Original Research Article

Aerobic Bacteriological Study with their Antibiogram in Children with Acute Diarrhoea in North East Karnataka, India

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A B S T R A C T

K e y w o r d s *Escherichia coli,* *Vibrio cholerae,* *Shigella species,* Antibiogram in Children with Acute Diarrhoea	Diarrhoea is endemic in India and accounts for morbidity and mortality among children aged 1-5 yrs, particularly in India due to the unprotected water supply, contamination of food and milk, improper disposal of human excreta, poor standards of personal hygiene, adverse climatic conditions and rampant malnutrition especially in rural areas. 100 cases of diarrhoea were enrolled in the study. Brief history and examination were done in a predesigned format. All were subjected for both macroscopic and microscopic examination, later hanging drop and gram's staining was done. Suspected pathogens were identified by standard methods and antibiotic sensitivity was done using Kirby Bauer disc diffusion method. Totally, 100 samples processed, the isolation rate of enteric pathogens were 84%. The common pathogens isolated was Entero Pathogenic *Escherichia coli* 32(33.68%), *Shigella* species 22(23.15%), *Vibrio cholerae* 19(20%). Entero Pathogenic *Escherichia coli* showed high prevalence in 13-24 months age group, *Shigella* in 25-36 months and *Vibrio* in 36-60 months age group. All the 3 species showed sensitivity to Ciprofloxacin, Gentamicin, Cephalosporins and Norfloxacin respectively. The most common etiology of acute diarrhoea in 1-5 year age group is Enteropathogenic *Escherichia coli*, *Shigella* species and *Vibrio cholerae* respectively.

Introduction

Diarrhoea is endemic in India and accounts for a high morbidity and mortality among children, particularly in developing countries like India due to the unprotected water supply, contamination of food and milk, improper disposal of human excreta, poor standards of personal hygiene, adverse climatic conditions and rampant malnutrition especially in rural areas.

Diarrhoea in children can be acute or persistent / invasive or secretory due to various causes like bacterial, viral, parasitic, fungal or combination of these. Among these causes, bacterial infection is the most common cause of acute diarrhea. The organisms causing bacterial infection are *E. coli*, *Vibrio cholerae*, *Salmonella*, *Shigella* and *Campylobacter,* etc.

It was observed that *E. coli* was found in 100% of cases, 23% were pathogenic followed by *Vibrio cholerae*, *Klebsiella pneumoniae*, *Proteus morgani*, *Pseudomonas aeruginosa*, *Campylobacter* and *Proteus mirabilis* in less than 5 yrs age group. The prevalence of *Vibrio cholerae* was31.7% and 23.4% occurred in the age group of less than two years, 41.4% in 2–5 years and 31.5% in more than 5–12 years (Aggarwal *et al.,* 1989).

Shigella was isolated from 56/634 diarrhoeal samples (8.8%) of which *S. sonnei* was the predominant species (55%). Occurrence of *Shigella* was highest in the 2–5 years age group (70.9%) and highest in summer (73.2%) with the most frequent clinical manifestation being abdominal pain (67.8%) Ezzat Ollah Ghaemi *et al.* (2007).

Hence the need for knowing the prevalence of pathogens in children was took up. The study was conducted to find out the prevalence of acute diarrhoeal diseases due to aerobic bacteria in children aged between 1-5yrs by isolation and identification of pathogenic bacteria from stool samples and to study the antibiotic sensitivity pattern of the isolates.

Materials and Method

The study was conducted at Basaveshwara Hospital, attached to M.R. Medical College, Gulbarga from Jan 2011 to Dec 2011.A total of 100 cases of acute diarrhoea, who came for the treatment to the above mentioned hospital were taken up for the study. Children with acute diarrhoea of less than 72hours duration were included in the study. Patients on antibiotic therapy during preceding three days and complicated illness were excluded. Children aged between 1 and 5 years were included in the study. The stool samples were examined macroscopically for consistency, colour, presence of mucus, blood. Grams stain and hanging drop were done and all the stools samples were subjected for plating in Mac Conkey agar, xylose lysine deoxycholate agar and thiosulphate citrate bile sucrose agar. Enrichment media's like selenite F broth and alkaline peptone water were used for *Salmonella*, *Shigella* and Vibrio and after 6hrs of incubation it was subcultured into selective media. The colonies were subjected to Gram's stain, hanging drop, biochemical reactions. Special tests were done for *Vibrio*, *Salmonella* and *Shigella*. Procedures were followed according to standard methods given in practical manual of medical microbiology (Mackie and McCartney). Serotyping was done for all the species isolated with specific antisera. The sensitivity was performed over Mueller Hinton agar plates by disc diffusion method recommended by Kirby – Bauer. Sensitivity was performed using control strains of *Staphylococcus aureus* ATCC25923, *E. coli* ATCC25922, and *Pseudomonas* ATCC 27853.

