Conventional Bacterial Pathogens in Patients with Diarrhea Attending a Tertiary Care Hospital in Goa

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ABSTRACT

Background: Diarrheal disease remains one of the largest health problems in many parts of the world.
Objectives: To evaluate bacterial etiological agents in cases of diarrhea and to observe correlation with age, gender and stool microscopic findings.
Methods: A prospective study involving microscopic and cultural analysis of all stool samples received in the Department of Microbiology, Goa Medical College, Bambolim, Goa.
Results: Amongst the 545 faeces samples processed, bacterial pathogens were isolated in 32 samples (5.9%). Bacteria isolated were Shigella (59.4%), Salmonella (28.1%), Aeromonas (9.4%) and Vibrio cholerae (3.1%). Culture positive cases included 62.5% adults and 56.2% females. Microscopic presence of pus cells was seen with Shigella (68.4%), Salmonella (55.6%) and all 3 cases of Aeromonas diarrhea.
Conclusion: While the present study documents the bacterial agents of diarrhea, monitoring should be undertaken to identify any change in etiological trends.

Introduction

Diarrheal diseases include a wide gamut of infective and non infective types and encompass clinical varieties such as diarrhea, gastroenteritis, dysentery and food poisoning.

These diseases form important causes of morbidity and preventable mortality. In developing countries, diarrheal diseases rank second among all deaths due to infectious diseases.[1] An estimated 1000 million episodes occur yearly, in children under 5 years of age, causing 5 million deaths among them, annually.[2]

Diarrheal pathogenesis and infective etiology is multifaceted. The list of pathogens causing enteric infections has lengthened greatly, over the decades, a substantial proportion of them, being caused by bacteria and viruses.[3]

The conventional bacterial pathogens causing diarrhea include Salmonellae, Vibrio, Camplylobacter jejuni, Clostridium, Bacteroides fragilis, Bacillus cereus, Staphylococcus aureus and the various classes of diarrheagenic Escherichia coli. Over the past few years, Aeromonas has assumed a great deal of interest as a human diarrheal pathogen.[3]

Dysentery is mainly caused by organisms belonging to the genus Shigella, which invade the intestinal mucosa and lead to bloody stools with mucus, as a presenting symptom. Apart from bacteria, parasites and fungi, viruses have also contributed to diarrheal illness.

Microbial etiology of diarrheal diseases is of great importance and stresses the need for cultural diagnosis, especially when antibiotic therapy has to be used in treatment. In addition, it aides in establishing the trend of bacterial enteric pathogens in a given locality, which may be governed by geographical and socioeconomic elements.[4] It also serves as a guide to improve safe water supply, sanitation and environmental conditions.

This study was carried out over a period of eight months (May to December, 2015), to assess the prevalence and types of various bacterial pathogens, from cases of diarrhea. A correlation of stool microscopic findings with isolation of bacterial pathogens was attempted. The isolation rate and the bacterial pathogens encountered in the present study is compared with that obtained during the same period, in the year 2006; to see a changing trend, if any.

Materials and methods

This study was carried out in the Department of Microbiology, Goa Medical College, Bambolim, Goa, over a period of 8 months, from May to December, 2015. A total of 545 stool specimen were processed from cases of diarrheal diseases.

All samples were subjected to gross and microscopic examination, prior to cultural studies. Gross examination was made for presence of blood, mucus and parasites. Saline and iodine wet mounts were prepared and examined microscopically for the presence of pus cells, red blood cells and ova/trophozoites/cysts/larvae of parasites.

The stool samples were then subjected to cultural analysis, which involved primary isolation and enrichment. The culture media employed for this purpose were MacConkey Agar, Desoxycholate Citrate Agar, Xylose Lysine Decarboxylase Agar, Thiosulphate Citrate Bile Salt Sucrose agar, Hektoen Enteric Agar, Selenite F broth and Alkaline Peptone water.

All media were incubated overnight at 37⁰C. Subcultures from enrichment media were made onto MacConkey Agar and Hektoen Enteric agar. Bacteriological analysis of suspected pathogens was done by standard laboratory techniques and biochemical tests.[5]

The identity of Shigellae, Salmonellae and Vibrio cholerae was confirmed by serology, using specific antisera.

All bacterial pathogens were subjected to antimicrobial susceptibility testing on Mueller Hinton agar, using Kirby Bauer disc diffusion technique.

Results

A total of 545 faeces samples were processed during the eight months study period, from May to December, 2015. Conventional bacterial pathogens were isolated in 32 stool samples; the percentage isolation being 5.9%. All bacterial pathogens were isolated as a single pathogen.

The isolation of various bacterial pathogens is depicted in Table 1. Organisms belonging to the genus Shigella were the predominant pathogens (59.4%) followed by Salmonella (28.1%). The species belonging to the genus Shigella were all identified as Shigella sonnei. Within the genus Salmonella, 5 isolates were S. typhimurium, 3 isolates were Salmonella Group D (non typhoi-

dal) and 1 isolate was Salmonella group C. All Aeromonas isolated were A. hydrophila.

Amongst the bacterial culture positive cases, adults accounted for 62.5% of the total. Male subjects were 14 in number (43.8%) and 18 (56.2%) were female patients. Correlation of type of bacterial pathogens with pediatric / adult distribution and gender is shown in Tables 2 and 3. Shigellae were isolated with equal frequency in both the groups and genders (Pediatric = 52.6%, Adult = 47.4%; Males = 52.6%, Females = 47.4%). However Salmonellae were more frequently isolated in adults (77.8%) and in females (77.8%). All Aeromonas and V. cholerae were isolated in adults.

