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Original Research Article

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Bacterial enteropathogens causing acute diarrhoea in children in a tertiary care hospital, Imphal

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ABSTRACT

Background: Diarrhoeal diseases are responsible for causing 3 million deaths worldwide every year especially among the children and also the commonest cause of morbidity and mortality in developing countries like India. Infective diarrhoea could be either bacterial, viral, parasitic or occasionally a combination of these.

Methods: A cross-sectional study was carried out in children below 12 years with acute diarrhoea in the Microbiology Department, RIMS, Imphal for a period of 2 years. Stool samples were subjected to routine microbiological examination, followed by culture and sensitivity. Data were collected in a predesigned data collection sheet.

Results: Majority of the diarrhoeal cases were seen among the age group of 1-3 years (44.3%), predominantly among the male children (66.2%) and mostly in summer. Out of 210 culture positive stool samples, Escherichia coli(83.3%) was the predominant enteropathogen with followed by *Shigella spp.* (12.9%), *Klebsiella spp.* (2.9%) and *Salmonella spp.* (1%). Serotyping revealed thirty five enteropathogenic *E. coli*, eighteen *Shigella flexneri*, seven *Shigellasonnei*, two *Shigella boydii* and two *Salmonella typhimurium*. Majority of the isolates showed high resistance to amoxicillin, ampicillin, ciprofloxacin, ofloxacin and cotrimoxazole.

Conclusions: Bacterial enteropathogens are an important cause of acute diarrhoea among children. Rehydration therapy remains the initial treatment. Though it is usually self-limiting, empirical and specific antimicrobial therapy can be considered in certain situations. Awareness of improving hygiene and infectious diseases may help reduce the burden of infection.

Keywords: Bacterial enteropathogens, Diarrhoeagenic E. coli, Diarrhoea in children

INTRODUCTION

Diarrhoea is defined as passage of loose or watery stools at least 3 times per day or more frequently than normal for an individual. Worldwide, more than 1 billion cases and at least 4 million deaths per year are attributed to diarrhoea. In India, one third of total paediatric admissions in hospitals are due to diarrhoeal diseases and 17 percent of all indoor paediatric patients deaths are diarrhoea related. Acute diarrhoea is caused by a variety of infectious agents which ranges from viruses, bacteria

and parasites. *Escherichia coli*, *Salmonella spp*, *Shigella spp*, *Vibrio cholerae*, *Campylobacter jejuni* and Aeromonas species are the common bacterial causes.³

The infectious agents causing diarrhoea are transmitted mainly by faeco-oral route i.e. ingestion of contaminated food, drinking contaminated water or direct contact with contaminated stool.⁴ Bacterial pathogens causing childhood gastroenteritis in developed and developing countries are quite different. Non-typhoidal Salmonella species is the prevalent bacterial pathogen in developed

countries whereas enteropathogenic Escherichia coli (EPEC) and Shigella species are common in developing countries.⁵ Diarrhoeagenic Escherichia coli (DEC) strains are most frequently associated with diarrhoea in children in developing countries which includes enterotoxigenic E. coli (ETEC), enteroaggregative E. coli enteropathogenic (EAEC), E. colienterohaemorrhagic E. coli (EHEC) or verocytotoxin producing E. coli, enteroinvasive E. coli (EIEC) and diffusely adherent E. coli (DAEC). In children below 5 Diarrhoeagenic E. coli, specifically enteropathogenic E. coli (EPEC) is the leading bacterial agent causing diarrhoea.6

In children, as acute diarrhoea mostly subsides spontaneously and are generally self-limiting, rehydration and adequate nutrition forms the basis of treatment. However, diarrhoea caused by Shigella spp, Vibrio cholerae, Salmonella spp, Giardia lamblia and Entamoeba histolytica are effectively treated by antimicrobial drugs.⁷ Antibiotic treatment of common bacterial infections plays a crucial role in reducing morbidity and mortality due to these diseases; however, this could be associated with increased development of antibiotic resistance due to overuse and misuse of antibiotics in the treatment of diarrhoea.8 Therefore, it is very important to know the exact pathogen causing diarrhoea and its antimicrobial sensitivity in that particular area for earlier and specific intervention so that we can decrease the financial load on the patient as well as on the health care and also prevent further development of antimicrobial resistance.

Objective of the study was to find out the various bacterial pathogens causing acute diarrhoea in children and study the antibiotic susceptibility pattern.

