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CLINICAL PROFILE OF ACUTE DIARRHEAL DISEASES BY AEROBIC BACTERIA IN A TERTIARY CARE CENTRE OF CENTRAL INDIA.

Community Medicine

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ABSTRACT

INTRODUCTION: Diarrheal disorders are the second most common cause of deaths in under 5 age group. 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.

AIM: To find the clinical profile of acute diarrheal diseases caused by aerobic bacteria Escherichia coli, salmonella spp., and shigella spp. in faecal samples of infants and children.

METHODOLOGY: The present study of bacteriology is a hospital based descriptive, cross sectional study, on aerobic bacteria causing watery diarrhea (< 15 days) in infants>1 month and children under 5 years of age. Total 120 samples were processed for direct microscopy and culture growth for identification of enteropathogen. Clinical profile of study population was analyzed (IBM SPSS version 21).

RESULTS: Diarrhea was more common in boys (65, 54.2%) as compared to girls (55, 45.8%). (M: F=1: 0.85).Of the total number of cases obtained, higher fraction was encountered during the months of April (11), May (18), June (20), July (18), August (17) and September (12).Stool culture for bacteria was carried out in all the study cases, and was positive in 50.66% of the cases. Among positive cases, Escherichia coli were found to be the predominant organism in 44.2% cases. Salmonella was 6.7%, Klebsiella 8.3% and Shigella 11.6%. Among the positive culture cases, 39 patients (45.9%) had fever at the time of admission and pus cells > 10/HPF in stools were present in 27(31.8%).

CONCLUSION: Isolation of bacteria as E coli and Klebsiella from the stools of patients does not always establish an etiological relationship but most of the time.

KEYWORDS

Acute diarrhoea, Dysentery, Enteropathogens.

INTRODUCTION

Acute gastroenteritis or infectious diarrhea is one of the leading causes of illnesses and deaths 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. The term **diarrheal disorders** is more commonly used to denote infectious diarrhea in public health settings, although several noninfectious causes of GI illness with vomiting and/or diarrhea are well recognized[1].

Diarrheal disorders are the second most common cause of deaths in under 5 age group. In developing countries, on an average every child suffers 3.3 episodes of diarrhea 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 diarrheal diseases accounts for about 8% deaths in under-5 years of age group. During the year 2011, about 10.6 million cases with 1,293 deaths were reported in India[3], children under 5 years of age suffer from 2-3 episodes of diarrhea annually [4].

Many microorganisms are implicated in the pathological mechanism of diarrhoea, the most common causes are: 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 *(Entamoebahistolytica, Giardia lamblia, Cryptosporidium* parvum, Strongyloidesstercoralis), *and fungal* (Candida albicans) [5-9].

There is a paucity of data in this regard from central India hence this study was conducted to elicit the clinical profile and nature of bacterial isolates (aerobic) among study population.

AIM

To find the clinical profile of acute diarrheal diseases caused by aerobic bacteria Escherichia coli, salmonella spp., and shigella spp. in faecal samples of infants and children.

OBJECTIVES

1. To find the clinical profile of acute diarrhea (by aerobic bacterias) in fecal sample of under 5- children.
2. To determine various enteropathogens causing dysentery in under 5 children.

MATERIAL AND METHODS

The present study of bacteriology is a hospital based descriptive, cross sectional study, on aerobic bacteria causing diarrhea and their antibiogram from stool samples of infants>1 month and children under 5 years of age, conducted in the Department of Microbiology and Pediatrics, Index Institute of medical science, Indore (MP)from September 2011 to September 2014.

All the Children of age group >1 month to 5 years attending the OPD and admitted in IPD from September 2012-September 2013, with watery diarrhea (<15 days) and those whose parents/guardians gave an informed consent were included in the study. Diarrheal cases with > 15 days duration, passage of frequently formed or pasty stools, diarrhea other than watery diarrhea, food poisoning and systemic infections where excluded. All study samples (120) taken only under aseptic precautions were processed. Ethical clearance was obtained. The results and names were kept confidential.

Equipments used were bio safety cabinet IIA, bacteriological Incubator maintained at 37°C, microscope, gloves, bunsen burner, glass slide, cover slip, culture media and antibiotic discs.

Freshly passed stool samples without any added preservatives were collected in sterile containers. All the specimens collected were transported without delay to the laboratory (< 24 hrs) and in case of expected delay Cary Blair transport media was used. Samples were labeled as required.
The collected sample was processed as below:

DIRECT MICROSCOPY

Stool sample was done by preparing saline wet mount and Iodine wet mount to look for leucocytes, RBC's, ova and cysts.

INOCULATION OF THE SAMPLES ON TO DIFFERENT CULTURE MEDIA FOR ISOLATION OF ORGANISMS.

