ORIGINAL RESEARCH PAPER

Microbiology

Aerobic Bacterial Isolates and their Autobiogram from Stool Samples of Infants and young Children with Acute Gastroenteritis in Patients Attending A Tertiary Care Hospital in Jharkhand

KEY WORDS: Diarrhoea, Bacterial isolates, Antibiogram.

Hassan A A	Tutor, Department of Microbiology, JLNMCH, Bhagalpur.
Singh S	Tutor, Department of Microbiology, AIIMS, Patna
Sarfraz A	Senior Resident, Department of Microbiology, AIIMS, Patna
Agrawal A K	Associate Professor and Head, Department of Microbiology, RIMS, Ranchi

ABSTRACT

Diarrhoea accounts for a major cause of morbidity and mortality in infants and young children. *Escherichia coli* is one of the leading causes of diarrhoea in young children. Drug resistance among the bacteria isolated from stool samples is on rise and is a major hurdle in proper treatment. Hence a study was done to find out the prevalence of acute diarrhoeal diseases due to aerobic bacteria in infants by isolation and identification of pathogenic bacteria from stool samples and to study the antibiotic sensitivity pattern of the isolates.

MATERIAL AND METHOD- A total of 120 cases of diarrhoea was studied from September 2012 to September 2013, in the Department of Microbiology at Rajendra Institute of Medical Sciences, Ranchi.

RESULTS - *Escherichia coli* was found to be the predominant organism present in 44.2% of the cases. This was followed by *Shigella* spp. which accounted for 11.6% of cases. Salmonella spp. *Klebsiella spp.* were isolated from 6.7 % and 8.3% of samples respectively. Furazolidone was the most sensitive antibiotic whereas nalidixic acid was least sensitive among all antibiotics used.

INTRODUCTION

Acute gastroenteritis or infectious diarrhoea is one of the leading causes of illnesses and deaths in infants and children throughout the world, especially in developing countries. It denotes the infections of the gastrointestinal tract caused by bacterial, viral, or parasitic pathogens including dietary abuses. Many of these infections are food borne illnesses.

Diarrhoeal disorders in childhood accounts for a large proportion, about 18% of childhood deaths, with 1.5 million deaths occurring per year globally, making it the second most common cause of child deaths worldwide. The World Health Organization (WHO) and UNICEF estimate that almost 2.5 billion episodes of diarrhoea occur annually in children <5 yr of age in developing countries, with more than 80% of the episodes occurring in Africa and South Asia[1] . In developing countries on an average, every child suffers 3.3 episodes of diarrhoea per year, but in some areas the average exceeds 9 episodes per year[2]. It causes about 11% of child deaths worldwide. In India acute diarrhoeal disease is responsible for about 8% of deaths in children who are under-5 years of age group. During the year 2011, about 10.6 million cases with 1,293 deaths were reported in India[3]. In India children under 5 years of age suffer from 2-3 episodes of diarrhoea annually[4].

Diarrhoea is defined as the passage of three or more loose or liquid stools per day or more frequent passage than is normal for the individual.[5] Many microorganisms are responsible for diarrhoea, the most common causes being Viruses (Rotavirus, Caliciviruses, Astrovirus, Enteric adenovirus, Noroviruses previously called Norwalk viruses), Pathogenic Bacteria *(Escherichia coli, Salmonella spp., Shigella spp., Vibrio cholerae, Campylobacter jejuni, Clostridium difficile, Yersinia enterocolitica, Aeromonas spp., Staphylococcus aureus, and Bacillus cereus), Parasites (Entamoeba histolytica, Giardia lamblia, Cryptosporidium parvum, and Strongyloides stercoralis), and fungus (Candida albicans)*[6]

MATERIAL AND METHODS

The study was conducted in the Department of Microbiology, Rajendra Institute of Medical Sciences, Ranchi from September 2012 to September 2013 to find out the prevalence of acute diarrhoeal diseases due to aerobic bacteria in infants by isolation and identification of pathogenic bacteria from stool samples and to study the antibiotic sensitivity pattern of the isolates.