Results and Discussion

A total of 100 stool samples were processed during the study period of one year for bacterial pathogens.

Table 1 shows that the highest number of cases was encountered between 13-24 months of age group. Male preponderance was noted. Of 100 cases, 59 were males and 41 females. Male: female ratio-1.43:1.

Table 2 shows out of 100 samples received, culture was positive in 95 cases and no growth was present in 5 cases. Out of the total 95 organisms, 36(37.89%) strains of *Escherichia coli*, 23(24.2%) strains of *Shigella*, 19(20%) strains of *Vibrio* and

other organisms like *Klebsiella, Enterobacter, Proteus, Pseudomonas* were also isolated.

Table 3 shows out of the 95 culture positive samples, 80 organisms were identified as possible etiological agents. The prevalence rate of the isolated organisms was 84%.The EPEC strains was 32(33.68%), *Shigella* sp's was 22(23.15%), *Vibrio cholera* was 19(20%) and others like *Klebsiella, Enterobacter, Proteus* and *Pseudomonas* were isolated.

Table 4 shows enteropathogens isolated from 95 culture positive showed highest incidence of EPEC in 13–24 months of age and the incidence showing lower in higher age group, followed by *Shigella* sp's in 25–36 months age group and *Vibrio* in 36–60 months age group respectively.

Table 5 reveals the antibiotic sensitivity pattern of EPEC, *Shigella* and *Vibrio* in the 80 pathogens isolated. It was observed EPEC strains are sensitive to ciprofloxacin, cefotaxime, gentamicin and tetracycline. Also *Shigella* was sensitive to ciprofloxacin, cefotaxime, gentamicin and nalidixic acid. Lastly *Vibrio* strains were sensitive to cefotaxime, gentamycin, tetracycline, ciprofloxacin, co-trimoxazole and furazolidine respectively.

Diarrhoea in children is important in leading countries since it is associated with both mortality and morbidity due to the changing pattern of organisms and their antibiotic sensitivity. Hence it is important to study the etiology and their isolation followed by antibiogram.

In the present series the isolation rate of enteric bacteria was 84%. These results are consistent with the studies shown by Mubashir (1988) and Ogbonnaya Ogbup *et al.* (2008) respectively.

Prevalence of *Shigella* in our study was 23.15%.These results are more as compared to reports by Mamatha Ballal *et al.* (1992) and Ezzat Ollah Ghaemi *et al.* (2007).

The isolation rate of *Vibrio cholerae* in our study was 20%, with highest attack rate of cholera in children above 2 years of age. The age -wise distribution of *Vibrio cholerae* correlates with that stated by WHO group.

All strains in our study showed biotype ElTor and serotype Ogawa. Seasonal variations were seen with maximum occurrence of cases in early monsoon period. These findings are slightly higher with reports like Khatua *et al.* (1984), Anand *et al.* (1996), Mamatha Ballal and Shivananda (2002). Other organisms like *Pseudomonas aeruginosa, Enterobacter, Proteus, Klebsiella* were isolated in 8.06% of cases. These organisms may be normal inhabitants of the intestine but it was present as predominant growth hence considered as pathogens.

The isolation rates in other studies vary from 10–33% as mentioned by Khanna (1977) and Naresh Gupta *et al.* (1985). Pathogenicity of these organisms in causation of diarrhoea is controversial. However, these suspected pathogens when isolated either in pure culture or in significant number and in the absence of other definitive pathogens their presence cannot be ignored.

In the present study, EPEC showed maximum resistance to ampicillin (91%), furazolidine (91%) and nalidixic acid (71%) and maximum sensitivity was observed to cefotaxime (100%), ciprofloxacin (69%) and norfloxacin (79%).