Stool samples were inoculated on nonselective MacConkey agar, for shigella moderately selective media was used ie. Xylose lysine deoxycholate and deoxycholate citrate agar. Enrichment was done in selenite f broth for salmonella and shigella. Oxidase test was done for

all the nonlactose fermenting colonies. Interpretation was done as follows:

The small and pale nonlactose fermenting colonies of *Shigella*or slightly larger colonies of *Salmonella*spp. and *E.Coli*show oxidase negative reactions, where as *Pseudomonas, Vibrio cholerae, Aeromonas* and *Pleisomonas* give oxidase positive reaction, so they were excluded. Recognised classes of Escherichia coli (EPEC, ETEC, EHEC. EAggEC, and EIEC) were not isolated due to lack of facility in the institution.

On 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 centers.

PRELIMINARY IDENTIFICATION ON CULTURE MEDIA:
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 recorded.

Bio-chemical tests - The isolates were confirmed for production of enzymes (Catalase, Oxidase, Urease, Arginine dihydrolase, Lysine decarboxylase, Ornithine decarboxylase, Nitrate reduction), for substrate utilization (Citrate test), for metabolism of proteins and amino acids (Indole production test for utilization of carbohydrates, inoculation on triple sugar iron Agar, Oxidation fermentation test using 1% glucose, Sugar fermentation tests, Methyl red and Voges Proskauer test).

The data was entered in excel sheets, expressed in percentages and fractions and analysed by IBM SPSS 21ST version.

RESULTS:
In the present study, maximum number of cases of acute diarrhea occurred in the age group of 6-24 months was 83 (69.2%) followed by under 6 months (23, 19.2%), in 25 to 36 months (7, 5.8%) and in 37 to 48 months (4, 3.3%). The least percentage infection was found in higher age group i.e. between 49 to 60 months (3, 2.5%).

Diarrhea was more common in boys (65, 54.2%) as compared to girls (55, 45.8%). (M: F=1: 0.85).

SEASONAL TRENDS:
Of the total number of cases obtained, higher fraction was encountered during the months of April (11), May (18), June (20), July (18), August (17) and September (12).

Majority of the children (74.1%) were breast fed combined with artificial feeds. Exclusive Breast feed was given in 16.7 % of the cases and exclusively artificial feed was given in 9.2% of the cases.

COLOR OF STOOLS:
The color of the stools in 75.8% of the cases was yellow, while 20% had green and 4.2% had clay colored or white stool.

Majority of the cases (85%) had watery stools. Semisolid consistency was present only in 15% of the cases. Majority of the cases (83.3%) had no blood or mucus in stools whereas 12.5% had only mucus in stools and 4.2% had both blood mixed with mucus.

CLINICAL FEATURES
Vomiting was present in 64.2% of the cases. Children vomited most often after taking medications. Vomitus was not mixed with blood in any of the cases.

Fever was recorded in 43.3% of the cases. Fever was of mild degree in majority.

ON MICROSCOPY: In stool, pus cells >10/HPF were seen in 31.7% of the cases and RBC's were seen in 12.5% of the cases. Egg of Ascaris lumbricoides was seen in 2 cases and Ancyclostoma duodenale ova were present in 1 case. No protozoa like Giardia or cyst of Entamoeba histolytica could are detected.

Stool culture for bacteria was carried out in all the study cases, and was positive in 50.66% of the cases. Among positive cases, Escherichia coli were found to be the predominant organism in 44.2% cases. Although Escherichia coli is a commensal of the gut but on occasions when there is an exacerbation it may become pathogenic. Among the isolates, Salmonella was 6.7%, Klebsiella 8.3% and Shigella 11.6%.

Among the positive culture cases, 39 patients (45.9%) had fever at the time of admission and pus cells > 10/HPF in stools were present in 27(31.8%).

DISCUSSION
In the present study most cases of acute diarrhea were found below 2 years (88.4%). Similar was reported by C:\roraMM.Arora and Pohowalla(72%)[10], Sood S[11] (91%), BeheraSK[12](77.5%), Joshi CK et al[13](73.8%) and Ansari S etal[14].

The highest incidence of diarrheal diseases in the first 2 years of life is related to early and faulty weaning, top feeds with its unhygienic accessories and storage of milk and food predisposing to higher incidence of infection, inadequate diet leading to malnutrition and thereby reducing the natural defense of the body. Khanduja et al[15] and Sood S[11] suggested mouthing habits as predisposing factor for these infections. Further, the weakened immune system of the body rendering it susceptible to an attack by the enteric pathogens and malnutrition worsening the situation.

The prevalence of acute diarrhea among male to female was found to be 1: 0.85. Similar observations were made by Karmakar et al[16], Victor Gardens et al[17], An sari S, et al[14].

According to Sood[11], Behera etal[12], Naruka BS et al[18]and Pavri[19] twice dominance of males was found. However Sethi[20]and Tanakmoni Devi etal[21]have reported no sex difference. It may be due to the fact that males are more prone to infections. It could possibly be attributed to gender discrimination in health seeking behavior.

Manchanda and Arora[22] reported that 31.2% of all diarrheal cases were exclusively breast fed, Naruka BS et al[18] found it to be 32.5% Sood[11] reported 23% while in our study it was 17.6%.