Inclusion criteria :
1. Children aged between 1 month to 5 years.
2. Diarrhoeal cases with watery diarrhoea for upto 15 days of

duration.

Exclusion criteria :
1. Diarrhoeal cases with greater than 15 days duration.
2. Passage of frequent formed stool or pasty stool were excluded.

Saline wet mount of fresh stool was made and examined under microscope for presence of pus cells and RBCs. Samples were subjected to hanging drop and Gram stain. Enrichment was done in Selenite F broth and incubated at 370C for 6 hrs. After enrichment, subculture was done on MacConkey Agar and Xylose lysine deoxycholate (XLD) agar and further incubated at 370C overnight. The organisms were identified by performing the following biochemical tests – Catalase, Oxidase, Indole test, Motility in semisolid agar, Citrate utilisation test on Simmon's citrate agar, Urease test on Christensen urea agar, Acid/gas/H2S production on Triple sugar iron agar, Nitrate reduction test. The sensitivity was performed on Mueller Hinton agar plates by Kirby Bauer disc diffusion method following the CLSI guidelines.

RESULT AND DISCUSSION

The study was extended over a period of 12 months, from September 2012 to September 2013. During the period of study, a total number of 120 cases were obtained according to the inclusion and exclusion criteria.

Age and Sex Distribution - In this study the maximum number of cases [83 (69.2%)] of diarrhoea occurred in 6-24 months of age group. This was followed by the lower age group of infants under 6 months with 23cases (19.2%). In 25 to 36 months age group, 7 cases (5.8%) and in 37 to 48 months age group 4 cases (3.3%) were seen. The least infection was found in higher age group between 49 to 60 months 3 (2.5%).

Age groups in months	Male		Female		Total	
	No. of cases	Percentage (%)	No. of cases	Percentage (%)	No. of cases	Percentage (%)
0-6	12	18.4	11	20.0	23	19.2
7 – 24	45	69.2	38	69.1	83	69.2
25 – 36	4	6.2	3	5.5	7	5.8
37 – 48	2	3.1	2	3.6	4	3.3
49 – 60	2	3.1	1	1.8	3	2.5

Boys had higher incidence of diarrhoeal cases which were 65 (54.2%) as compared to girls with 55 cases (45.8%). The male to

female ratio was 1: 0.85. Pus cells > 10/HPF was seen in 31.7% of the cases and RBC's were seen in 12.5% of the cases.

Bacteriology - Stool culture for bacteria was carried out in all the study cases, and pathogenic organisms were seen in 70.8% of the cases. *Escherichia coli* was found to be the predominant organism present in 44.2% of the cases. This was followed by Shigella spp. which accounted for 11.6% of cases. *Salmonella spp. Klebsiella spp.* were isolated from 6.7 % and 8.3% of samples respectively. In 29.2% of the remaining cases, no enteropathogenic bacteria could be detected.
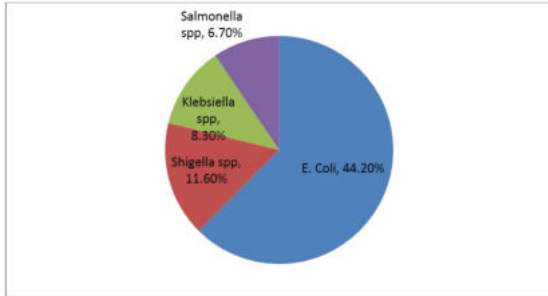

Antibiogram - *Escherichia coli* (n=53)

Among *Escherichia coli* isolates majority of the strains were sensitive to chloramphenicol (98.1%). The isolates were resistant to cotrimoxazole (41.5%) ciprofloxacin (39.6%), and nalidixic acid (30.2%).

