Enteric pathogens in pediatric patients- A Three Year Study from a tertiary care Hospital of North India

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ABSTRACT

Acute diarrhea is a common problem in children in developing countries like India leading to hospitalization. The objective of the study was to assess the etiological agents causing diarrhea in children and their sensitivity patterns to various antimicrobials. This study was carried out from January 2007- December 2009 on 860 pediatric patients attending the out patients department or admitted in the wards of Lok Nayak Hospital, New Delhi with gastroenteritis. Eight hundred and sixty stool samples were collected and examined for various pathogens.

Enteric pathogens were isolated from 321 (37.2%) samples. Parasitic pathogens were observed in 67 (7.79%) stool samples. *V.cholerae* was the most commonly isolated bacteria 134/321 (41.74%), followed by *Shigella* 90/321 (28.03%) and *E.coli* 86 (27.79%). *Salmonella spp.* (1.5%) and *Aeromonas hydrophila* (1.86%) were isolated less frequently. Resistance to amoxycillin was observed in 99% of *Vibrio*, 95% of *E. coli*, 58% of *Shigella* and 20% of *Salmonella* species. Variable degree of resistance ranging from 0% in *Salmonella* to 69% in *E. coli* was observed against ciprofloxacin, while resistance to nalidixic acid was observed in more than 90% of all types of bacterial pathogens. Resistance to ceftriaxone, cefotaxime and gentamicin was less common. Identification of the etiological agent of diarrhea in children is very important as it can help in the institution of appropriate therapy and the reduction of morbidity and mortality in these patients.

Keywords : *Salmonella, V.cholerae,* paediatric.

INTRODUCTION

Diarrheal diseases are a leading cause of morbidity and mortality in children in developing countries like India. More than 4 million children under 1 year of age die each year of infectious diarrhea, worldwide.[1] In India, one third of the total pediatric admissions in hospitals are due to diarrheal diseases and 17% of all deaths in indoor pediatric patients are diarrhea related.[1,2] Although fluid and electrolyte replacement is recommended for management of acute diarrhea, but antibacterial agents are often administered for treatment of *Salmonella* gastroenteritis and Shigellosis in children or even diarrhea due to *E. coli* or *Vibrio cholerae*. In India, most diarrhea infected patients are often treated with antibiotics prior to receiving definitive laboratory results. Indiscriminate use of these agents has resulted in the development of multidrug resistant organisms. The progressive increase in antimicrobial resistance among enteric pathogens in developing countries has become a major issue of concern.[3,4] Therefore, knowledge of the pattern of the infections and antibiotic susceptibility patterns of the etiological agents can often guide therapy when resource limitations hamper the exact diagnosis of diarrheas in pediatric patients. In the present study, we determined the prevalence of bacterial and parasitic pathogens in children less than 12 years of age and the antimicrobial susceptibility of important bacterial isolates over a period of three years (January 2007 to December 2009).

MATERIAL AND METHODS

An episode of acute diarrhea was defined as the passing of at least four loose stools in a 24 hours period for infants <1 month old and at least 3 times in a 24 hour period for older infants. Dysentery was defined as a diarrhea lasting longer than 10 days with significant fever or systemic complaints and has inflammatory cells or blood in the stool.[1,5]

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Stool samples were collected from 860 infants and children (less than 12 years) with acute diarrhea presenting to the outpatient department or admitted in the ward of Lok Nayak hospital, during January 2007 to December 2009. Single stool sample was taken from each patient. Routine stool microscopy of saline and iodine preparations was done for intestinal parasites following the standard formol ether method of concentration. A battery of culture media- alkaline peptone water, selenite F broth, Mac Conkey's agar, bile salt agar (BSA), xylose lysine deoxycholate (XLD), were used for isolation of bacterial pathogens. All the isolates were subjected to standard biochemical tests for identification. *Salmonella, Shigella*, *E. coli*, and *V. cholerae* isolates were serotyped by slide agglutination test using commercially available specific antisera (Denka Seiken Co. Ltd.). Antimicrobial susceptibility testing was performed on Mueller Hinton Agar (MHA) by Kirby Bauer's disc diffusion method.[6] The following standard antimicrobial discs were tested: amoxycillin (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), ceftriaxone (30µg), cefotaxime (30µg), nalidixic acid (30µg) and tetracycline (30µg).

RESULTS

Amongst the 860 children (1-12 years), 434 (50.46%) were of less than 2 years of age. The youngest was three months and the oldest was eleven years. The mean age of the diarrheal cases was 17.1 months. Out of 860 fecal specimens, 321 (37.2%) isolates were obtained. Amongst the 860 specimen, parasitic pathogens were observed in 67 stool samples. *Giardia lamblia* was the most frequently 21/67 (31.34%) observed non-bacterial enteric pathogen. *Ascaris lumbricoides* was found in

19/67 (28.35%) samples, *Ancylostoma duodenale* in 16/67 (23.8%) and *H. nana* was found in 7 of 67 diarrheal stools positive for parasites (Table I). *Cryptosporidium* was found in 4/67(5.9%) parasite positive diarrheal stools. However, HIV status of these 4 patients could not be evaluated due to loss of follow up.

