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Aerobic Bacterial Isolates and Their Antibiogram from Stool Samples of Infants and Young Children with Acute Gastroenteritis in Patients Attending a Tertiary Teaching Hospital in Central India

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Abstract: ***Introduction:*** There are multiple etiologies responsible for infectious gastroenteritis causing acute diarrhea which are often under diagnosed. Also acute diarrhea is one of the major causes of morbidity and mortality among children less than 5 years of age. ***Materials & Methods:*** The present study of bacteriology on aerobic bacteria causing diarrhea and their antibiogram from stool samples of infants and children less than 5 years of age was carried out in the Department of Microbiology, IIMSAR, and Indore from January 2018 to November 2018. Microscopic examination of stool sample was made by paring saline wet mount. Preliminary identification on culture media as required, biochemical tests and antibiotic sensitivity were done all the cases. ***Results:*** During the period of study the total numbers of 120 cases were included. In the present study the maximum number of cases of diarrhea occurred in 6-24 months of age group 83 (69.2%). Male child or boys had higher diarrhea cases 78 (65%) as compared to girls 42 (35%). Majority of the children 84 (70%) were breast fed combined with artificial feeds. Escherichia coli were found to be the predominant organism found in 42.5% of the cases. Salmonella was isolated from 7.5% of cases. Klebsiella 6.67% and Shigella 11.67% of the cases were isolated. Among Escherichia coli isolates majority of the strains were sensitive to chloramphenicol (94.12%) amikacin (92.16%) and gentamycin (84.31%). ***Conclusion:*** In the present study, E. coli was the commonest microorganism followed by Shigella. Thus, the importance of safe water and food hygiene would be most important intervention to prevent acute gastroenteritis in children.

Keywords: Acute gastroenteritis, Infectious diarrhea, Children, Antibiogram, Stool samples.

INTRODUCTION

Acute gastroenteritis or infectious diarrhea is one of the leading causes of illnesses and death in infants and children throughout the world, especially in developing countries. It denotes infections of the gastrointestinal tract caused by bacterial, viral, or parasitic pathogens including dietary abuses. Many of these infections are food borne illnesses. The most common manifestations are diarrhea and vomiting, which can also be associated with systemic features such as abdominal pain and fever [1, 2].

Global and national estimates clearly indicate that diarrheal disease is a major public health concern [3]. According to the World Health Organization (WHO), diarrheal diseases are the second leading cause of death (~760,000 per year) in children <5 years of age. Recent studies suggested that diarrheal diseases are the leading cause of childhood deaths in developing countries. Infectious agents, viz., viruses (Rotavirus, Adenovirus, Hepatitis A&E, and Norwalk), bacteria (E. coli, Salmonella, Shigella, and Vibrio), protozoa (Giardia, Cryptosporidium, Cyclospora, Microsporidia, Isospora), etc., are usually responsible for serious diarrheal disease outbreaks [4, 5]. Among these pathogens, diarrheagenic E. coli (DEC), Rotavirus, and Shigella spp. are the major contributors of childhood morbidity and mortality [6, 7].

Diarrhoeal disease is the second leading cause of death in children under five years old, and is responsible for killing around 525 000 children every

year. Diarrhoea can last several days, and can leave the body without the water and salts that are necessary for survival. In the past, for most people, severe dehydration and fluid loss were the main causes of diarrhoea deaths. Now, other causes such as septic bacterial infections are likely to account for an increasing proportion of all diarrhoea-associated deaths. Children who are malnourished or have impaired immunity as well as people living with HIV are most at risk of life-threatening diarrhoea [8].

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). Frequent passing of formed stools is not diarrhoea, nor is the passing of loose, "pasty" stools by breastfed babies. There are three clinical types of diarrhoea [8].

- Acute watery diarrhoea – lasts several hours or days, and includes cholera;
- Acute bloody diarrhoea – also called dysentery; and
- Persistent diarrhoea – lasts 14 days or longer.

Gastrointestinal infections are classified into three major syndromes: watery diarrhoea (which is known as gastroenteritis, appears when the infection occurs in the small intestine), dysentery (a type of gastrointestinal infections in large intestine represented with small faecal volume with mucus and blood). Diarrhoea can also be classified based on many other factors, such as duration of illness (acute vs. chronic), pathophysiological mechanisms (eg. osmotic vs. secretory), volume (eg. small vs. large), and stool characteristics (watery, fatty, and bloody)[9, 10].

