacterial pathogens. Among 46 bacterial pathogen positive cases 36 (78.3%) were in the age between 6-24 months, followed by 7 (15.2%) in the age less than 6 months. Similar result was also found by Moyo et al.[15]

Escherichia coli were detected in 12 (2.3%) of cases with EPEC constituting 66.6% followed by ETEC and EHEC each 16.7%. Similar results were also reported by several other investigators.[4,13,22,25,26]

In this study, the *Shigella* species constituted 24 (4.6%) in cases of diarrhea (P<0.01). Similarly Moyo et al.[15] in Tanzania found a prevalence of *Shigella* species to be 5.7% and other studies also have found the similar prevalence of 7.7% in Calcutta (India),[17] 3.2% in Mumbai (India)[18] and 4.9% in Jordan.[19] In present study among *Shigella* spp., *Shigella boydi* constituted 12 (50.0%), *Shigella sonnei* 6 (25%), *Shigella flexneri* 5 (20.8%), and *Shigella dysenteriae* 1 (4.2%) which was in consistent with study by Sherchand et al. in Nepal,[13] Ghaemi et al. in Iran[20] and Bodhidatta et al. in Thailand.[21]

The *Salmonella* spp. causing diarrhea in our study was reported in 10 (1.9%) cases. The finding of our study falls within the reported range of 1-5% of gastroenteritis cases in most developing countries.[15] Our result concurs with different investigators from several places.[18,19,22,23] Among *Salmonella* spp., *Salmonella* Typhi was reported in 2 (20.0%), *Salmonella* paratyphi A was reported in 1 (10.0%) and other *Salmonella* spp. were reported in 7 (70.0%) cases.

No *Vibrio* spp. were isolated in this study. The spread pattern of *Vibrio* spp. suggested water borne infection in rainy seasons, although the quantity of water available is large in rainy season most water sources

are contaminated with excreted microorganisms from surface water runoff.[24]

While discussing about the antibiotic sensitivity profile, among *Escherichia coli* 100% isolates were found to be susceptible to Chloramphenicol while 11 (91.7%) isolates were susceptible to Gentamicin, Fluoroquinolones, and 3rd generation Cephalosporin and 9 (75.0%) isolates were found to be susceptible to Tetracycline whereas very few isolates were resistant to various antibiotics among them 5 (41.7%) isolates were resistant to Ampicillin. According to Kaminski et al,[31] Ciprofloxacin was 100% effective against *E. coli* and 50% of isolates were resistant to Ampicillin and Al Gallas et al,[10] found 71.4% isolates sensitive to Tetracycline and 20.4% isolates resistant to Ampicillin. Out of total *Shigella* species 22 (91.7%) were found to be sensitive to Gentamycin followed by 21 (87.5%) sensitive towards Fluoroquinolones and Amikacin whereas 13 (54.2%) isolates were resistant to Nalidixic acid. According to Das et al,[27] from north Indian patients the strains of *Shigella* spp. isolated were highly susceptible to Norfloxacin, Gentamycin and increasing resistance to Nalidixic acid, according to Bodhidatta et al,[21] Total 100% isolates of *Shigella* spp. were sensitive to Ciprofloxacin which concurs with our finding. Similar study by Sonawala et al,[18] in India showed that *Shigella* spp. were 100% sensitive to Nalidixic acid which is much higher than our study and 98.8% of isolates were sensitive to Gentamycin which is consistent with our study.

Chloramphenicol and Tetracycline were the most effective antimicrobials for *Salmonella* spp. with 9 (90.0%) isolates susceptible followed by Cotrimoxazole 8 (80.0%) and 7 (70%) of *Salmonella* isolates were susceptible to Fluoroquinolones whereas 7 (70.0%) isolates were resistant to Ampicillin followed by 6 (60.0%) isolates resistant to Nalidixic acid (NARS). Mandomando et al,[22] reported 92% of *Salmonella* isolates were sensitive to Chloramphenicol, 85% isolates were sensitive to Tetracycline and 62% isolates of *Salmonella* spp. were resistant to Ampicillin. According to Patel et al,[16] 10% of the *Salmonella* isolates were resistant to Ampicillin and 7.1% were resistant to Nalidixic acid. This finding is much lower than our study. This may be due to empirical use of these antibiotics and development of resistance in our context. Dahifar et al from Tehran found 67% of *Salmonella* isolates were resistant to Nalidixic acid (NARS) which is consistent with our study.[28] Patients infected with nalidixic acid-resistant isolates (NARS) respond less well to fluoroquinolones, even when the isolates' MICs are within the susceptible range for fluoroquinolones.[29,30]

According to our study Chloramphenicol, Fluoroquinolones, Gentamycin, and 3rd generation cephalosporin were the most effective antimicrobial for the bacterial pathogens. However, the use of Chloramphenicol and Fluoroquinolones are not recommended in younger children.[22] Because of the abuse of antimmicrobial drugs, the antibiotic resistance of *Salmonella* strains from humans is expected to increase. It has been reported that there has been an increase in antibiotic resistance of *Salmonella* isolates from humans.[32] In this study too, 7 (70.0%) of *Salmonella* spp. were MDR.

CONCLUSIONS

The study indicated that the frequency of diarrhea was higher in male children compared to female children. Chloramphenicol, Gentamicin and Fluoroquinolones were the most effective antibiotics while Ampicillin and Nalidixic acid were the least effective antibiotics in vitro against the bacterial isolates. *Shigella* species were common among the bacterial pathogen causing acute diarrhea in children under 5 years of age. These children can become a source of outbreaks. So the awareness on prevention of the infectious diseases, improving hygiene should be implicated to reduce the burden of infectious diseases.

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