METHODS

This cross-sectional study was conducted at the Department of Microbiology, RIMS, Imphal, Manipur, India from September 2018 to August 2020. The study was approved by Research Ethics Board, RIMS. A total of 210 children under 12 years of age who attended Paediatric Department with acute diarrhoea and whose stool culture was positive were included in the study. Children with diarrhoea due to malnutrition, those who are immuno-compromised and who have received antibiotics were excluded from the study. An informed consent was taken from parents or guardians after explaining them all prospects of study. A proforma was used to record history and data.

Fresh stool specimens were collected in a sterile wide mouthed, dry, leak proof screw capped container and processed as soon as possible. Routine macroscopic and microscopic examination of the stool sample was done followed by culture and sensitivity testing.

Culture

The stool samples were inoculated on MacConkey agar, XLD (Xylose Lysine Deoxycholate) agar and SF (Selenite-F) broth. For suspected *Vibrio cholerae*, stool sample was inoculated on alkaline peptone water (APW) and after four hours of incubation at 37°C, a subculture was done on Thiosulphate citrate bile salt sucrose (TCBS) agar. For suspected Campylobacter species, Skirrow's media (Hi Media) was used under the micro aerophilic condition in the candle jar at 42°C for 48hrs. The colony morphology were studied and subjected to gram staining, motility testing and further biochemical tests for the identification of the causative organisms.

Serotyping of the *Escherichia coli* isolates for enteropathogenic (EPEC) were done using *E. coli* OK O pool antisera pool 1 (O26, O103, O111, O145, O157), pool 2 (O55, O119, O125, O127, O128) and pool 3 (O86, O114, O121, O126, O142) procured from SSI Diagnostica. Serotyping for Salmonella and Shigella species were done using antisera procured from Biorad company.

Antibiotic susceptibility testing

Antimicrobial sensitivity testing (AST) was done on Mueller-Hinton agar by Kirby-Bauer disc diffusion method for the following antibiotics- ampicillin (10μg), amoxicillin clavulanate (30μg), amikacin (30μg), gentamicin (10μg), cotrimoxazole (25μg), ciprofloxacin (5μg), ofloxacin (5μg), ceftriaxone (30μg), piperacillintazobactum (100/10μg), imipenem (10μg). Azithromycin (15μg) only for Salmonella and *Shigella spp*.

For colistin, minimum inhibitory concentration (MIC) was determined by microbroth dilution method as per CLSI guideline. Solates having MIC of $\leq 2~\mu g/$ ml was considered as susceptible and $> 2~\mu g/$ ml as resistant. VITEK 2 was used whenever required for confirmation of the organism isolated.

Statistical analysis

Data obtained were statistically analysed by using SPSS software version 21(IBM corp, Armonk, NY, USA). Data was summarised using descriptive statistics such as frequency and percentage.

RESULTS

Out of 210 total cases, majority of the cases belong to the age group of 1-3 years i.e 93 (44.3%) followed by age group of 6 months-1year 48 (22.9%), least number of cases were seen in the age group of 5-7 years 9 (4.3%) and 7-9 years 9 (4.3%) as shown in Figure 1.

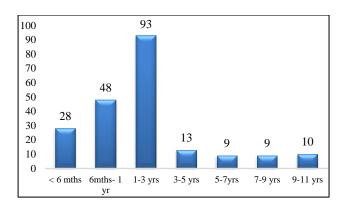


Figure 1: Age wise distribution.

Maximum number of cases were seen among males i.e 66.2% (139) and remaining 33.8% (71) were females. Bacterial diarrhoea were seen more commonly among the Hindu population (73.80%) followed by Christian (13.30%) and Muslim (12. 90%). 79 (37.6%) cases were from inpatient department and 131 (62.4%) belonged to

outpatient department. Majority of the cases were seen among the urban population (70%) than in the rural population (30%) and bacterial diarrhoea due to *Salmonella infection* were seen among the rural population only. Macroscopic and microscopic examination showed presence of blood in 6.2% (13) and mucus in 23.3% (49) of the samples. RBC and pus cell was seen in 13.8% (29) and 27.6% (58) of the samples respectively. *Escherichia coli* was the most predominant enteropathogen isolated 175 (83.3%) followed by *Shigella, Klebsiella* and *Salmonella species* with 12.9%, 2.9% and 1% respectively as shown in Table 1.

Table 1: Prevalence of various bacterial enteropathogens.

Bacterial agent	No. (n=210)	Percentage
Escherichia coli	175	83.3
Klebsiella pneumoniae	6	2.9
Shigella species	27	12.9
Salmonella species	2	1.0

Table 2: Age related prevalence of various bacterial enteropathogens causing acute diarrhoea in children.