Study was done to estimate the prevalence of acute diarrheal diseases caused by aerobic bacteria Escherichia coli, salmonella spp., and shigella spp. in faecal samples of infants and children and to see the antibiotic sensitivity pattern of the isolates, since these pathogenic bacteria exhibit changing pattern of antibiotic sensitivity.

MATERIALS AND METHODS

The present study of Bacteriology on aerobic bacteria causing diarrhea and their antibiogram from stool samples of infants and children less than 5 years of age was carried out in the Department of Microbiology, IIMSAR, and Indore from January 2018 to November 2017. Institutional Ethics committee permission was taken.

Inclusion criteria

- Children aged between 1 month to 5 years
- Diarrheal cases with watery diarrhea for upto 15 days of duration
- Only those samples that are taken under aseptic precautions

Exclusion criteria

- Diarrheal cases with greater than 15 days
- Passage of frequent formed stools and pasty stools
- Diarrheal cases with other causes and also food poisoning
- Children with other systemic infection

Collection of Samples

Stool samples of all admitted and O.P.D. attending children between 1 month to 5 years of aged presenting with acute diarrhea and fulfilling the inclusion and exclusion criteria were included in the present study. Freshly passed stool samples without any added preservatives were collected in sterile containers. Cases are patients attending Pediatric OPD at IIMSAR, Indore for diarrhea (defined as the passage of 3 or more loose or liquid stools per day). All the specimens collected were transported without delay to the laboratory for further processing in the sterile container. Samples were completely labeled by the necessary data (date and time of collection, patient name, and sample type).

The samples collected were processed as follows:

- Direct microscopic examination of the specimen collected.
- Inoculation of the samples onto different culture media for isolation of organisms.
- Preliminary identification on culture media as required
- Biochemical tests
- Antibiotic sensitivity

Direct Microscopy

Microscopic examination of stool sample was made by paring saline wet mount and Iodine wet mount to look for leucocytes, RBC's, ovas and cysts.

Culture

Stool samples were inoculated on MacConkey Agar, xylose lysine deoxycholate (XLD) agar and deoxycholate citrate agar (DCA) Enrichment was done in Selenite F broth and incubated at 37°C for 6 hrs. After enrichment subculture was done in the above media and further incubated at 37°C overnight.

For isolation of most bacterial pathogens from stool, the stool pecimen was directly plated on to a nonselective medium MacConkey's agar (MAC), a moderately selective medium for Salmonella Shigella (XLD agar) and (DCA agar). Enrichment culture was done in selenite F broth for *Salmonella* and *Shigella.* Generally 2 loopfuls (larger amount are taken since there are only a few -athogen present and their growth can be overcome by the normal commensals present in stool) were inoculated on nonselective media, 3-4 loopfuls on highly selective media and 2-3 loopfuls into enrichment broths.

MacConkey's Agar

Oxidase test was done for all the non lactose fermenting colonies.

- The small and pale non lactose fermenting colonies of *Shigella* or slightly larger colonies of *Salmonella* spp. and *E.Coli* show oxidase negative reactions, whereas
- *Pseudomonas, Vibrio cholerae, Aeromonas* and *Pleisomonas* give oxidase positive reaction. So that they are excluded.

XLD

Pink colonies with black centres suggestive of Salmonella or pink colonies without black centre suggestive of *Shigella* and non H_2S producing *Salmonella.* Most Escherichia colonies were seen as yellow colonies. Proteus forms yellow colonies with black centres.

Preliminary identification

Staining from culture

Colonies on MacConkey's agar and XLD agar plates were stained by gram's staining method and the morphology, gram reaction and arrangement of the microorganisms were noted.

Motility: Colonies from the solid media was inoculated in peptone water and incubated for 2 hours. Hanging drop method was used for demonstration of motility. A drop of the broth culture was taken on the centre of a cover slip and a cavity slide placed over it with the cavity covering the centre of cover slip and inverted. The preparation was then observed under low and high power lens of the microscope.

Bio-chemical tests - The isolates were confirmed by for production of enzymes by biochemical tests like Catalase, Oxidase, Urease, Arginine dihydrolase, Lysine decarboxylase, Ornithine decarboxylase and Nitrate reduction. For substrate utilization Citrate test was done.

For metabolism of proteins and aminoacids

- Indole production test Utilization of carbohydrates
- Inoculation on triple sugar iron Agar
- Oxidation fermentation test using 1% glucose (Hugh Leifson method)
- Sugar fermentation test using following sugars : Glucose, sucrose, lactose, maltose, mannitol, xylose, dulcitol, arabinose
- Methyl red and Voges Proskauer test
- Antibiotic sensitivity testing by Kirby Bauer disc diffusion test.