Table.1 Age distribution of cases

Age in months	Males		Females		Total		M:F
	No.	%	No.	%	No.	%	
13-24	26	26.0	22	22.0	48	48.0	1.18:1
25-36	17	17.0	11	11.0	28	28.0	1.54:1
36-60	16	16.0	8	8.0	24	24.0	2:1
Total	59	59.0	41	41.0	100	100.0	1.43:1

Table.2 Showing various organisms isolated in 95 cases

Organism	Number of organisms isolated	Percentage (%)
Escherichia coli	36	37.89
Shigella	23	24.21
Vibrio	19	20.0
Klebsiella	2	2.10
Enterobacter	6	6.31
Proteus	6	6.31
Pseudomonas	3	3.15
Total	**95**	**100**

Table.3 Pathogens isolated in 95 cases of stool samples

Pathogen	No. isolated	Percentage (%)
EPEC	32	33.68
Shigella sp	22	23.15
Vibrio cholera O1	19	20.0
Klebsiella pneumoniae	2	2.10
Enterobacter aerogenes	2	2.10
Proteus mirabilis	2	2.10
Pseudomonas aeruginosa	1	1.05
Total	**80**	**84**

Table.4 Isolation rate of enteropathogens in different age groups

Pathogens	Age in months						Total	
	13-24		25-36		37-60			
	No	%	No.	%	No.	%	No.	%
EPEC	26	27.36	5	5.26	1	1.05	32	33.68
Shigella sp	4	4.21	15	15.78	3	3.15	22	23.15
Vibrio cholerae O1	0	0	8	8.42	11	11.57	19	20
Klebsiella pneumoniae	0	0	2	2.10	0	0	2	2.10
Enterobacter aerogenes	2	2.10	0	0	0	0	2	2.10
Proteus mirabilis	2	0	0	0	0	0	2	2.10
Pseudomonas aeruginosa	1	1.05	0	0	0	0	1	1.05

Table.5 Table showing susceptibility pattern of isolates

Antibiotics	EPEC(32) (No./%)	*Shigella*(22) (No./%)	Vibrio(19) (No./%)
Ampicillin	3(9.37)	0(0)	11(57.89)
Chloramphenicol	9(28.12)	7(21.87)	13(68.42)
Co-Trimoxazole	16(50)	5(15.62)	15(78.94)
Ciprofloxacin	22(68.75)	21(95.45)	16(84.21)
Furazolidine	3(9.37)	14(63.63)	16(84.21)
Tetracycline	19(59.37)	0(0)	14(73.68)
Gentamycin	19(59.37)	22(100)	14(73.68)
Cefotaxime	32(100)	22(100)	16(84.21)
Nalidixic acid	9(28.12)	21(95.45)	0(0)
Norfloxacin	25(78.12)	10(45.45)	14(73.68)

Similar susceptibility pattern were observed by Lankeshwar Tewari and Surendra (1982) and Usha P. Kamlakar (1995) *Shigella* strains are particularly noted for their multidrug resistance which may result from the selection of resistant mutants because of wide spread use of antimicrobials. In the present study, *Shigella* species were found to be resistant to ampicillin (100%), tetracycline (100%), co-trimoxazole (15%), and norfloxacin (45.45%). Multidrug resistant *Shigella* has been reported from different parts of India (Pal, 1984; Panigrahi *et al.,* 1984). A significantly low resistance to Nalidixic acid (4.5%), third generation cephalosporins (0%) and gentamicin (0%) was noted. Continuing monitoring of the susceptibility pattern of *Shigella* species is important to notice the emergence of drug resistance.

In the present series *Vibrio cholerae* showed resistance to nalidixic acid (100%), ampicillin (57%) and sensitive to cephalosporins (84.21%), tetracycline (73.61%) and ciprofloxacin (84.21%). This was comparable with study done by Mamatha Ballal and Shivananda (2002). Multidrug resistance with ElTor strains of *Vibrio cholerae* was reported as early as 1977−1978 from Tanzania by Mhalu *et al.* (1979).

In the present study, it is important to state that our inability to screen for the other potential enteric pathogens particularly (viruses, parasites, anaerobes), the low population size of cases and the limited number of antibiotics used in the study were among the limitations of this study.

Hence we conclude that many organisms other that EPEC are also the causative agents of acute diarrhoea in children above 1 yr, possible methods have to be made to isolate them and antibiotics should be used judiciously to control the emergence of resistant strains and limit the acquisition of additional antibiotic resistant genes in the existing strains.

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