Age group	Escherichia coli n = 175 (%)	Shigella spp. n = 27 (%)	Salmonella spp. n =2 (%)	Klebsiella spp. n = 6 (%)
< 6 months	22 (12.6)	0	0	6 (100)
6 months-1 year	47 (26.9)	1 (3.7)	0	0
1-3 years	80 (45.7)	13 (48.1)	0	0
3-5 years	7 (4)	6 (22.2)	0	0
5-7 years	5 (2.9)	2 (7.4)	2 (100)	0
7-9 years	7 (4)	2 (7.4)	0	0
9-11 years	7 (4)	3 (11.1)	0	0

Majority of the bacterial isolates showed peak incidence in the age group of 1-3 years with 45.7% for *E. coli* and 48.1% for *Shigella spp. E. coli* was the only agent distributed among all age groups showing 39.5% incidence among infants, 49.7% in the age group of 1-5 years and least in age group of >5 years (10.9%). *Shigella spp.* had least incidence among infants (3.7%) and incidence increases with age showing peak in the age group of 1-5 years (70.3%). *Salmonella spp.* showed no incidence among infants and incidence increases with age showing peak in the age group of 5-7 years (100%). Klebsiella spp. has peak incidence among infants (100%) and showed no isolates in age group >5 years.

Predominant clinical feature associated was vomiting (36.7%) followed by fever (27.1%) and dehydration in 23.8% of the cases. Only 6.1% of the cases presented with passage of blood stool. Children with diarrhoea due to *Shigella spp.* presented mostly with passage of blood in stool (40.7%) and those with *Salmonella spp.* presented mostly with fever (100%) and passage of blood in stool (50%). Maximum number of cases occurred in summer. Most of the cases of diarrhoea caused by *E. coli, Klebsiella, Shigella* and *Salmonella* species occurred

mostly in summer with 71%, 21%, 2% and 6% respectively. Least number of isolates were seen during autumn.

Antimicrobial sensitivity testing showed high rate of resistance to ampicillin (100%), amoxicillin-clavulanic acid (91.4%) and fluoroquinolones (>80%) among the Escherichia isolate but were highly sensitive to aminoglycosides (>80%) and imipenem (>95%). Isolates from both the OPD and IPD samples also showed similar pattern of susceptibility except that imipenem resistance was seen in 4.3% of the *E. coli* isolates from OPD.

High rates of resistance to fluoroquinolones (>80%), cotrimoxazole (100%), amoxicillin clavulanic acid (100%) and ampicillin (100%) were seen among *Klebsiella spp*. isolates from the IPD sample. Also, 16.7% of imipenem resistance was seen among the *Klebsiella isolates*. *Shigella spp*. isolates were sensitive to all the antibiotics tested but showed resistance to amoxicillin-clavulanic acid (100%), ampicillin (>85%), fluoroquinolones (>90%) and co- trimoxazole (80%). *Salmonella spp*. isolates were found to be multi-susceptible to almost all the antibiotics except some resistance was found against

amoxicillin (50%) and co-trimoxazole (50%). All the isolates were susceptible to colistin with MIC $\leq 2\mu g/ml$.

DISCUSSION

Acute diarrhoeal disease caused by enteropathogenic bacteria is an important public health problem and a major burden in developing countries like India. Diarrhoea in children is associated with both mortality and morbidity due to the changing pattern of organism and their antibiotic sensitivity. Therefore, it is important to study the etiology and identify the causative organism followed by antibiogram.

In this study, males (66.2%) were the major sufferer of bacterial diarrhoea than females (33.8%) with a ratio of 2:1 which is similar to the study done by Ansari et al. 10 Boys are more vulnerable to diarrhoeal disease than girls as boys are likely to wander off in unsanitary surroundings as compared to girls as stated by Siziya et al in his study. 11 Another reason could be due to cultural beliefs that value boys more highly, therefore making them more likely to receive timely diagnosis and treatment. Also, it was found that diarrhoeal cases were more common among the Hindu population as Imphal city is mostly populated by Hindus.