Identification of Shigella was done on the basis of Oxidase test, hanging drop test, Mannitol fermentation test, Nitrate reduction test, TSI test and Methyl red test.

Antibiotic sensitivity testing

Antibiotic sensitivity testing was done by modified Kirby-Bauer disc diffusion method as per the Clinical Laboratory Standards Institute guidelines.[11] The appropriate antimicrobial-impregnated disc procured from Himedia was placed on the surface of the agar, using forceps to dispense each antimicrobial disk one at a time. The disks were pressed down with forceps to ensure complete contact with the agar surface. Discs were applied evenly and no closer than 24 mm from each other and 15 mm from the plate margin. A maximum of 5 sec were placed in a 90 mm plate.

RESULTS

The study was extended over a period of 11 months, from January 2018 to November 2018. During the period of study the total numbers of 120 cases were included. In the present study the maximum number of cases of diarrhea occurred in 6-24 months of age group 83 (69.2%) followed by the lower age group of infants under 6 months was 23 (19.2%), in 25 to 36 months age group 7 (5.8%) cases and in 37 to 48 months age group 4 (3.3%). The least infection was found in higher age group between 49 to 60 months 3 (2.5%).

Table-1: Age and sex distribution of cases

Age groups in months	Male		Female		Total	
	No. of cases	Percentage	No. of cases	Percentage	No. of cases	Percentage
< 6	18	23.08	8	19.05	26	21.67
5 - 24	42	53.85	28	66.67	70	58.33
25 - 36	8	10.26	3	7.14	11	9.17
37 - 48	6	7.7	2	4.76	8	6.67
49 - 60	4	5.13	1	2.38	5	4.17
Total	78	100	42	100	120	100

Male child or boys had higher diarrhea cases 78 (65%) as compared to girls 42 (35%). The male to female ratio was 1.86:1. Majority of male (53.85%) and female (66.67%) child admitted with diarrheal problems of 5-24 months category [Table 1]. Seasonal variation of this study sample (120 cases) could not be ascertained as they were selected from the total diarrhea cases attending outpatient department (OPD) and from

the ward, fulfilling the inclusion and exclusion criteria. However among the total diarrhea cases during the period of this study, majority of them occurred during Jan-March 18 (14.17%), April-Jun (47.5%), July-Sept (28.33%) and Oct-Nov12 (10%).

Table-2: Types of breast feeding in diarrheal cases

Types of breast feeding	No. of cases	Percentage
Exclusive Breast feeding	25	20.83 %
Exclusive artificial feeds	11	9.17%
Breast with artificial feeds	84	70%
Total	120	100%

Majority of the children 84 (70%) were breast fed combined with artificial feeds. Exclusive Breast feeding was given in 25 (20.83 %) of the cases and only artificial feeds were given in 11 (9.17%) of the cases [Table 2].

Table-3: Color and consistency of the stool in diarrheal cases

Color of the Stool	No. of Cases	Percentage
Yellowish	101	84.17
Greenish	11	9.17
White or Clay Colored	8	6.67
Consistency		
Watery	98	81.67
Semisolid	22	18.33
Total	120	100

The color of the stools in 84.17% of the cases was yellow, while 9.17% of the cases had green stools and 6.67% had clay colored or white stool. Majority of the cases (81.67%) had watery stools. Semisolid consistency was present only in 18.33% of the cases [Table 3].

Table-4: Other features of stool in diarrheal cases

Other Features of Tools	No. of Cases	Percentage
No blood or mucus	99	82.5
Only mucus in stools	15	12.5
Both blood and mucus	6	5
Total	120	100

Majority of the cases (82.5%) had no blood or mucus in stools whereas 12.5% had only mucus in stools and 5% had both blood mixed with mucus [Table 4].

Table-5: Clinical presentation in diarrheal cases

Clinical presentation	No. of Cases	Percentage
Vomiting	69	57.5
Fever	50	41.67
Abdominal pain	37	30.83

Clinical presentations were associated with diarrhea like vomiting (57.5%), fever (41.67%) and abdominal pain (30.83%) [Table 5].

Stool examination

Microscopic examination of stool was carried out soon after admission. Fresh stool was taken and was examined under microscope with saline and iodine preparations. Pus cells >10/HPF was seen in 27 (30.83%) of the cases and RBC's were seen in 21 (17.5%) of the cases. *Ancyclostoma duodenale* ova were present in 1 of the case and egg of *Ascaris lumbricoides* was seen in 2 cases. Protozoa *like Giardia* or cyst of *Entamoeba histolytica* was detected 4 cases.