The most frequently isolated bacterial agent was *V. cholerae* 134/321(41.74%). *V. cholerae* O1 El Tor 126/134 (94.02%) was more common than *V. cholerae* O139 1/134 (0.74%). However, 7/134(5.22%) isolates were found to be non O1 nonO139 serotypes. Serotype Ogawa 106/126 (84.12%) was more predominant than serotype Inaba 20/126 (15.87%). *Shigella* was isolated in 90/321 (28.03%) samples obtained from cases with dysentery. Amongst the *Shigella* species isolated, *Shigella flexneri* was most common (n= 66/90, 73.3%) isolate, followed by *Shigella dysenteriae* (n= 14/90, 15.5%), *Shigella boydii* (n= 8/90, 8.8%), and *Shigella sonnei* (n=2/90, 2.2%). All the *E.coli* isolates 86 (26.79%), seen as pure growth and abundant colonies were subjected to the serotyping. EPEC was most common, accounting for 45/86 (52.3%) of the *E.coli* isolated from diarrheal stools. While, enterotoxigenic, enterohemorrhagic, enteroinvasive *E. coli* were isolated in 20 (23.2%),14 (16.27%), and 7 (8.13%) cases respectively. *Salmonella* was isolated from 5 (1.5%) samples (4 *Salmonella* Typhimurium and one *Salmonella* Typhi), while *Aeromonas* was isolated from 6 (1.86%) samples (Table I).

The resistance pattern of these isolates is shown in table II. *V.cholerae* isolates showed more than 91% resistance to amoxycillin and nalidixic acid. However

Table I
Year wise distribution of enteropathogens isolated/ observed

Bacterial Pathogens	2007	2008	2009	Total	Cumulative total
Vibrio	55	27	52	134 (41.74%)	134
Shigella	30	22	38	90 (28.03)	224
E. coli	25	28	33	86 (27.79)	310
Salmonella	3	1	1	5 (1.55)	315
Aeromonas	2	3	1	6 (1.86)	321
Parasitic Pathogens					
Ascaris	8	6	5	19 (28.3)	19
Giardia	5	7	9	21 (31.3)	40
H. nana	3	2	2	7 (10.4)	47
Hookworm	4	5	7	16 (23.8)	63
Cryptosporidia	0	3	1	4 (6)	67

Table II
Resistance patterns observed in bacterial pathogens

Organism	Cipro- floxacin	Nalidixic Acid	Amoxy- cillin	Cefo- taxime	Ceftria- xone	Genta- micin	Tetra- cycline
Vibrio	5%	91.7%	99%	3.8%	0.5%	2%	9%
Shigella	4.7%	97%	58%	3%	2%	4%	-
E. coli	69%	91%	95%	5%	0%	11%	-
Salmonella	0%	20%	20%	20%	20%	20%	-
Aeromonas	19%	95%	97%	6%	5%	15%	-

resistance to ceftriaxone, ciprofloxacin, gentamicin, cefotaxime, and tetracycline varied from 0.5% to 9%. The isolated strains of *Shigella* were highly resistant (97%) to nalidixic acid, while resistance to amoxicillin was demonstrated by 58% strains. The percentage resistance shown against gentamicin, ciprofloxacin ceftriaxone and cefotaxime, was 4%, 4.7%, 2% and 3% respectively. Amongst 5 salmonella isolates (4 *Salmonella* Typhimurium and one *S*. Typhi), only one (*S*. Typhimurium*)* showed multidrug resistance. This strain was resistant to amoxycillin, gentamicin, ceftriaxone, cefotaxime, and nalidixic acid, while sensitive to ciprofloxacin. Rest four isolates were all sensitive to all antibiotics. The resistance pattern of *Aeromonas* revealed 5-19% resistance to ciprofloxacin, gentamicin, ceftriaxone and cefotaxime, whereas resistance to amoxicillin and nalidixic acid was 95% and 97% respectively.

DISCUSSION

Our hospital is a 1690 bedded tertiary care hospital with thickly populated catchment areas and most of the patients fall in the low and middle socio economic status. This could have led to higher isolation rate of bacteria and parasites in this study (40.4%) as compared to another study from Delhi.[1] In agreement with other reports, *Shigella* and *E. coli* were the most common isolates in children.[7,8] The prevailing serotype of *E. coli* leading to childhood diarrhea in our population was EPEC serotype O111 (10.5%) and serotypes O119 (8%). The association between EPEC and infantile diarrhea has already been established[9] and our findings reconfirm this fact. In various studies, the susceptibility pattern of diarrhoea-causing bacteria, including *Shigella, E. coli, Vibrio cholerae* and *Salmonella* to gentamicin, nalidixic acid and

norfloxacin was found to be low while resistance to ampicillin and tetracycline was found to be more than 50%.[10,11] In our study, we also noted very high degree of resistance to amoxycillin and nalidixic acid. However resistance to gentamicin, amikacin and third generation cephalosporins was low. Hence it is required to keep these drugs in reserve and to minimize their overuse to prevent development of resistance to theses therapeutic agents.

Besides the well-known causes of enteric infections and diarrheal diseases, parasites and viruses are important etiological agents, which are often overlooked. On stool examination for ova and cyst. *Ascaris* was found in 28.35% (19/67) , *G. lamblia in* 31.34 % (21/67), *A. duodenale* in 23.8% (16/67), and *H. nana* in 10.4% (7/ 67) of the cases. Cryptosporidia were found in only four cases. These findings correlate with the findings from other workers in India.[12] It is important to rule out parasitic causes, since unnecessary treatment of such cases with empirical antibiotics will needlessly increase the burden of drug resistance.[1] Although bacterial agents are important causes of diarrhea in children but still indiscriminate use of potent antibiotics should be avoided as it can lead to acquisition of multiple drug resistance. Increasing antibiotic resistance in bacterial pathogens is now a common phenomenon in developing countries, including India. This is attributed to the injudicious use of antibiotics and frequent use of over-the-counter drugs without proper medical supervision.[1,3] Recently few studies have reported the circulation of highly resistant -lactamase-producing bacteria in public water supplies and sewage water samples in New Delhi, which is a major health concern.[13] This again may be related to imprudent overuse of antibiotics in our set up. Hence

regular monitoring of antimicrobial resistance profiles should be done and an antibiotic policy should be formulated in every hospital, to prevent development and spread of highly resistant strains.

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