The maximum sufferers were between 1 and 3 years of age i.e 44.3%. A similar pattern of age distribution has been found in study done by Poo et al.¹² This high incidence may be related to faulty weaning, unhygienic handling and storage of milk and food, development of mouthing habits at this age, increased mobility of children due to their recently acquired walking ability and immaturity of the immune system of the body rendering it susceptible to infection by enteric pathogens. In our study, Escherichia coli was recorded as the predominant pathogen with 83.3% prevalence followed by Shigella species, Klebsiella pneumoniae and Salmonella species with 12.9%, 2.9% and 1% respectively. This pattern was similar to other studies done in India by Rathaur et al and Subhashkumar et al which had shown higher incidence of diarrhoeagenic E. coli, supporting the well documented role of *E. coli* in diarrhoeal disease. 12,13 Age related prevalence of various enteropathogens causing acute diarrhoea in children showed that diarrhoea due to enteropathogenic E. coli (EPEC) was commonly seen in the age group of 1-3 years. Similar findings were also reported by Mani et al where they have isolated 35 EPEC isolates mostly from children with diarrhoea below 2 years of age.14

In the present study, 35 *E. coli* isolates belonged to enteropathogenic *E. coli*. Serotype O55, O119, O125, O127, O128 was the most common serotype followed by serotype O26, O103, O111, O145, O157. According to Muni et al, EPEC serotypes O111, O26, O55 were associated with diarrhoea and serotype O26 was the most common serotype. ¹⁵ *Klebsiella species* mostly affected children of age group of less than six months which was

also seen in study done by Rathaur et al. Shigella flexneri (8.6%) was the most common serotype isolated followed by Shigella sonnei (3.3%) and Shigella boydii (1%). No Shigella dysenteriae was isolated in this study. Kale et al also found Shigella flexneri as the predominant serotype causing bacterial diarrhoea in children. 16

Vomiting was found to be the predominant clinical feature associated with diarrhoea followed by fever (27.1%), dehydration 23.8% and passage of blood in stool. In case of diarrhoea due to *Shigella species*, 40.7% presented with passage of blood in stool associated with vomiting and dehydration in 18.5% of the cases. All 100% patients who had diarrhoea due to Salmonella species suffered from fever. Vomiting, dehydration and passage of blood in stool were seen in 50% of the cases deciphering that fever is a common clinical feature in diarrhoea with Salmonella species occupying the majority with all the cases presenting with fever.

Seasonal distribution of bacterial diarrhoea showed majority cases in summer probably owing to the increase in temperature. Similar study conducted by Dallal et al indicated that most of the cases of diarrhoea caused by *E. coli, Shigella, Salmonella* and *Campylobacter spp.* occurred in summer though Campylobacter species was not isolated in our study.¹⁷

In this study, all the bacterial isolates showed 97.6% resistance to ampicillin, 93.8% to amoxicillin, 61.4% to ceftriaxone, 73.3% to co-trimoxazole and > 80% to both ciprofloxacin and ofloxacin. The highest antimicrobial resistance rates were found against ampicillin (97.6%) and amoxicillin (93.8%). The isolates showed maximum sensitivity to colistin (100%), imipenem (97.1%), amikacin (91.9%) followed by gentamicin and piperacillin-tazobactum. Similar pattern of resistance of the bacterial isolates to the antibiotics was also seen in study done by Manikandan et al in Tamil Nadu. 18 Jarousha et al also found that bacterial enteropathogens causing diarrhoea were susceptible tomeropenem, ceftriaxone, followed by amikacin and ciprofloxacin and almost allenteropathogens were resistant to ampicillin and amoxicillin.19

Antibiotic susceptibility pattern among the isolates from OPD and IPD samples were almost similar with not much significant difference in the sensitivity and resistance pattern. Therefore, overall high sensitivity of all the causative bacterial agents were seen against aminoglycosides, imipenem and colistin in our study. Almost all the isolate showed high resistance to amoxicillin, ampicillin, ciprofloxacin, ofloxacin and cotrimoxazole.

The knowledge of common bacterial enteropathogens causing acute diarrhoea and its resistance pattern in a particular area can help practitioners to choose an adequate antimicrobial drug to start empirical therapy in a

patient with severe diarrhoea without knowledge of a specific pathogen.

CONCLUSION

This study highlights the significant burden of bacterial diarrhoea as a cause of acute diarrhoea in children and the role of Escherichia coli as an important agent causing bacterial diarrhoea. Routine use of antibiotics for infectious diarrhoea is not routinely recommended as most of the bacterial diarrhoea are self- limiting and is associated with the risk of increasing antimicrobial resistance. The choice of the antimicrobial agent has to be made empirically for those selected cases which may require antimicrobial therapy. Lastly, since the organisms causing diarrhoea are transmitted through faeco-oral route, the ultimate approach against diarrhoea in developing countries rests on the need for improving sanitary conditions, proper hand hygiene, maintaining exclusive breastfeeding until the sixth month of life and developing safe and effective vaccines for immune prophylaxis, along with systematic parental education.

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Ethical approval: The study was approved by the

Institutional Ethics Committee

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