Shigella spp. (n=14)

Majority (92.9%) of *Shigella* strains were found to be sensitive to ciprofloxacin. The isolates were mostly resistant to nalidixic acid (57.1%) followed by co trimoxazole 50%.
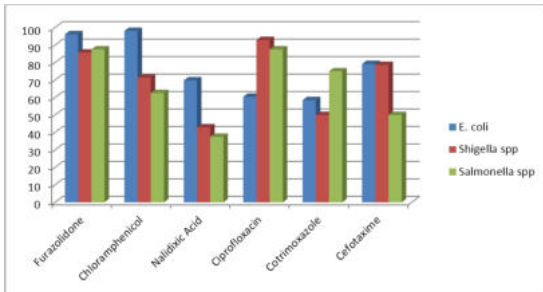
Salmonella spp. (n=8)

Among the *Salmonella* spp. isolated, most of the strains were sensitive to Furazolidone (87.5%). The isolates were also sensitive to ciprofloxacin in 87.5% followed by chloramphenicol (62.5%). In the present study resistance to nalidixic acid, cefotaxime and cotrimoxazole was 62.5%, 50% and 25% respectively.

DISCUSSION

Most diarrhoeal episodes occur during the first 2 years of life, and the incidence of acute diarrhoea below 2 years was 88.4%. A similar highest incidence during the first 2 years was reported by Sood [7] (1963) 91%, Joshi CK, et al [8] 73.8% Ansari S, et al [9].

In the present study the male to female ratio was 1: 0.85. Similar observations were made by Karmarkar et al [10] (M; F - 1:0.83), Ansari S, et al [9] (M:F- 1:0.56).

In the most carefully conducted studies of acute diarrhoeal diseases in different parts of the world, enteropathogenic bacteria have been detected in 30 to 67%   Bhat *et al* [11]; D.Sen et al [12]; Cravioto *et al* [13]; A.R.Ghosh *et al* [14]. In our study *E. coli* was isolated in 44.2% of the cases. A.R.Srivatsava *et al* [15] and Rao and Murthi [16] isolated diarrhoegenic *E.coli* in 37.27% and 38.67% cases respectively which is comparable with this study.

Shigella spp. was isolated in 14 cases (11.7%) out of 85 culture positive cases. In a study by Thapa et al [17] at Chandigarh, *Shigella*

spp. was isolated in 10% of cases. Srivastava et al [15] reported Salmonella infections in 4.4% of their cases. In a similar study by Naruka et al [18] Salmonella was isolated in 7.5% of patients which is concurrent with this study (7.3%).

E. coli isolates in the present study showed less susceptibility to first line antibiotics, such as nalidixic acid, cotrimoxazole. However, majority of strains were susceptible to chloramphenicol (98.1%). High degree of resistance to commonly used antibiotics was also noted by Das et al.[19] who reported 33% of *E. coli* strains to be resistant to norfloxacin, 33% to gentamicin, 44% of the resistant to cefotaxime and 77% to nalidixic acid. Ansari S et al [9] reported 33.3% of the isolates resistant to nalidixic acid, cotrimoxazole and amikacin each.

Resistance to nalidixic acid has increased whereas resistance to furazolidone has decreased over the years. This is due to widespread use of nalidixic acid as the first line agent for empirical treatment of infectious diarrhoea in children and ever decreasing use of furazolidone. Amikacin was most effective and an overall resistance of amikacin was 3.1%.

Krishnan R et al [20] reported a majority (95.6%) of *Shigella* strains sensitive to cefotaxime and amikacin. According to Ansari S et al 9, 87.5% of Shigella isolates were susceptible to fluoroquinolones and amikacin.

Majority of *Salmonella* strains isolated in this study were found to be sensitive to furazolidone and ciprofloxacin.

CONCLUSION

Childhood acute diarrheal diseases remain a public health concern over the years. Improvement of environmental sanitation and domestic hygiene and raising the socioeconomic status of the population will contribute to the elimination of the underlying causes of acute diarrhoea. The incidence is more during the first 24 months of life so infants should be breastfed. The antimicrobial susceptibility patterns should be assessed periodically to guide antimicrobial therapy.

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