In the present study, stools were more often yellowish (75.8%).similar results were found in Narukaet al[18] (76.4%) and Mittal SK et al[23] (60.0%).

Also, similar results regarding consistency of stools were noted by Narukaet al[18], Reddiah VP et al[24], Devignam N et al[25]and Kamala CS et al[26], who quoted figures of 83%, 76.6%, 86.137%and 91.84% respectively.

Most of the cases of diarrhea were found from May to September this was also found in other studies.[18,27]The higher incidence in summer was found may be due to free growth of saprophytic organisms in the contaminated food along with multiplication of bacteria in milk and food. The high incidence in the beginning of the rainy season is said to be due to greater breeding of flies seen during and after light rains.

Stool findings in the present study, had no blood or mucus (83.3%), 12.5% had only mucus, and 4.2% had both blood and mucus. These findings are similar to those reported by Naruka et al[19] and Mittal SK et al[23]. In a study by Naruka et al[18], 64.7% showed no mucus or blood in stools, 28.3% had only mucus while both mucus and blood was present in 5.8% of cases.

Fever was recorded in 43.3% of the cases. Naruka BS et al[18] and Manchanda and Arora[22]reported fever in 55.5% and 53.8% respectively. Chaoinin Wan el[28] al quoted 75.4% and Srivastava JR[27]in 48.2%.The different organisms isolated were Escherichia coli (44.2%); Salmonella spp. (6.7%); Klebsiella spp. (8.3%) and Shigella spp. (11.7%) in our study.

J.R.Srivatsava et al[27]and Rao and Murthi et al[36] isolated diarrhoegenic E.coli in 37.27% and 38.67%.this is comparable to our study (44.2%).In the series of AR. Ghosh et al[29] diarrhoeagenic E.coli were the most frequently detected organism (57.4%) in the faces of hospitalized infants.

P. Dutta et al[30] reported that enteropathogenic E.coli was the most predominant organism constituting 69.6% of their culture positive cases. Monto AS and Koopman[31] 1980, Sen D et al[32]1984 and Ganguly et al[33] reported that among the five classes of E.coli, EPEC and ETEC

were commonly associated with pediatric diarrhea.

Shigella sp. was isolated in 14 (11.7%) cases. While in a study by Thapa BR et al[34]at Chandigarh shigella spp. was isolated in 10% of cases.

In a study by Krishnan R et al[35] in teaching hospitals in Delhi, isolated 38.3% cases and Deepa P Patil[37] 10.1% cases

A large number of serotypes and subtypes of salmonellae are known to cause diarrheal disease. In our country Srivastava et al[27] reported salmonella infections in 4.4% of their cases, Naruka et al[18] (7.5%), Khanduja et al[15] (2.4%), and Dutta P et al[30] (2.6%).

Klebsiella is a ubiquitous organism which is isolated in a large proportion of both diarrheal and non diarrheal stools, so it is difficult to define the etiological role of this organism in diarrheal diseases. In our study these were isolated in 8.3% cases and is nearly same as Maiya PP et al[38](2%) and Deepa P Patil et al[28] (20.3%).

IMPLICATIONS

1. Inadequate clean water supply, improper excreta disposal and poor domestic and food hygiene may favor the spread of communicable diseases, especially infectious diarrhea, which is one of the leading causes of morbidity in the country. Infectious diarrhea affects mainly malnourished children, which is common in Madhya Pradesh.
2. Isolation of bacteria as E coli and Klebsiella from the stools of patients does not always establish an etiological relationship but most of the time.

TABLE1: TABLE SHOWING GENDER DISTRIBUTION OF STUDY POPULATION

Age group in months	MALE	FEMALE	TOTAL
< 6	12(18.4)	11(20)	23(19.2)
6-24	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)
Total	65(100)	55(100)	120(100)

*() percentages are indicated in parenthesis.

TABLE 2: TABLE SHOWING CLINICAL PROFILE OF STUDY POPULATION.

Study variables	No. of Cases	Percentage (%)	
AGE GROUPS (in months)	< 6	23	19.2
	MAY-24	83	69.2
	25 – 36	7	5.8
	37 – 48	4	3.3
	49 – 60	3	2.5
GENDER	MALE	65	54.2
	FEMALE	55	45.8
FEEDING PATTERN	EXCLUSIVELY BREAST FEED	20	16.7
	BREAST FEED AND ARTIFICAL FEED	89	74.1
	ONLY ARTIFICIAL FEEDS	11	9.2

	NO. OF CASES	PERCENTAGE (%)	
COLOUR OF STOOL	YELLOWISH	91	75.8
	GREENISH	24	20
	WHITE OR CLAY COLOURED	5	4.2
CONSISTENCY	WATERY	102	85
	SEMISOLID	18	15
OTHER FEATURES OF TOOLS	NO BLOOD OR MUCUS	100	83.3
	ONLY MUCUS IN STOOLS	15	12.5
	BOTH BLOOD AND MUCUS	5	4.2

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