Stool culture

Stool culture for bacteria was carried out in all the study cases, and was positive in 68.33of the cases. Escherichia coli were found to be the predominant organism found in 42.5% of the cases. Salmonella was isolated from 7.5% of cases. Klebsiella 6.67% and Shigella 11.67% of the cases were isolated. In 31.67% of the remaining cases no enteropathogenic bacteria could be detected [Table 6].

Table-6: Stool culture isolation in diarrheal cases

Culture Positive -Organism Isolated	No. of Cases	Percentage
Escherichia Coli	51	42.5
Shigella Spp.	14	11.67
Salmonella Spp.	9	7.5
Klebsiella Spp.	8	6.67
Total	82	68.33
Culture Negative	38	31.67

Amongst the culture positive cases 39 patients (45.9%) had fever at the time of admission. Pus cells > 10/HPF in stools were present in 27(31.8%) of the culture positive cases. The antimicrobial profile for isolated enteropathogenic bacteria to various antimicrobial drugs was determined by the disc diffusion method following the recommendations of the clinical and laboratory standards institute (CLSI).

Table-7: Antimicrobial susceptibility pattern of *Escherichia coli* isolated from diarrhoea cases

Number of isolates =51				
Sensitivity pattern ►	Susceptible		Resistant	
Antimicrobial Agents ▼	No.	%	No.	%
Amikacin	47	92.16	4	7.84
Amoxycillin	7	13.73	44	86.27
Ampicillin	29	56.86	22	43.14
Cefotaxime	39	76.47	12	23.53
Co-trimoxazole	33	64.71	18	35.29
Chloramphenicol	48	94.12	3	5.9
Gentamicin	43	84.31	8	15.69
Ciprofloxacin	27	52.94	24	47.1
Nalidixic acid	33	64.71	18	35.29

Among *Escherichia coli* isolates majority of the strains were sensitive to chloramphenicol (94.12%) amikacin (92.16%) and gentamycin (84.31%) followed by cefotaxime (76.47%). The isolates were highly resistant to amoxycillin (86.27%) followed by ciprofloxacin (47.1%), ampicillin (43.14%), cotrimoxazole (35.29%) and nalidixic acid (35.29%) [Table 7].

Table-8: Antimicrobial susceptibility pattern of Shigella spp. isolated from diarrhea

Number of isolates =14				
Sensitivity pattern ►	Susceptible		Resistant	
Antimicrobial Agents ▼	No.	%	No.	%
Amoxycillin	6	42.86	8	57.14
Cefotaxime	12	85.71	2	14.29
Co-Trimoxazole	8	57.14	6	42.86
Chloramphenicol	11	78.57	3	21.43
Amikacin	11	78.57	3	21.43
Gentamicin	11	78.57	3	21.43
Ciprofloxacin	10	71.43	4	28.57
Nalidixic acid	5	35.71	9	64.29
Furazolidone	10	71.43	4	28.57

Majority of *Shigella* strains were found to be sensitive to cefotaxime (85.71%), chloramphenicol and gentamicin (78.57%) ciprofloxacin (71.43%) and amikacin (78.57%). About 64.29 of strains were resistant to nalidixic acid and amoxycillin (57.14%) respectively [Table 8].

Among the salmonella sp. isolated, amikacin was sensitive in all cases, most of the strains are sensitive to amikacin (88.9%), co-trimoxazole (77.8%) and ciprofloxacin (66.7%). Furazolidone is also less sensitive in 55.6% than cefotaxime (66.7%) and chloramphenicol (66.7%). In the present study resistance to amoxycillin, nalidixic acid, gentamicin and cefotaxime was 55.6, 55.6, 55.6 and 33.33 per cent respectively [Table 9].

Table-9: Antimicrobial susceptibility pattern of Salmonella spp. isolated from diarrhea

Number of isolates =9				
Sensitivity pattern►	Susceptible		Resistant	
Antimicrobial Agents ▼	No.		No.	
Amikacin	8	88.9	1	11.11
Amoxycillin	4	44.44	5	55.6
Cefotaxime	6	66.7	3	33.33
Co-trimoxazole	7	77.8	2	8
Chloramphenicol	6	66.7	3	33.33
Gentamicin	4	44.44	5	55.6
Ciprofloxacin	6	66.7	3	33.33
Nalidixic acid	4	44.44	5	55.6
Furazolidone	5	55.6	4	44.44

DISCUSSION

The etiological pattern of bacteria causing acute diarrhea depends on geographical area. In developing countries, more than half a million infants and young children die each year because of AGE, and *Vibrio cholerae* still causes epidemics, but the most common bacterial agent is *Shigella* [12]. In India, *E. coli* was the most common agent of AGE (31%) followed by *Shigella* (24%). Infections with two or more pathogens were observed in 34% of cases, with a predominant incidence in children younger than 2 years old [13]. Bacterial pathogens account for 80% of cases of traveler's diarrhea [14]. ETEC, enteroinvasive *E. coli* (EIEC), and EAEC are implicated in the majority of cases, but also *Campylobacter*, *Salmonella*, and *Shigella* play a substantial role.

Study by Shrivastava AK *et al*. showed that Escherichia coli was detected to be the major etiological agent (30.07%) followed by Rotavirus (26.15%), Shigella (23.84%), Adenovirus (4.61%), Cryptosporidium (3.07%), and Giardia (0.77%). Concurrent infections with two or more pathogens were observed in 44 of 130 (33.84%) cases with a predominant incidence particularly in <2-year-old children (65.90%) compared to children of 2–5 years age group (34.09%). An overall result showed significantly higher detection rates among children with diarrhea in both combinations of two as well as three infections concurrently (p = 0.004915 and 0.03917, respectively) [13].

Study by Dedwal *et al*. revealed that seventy-four percent of children with diarrhea were in the age group of 7 to 12 months. Watery diarrhea (94%) was the commonest clinical presentation, followed by vomiting (78%), fever (78%), and dehydration (74%). Pathogenic bacteria were isolated in 51% of samples. *Escherichia coli* was most common (48%) followed by *Shigella flexneri* (2%) and *Vibrio cholerae (1%).* The most prevalent *E. coli* type was ETEC (20.8%) followed by EPEC (16.7%), EHEC (4.1%), and STEC (2.1%). The most prevalent serotypes of ETEC were O27, O23, *and O169. Among* EPEC *most* prevalent serotypes were O90, O26. The most prevalent *EHEC* strain found in this study was O71. *Rotavirus* was detected in 35% of patients. Most prevalent *Rotavirus* genotype was G9P [4] (28.6%) followed by G2P [4] (21.4%), G1P [8] (21.4%), G12P [6] (14.3%), G9P [8] (7.1%). Parasitic etiology was detected in 5% of cases. Coinfection of *E. coli* and *Rotavirus* was detected in 23% of children. Rotavirus was most commonly associated with *EPEC* (25.7%) followed by ETEC (17.1%) [15].

In the present study male child or boys had higher diarrhea cases 78 (65%) as compared to girls 42 (35%). The male to female ratio was 1.86:1. Another study showed that total diarrheal deaths in India among children aged 0-6 years were estimated to be 158,209 and proportionate mortality due to diarrhea in this age-group was 9.1%. Average estimated incidence of diarrhea in children aged 0-6 years was 1.71 and 1.09 episodes/person/year in rural and urban areas [14]. According to National Family Health Survey-3 (NFHS-3) report, 9% of all under-five children were reported to be suffering from diarrhea in last 2 weeks [16]. Studies have shown that the incidence of acute diarrheal diseases was as low as 1 episode/child/year in some urban areas [3, 17].

Ahmed SF *et al.* showed the overall period prevalence (last 15 days recall) of diarrhoeal diseases observed was 25.2%. Period prevalence was found to be highest during summer months (42.6%) and lowest during winter months (13.5%). The same was found to be (24.4%) and (19.7%) in spring and autumn seasons respectively. The period prevalence also varied significantly (P<0.05) across various seasons. The sex of the child had no significant effect on period prevalence rate of diarrhoea whereas in point prevalence, male gender (10.4%) was more prone to diarrhoea than that of females (8.1%) which were significant [18]. WHO Report reveals that distinct seasonal patterns of diarrhoea occur in many geographical areas. In temperate climate, bacterial diarrhoea occurred more frequently during the warm season, whereas viral diarrhoea, particularly diarrhoea caused by Rota virus, peaked during the winter. In tropical areas, Rota virus diarrhoea occurred throughout the year, increasing in frequency during the drier, cool

months, whereas bacterial diarrhoeas peaked during the warmer, rainy seasons. The incidence of persistent diarrhoea followed the same seasonal patterns as that of acute watery diarrhea[19].

CONCLUSION

In the present study, *E. coli* was the commonest microorganism followed by Shigella. Thus, the importance of safe water and food hygiene would be most important intervention to prevent acute gastroenteritis in children.

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