Table 4 gives the relationship of bacterial isolates to microscopic findings of presence of pus cells and red blood cells. In stool samples which yielded Shigellae, microscopically, more than 10 pus cells per high power field were seen in 68.4% cases and red blood cells in 42.1%. In Salmonella stools, pus cells were seen in 55.6% cases.

Antimicrobial sensitivity pattern of Shigellae (Table 5) showed resistance to Quinolones. However, resistance was not a problem in Salmonella.

The isolation rate of bacterial pathogens in the present study (2015) was 5.9% (32 out of 545 stools processed), while in the same period of the year 2006, it was 6.9% (32 out of 466 samples processed). In both the years, Shigellae, Salmonellae and Aeromonas were isolated; Shigellae predominating. However, Vibrio cholerae which was isolated in 2006 was rare in the present study (2015) (Table 6).

Discussion

Diarrhea is exceedingly common and exerts an enormous toll, in terms of morbidity, loss of work, productivity and consumption of medical resources.

The isolation rate of conventional bacterial pathogens in the present study was 5.9% and is comparable with the isolation rate of 4.6% encountered in the study of Das et al.[6] However, Mishra et al observed a higher isolation rate of 23.2% in their study.[7]

A low isolation of faecal pathogens in the present study could be attributed to the non-inclusion of Escherichia coli as a diarrheal pathogen. It could also be a reflection of extensive self administration of antibiotics for even a mild illness in this locality. On the other hand, as a remote possibility, the low isolation rate could be a true reflection of bacterial etiology of diarrheal diseases in this locality, considering the presence of increased health care awareness, in general.

The pattern of isolation of etiological agents of diarrheal diseases, varies from place to place, depending on geographic, demographic and socioeconomic factors.[4] In addition, the use of special and selective media will help in increasing the isolation of bacterial pathogens that are not frequently reported, common among them being Campylobacter species. In the event of outbreaks, the isolation rate of the involved pathogen increases, as was observed by Taneja et al, who reported an outbreak of Vibrio cholerae O1 Ogawa, in and around Chandigarh.[8]

In the present study, out of 32 stool pathogens isolated, the percentage isolation of Shigella was 59.4%. Shigellosis continues to be a persistent endemic problem in India. Most workers have consistently isolated Shigella flexneri as the predominant species.[6,9] Shigella sonnei, isolated in the present study, is a hardier organism, contaminating inanimate objects. In general, it is a disease of overcrowding, insanitary conditions and poor personal hygiene. Human carriers play a prime role in spread and perpetuation of the disease in a smoldering endemic form.[10]

Non typhoidal Salmonella gastroenteritis is still well recognized and can be accompanied by septicemia and mortality. Salmonellae were isolated with a frequency of 28.1%. This is similar to that obtained by Khanna et al[11] i.e. 20%. Salmonellosis has been recognized as a worldwide problem in both man and animals and bears important public health implications.

The isolation of Aeromonas and its significance in stool samples should be interpreted with caution and must rely on clinical correlation as faecal carrier rate is known.

Although diarrhea can affect all ages, adults accounted for 62.5% of the total, in the present study. However, it may pose an important and serious problem in infants and young children. Although Shigella isolation showed no difference in adults and pediatric age group, Salmonellae were isolated more frequently in adults, in the present study.

Shigella dysentery is self limiting and may not require antibiotic therapy. However, Salmonellosis, especially non typhoidal, may merit antibiotic usage. Fortunately, in these cases, resistance to antimicrobials did not appear to pose a problem in treatment, in the present study. On clinical suspicion of cholera, the patient is empirically started on Doxycycline. The single isolate of Vibrio cholerae was sensitive to Tetracycline and Doxycycline.

Correlation of microscopic findings of stool with isolation of bacterial pathogens revealed presence of pus cells in stools yielding Shigella, Salmonella and Aeromonas, in the present study. Khanna et al, in their study in Amritsar, also found a similar association.[11] A parameter of 10 leucocytes per field can help differentiate Shigella infection from Enteroinvasive and Enterotoxigenic Escherichia coli, Campylobacter and Rota virus diarrhea.[12]

Comparison of isolation rate and bacterial pathogens in the present study was similar to the findings obtained in 2006. However, a glaring difference is in the low isolation of Vibrio cholerae in the present study. The cases reported in 2006 (unpublished data) were during the immediate post monsoon period and could have led to an epidemic form, had it not been the active intervention of Governmental Health Services.

Conclusion

The present study documents the causative bacterial agents of diarrhea. A regular monitoring of enteric pathogens is essential to determine any change in etiology of bacterial diarrheas. Antimicrobial susceptibility testing should be conducted and compared periodically. An evaluation of seasonal distribution needs to be undertaken, to evaluate the relative frequency of specific pathogens throughout the year. Morbidity due to diarrheal diseases can be reduced by clear initiatives in the areas of sanitation, fly control and knowledge of disease transmission.

Tables

TABLE 1: ISOLATION OF VARIOUS GENERA OF BACTERIAL PATHOGENS

Pathogens isolated	Number of Strains	Percentage
Shigella	19	59.4
Salmonella	9	28.1
Aeromonas	3	9.4
Vibrio cholerae	1	3.1
Total	32	100

TABLE 2: PAEDIATRIC/ADULT DISTRIBUTION WITH RELATION TO TYPE OF BACTERIAL PATHOGENS

Group	Bacterial pathogens isolated				Total (n=32)
	Shigella (n=19)	Salmonella (n=9)	Aeromonas (n=3)	V. cholerae (n=1)	
Pediatric	10 52.6%	2 22.2%	-	-	12 37.5%
Adult	947.4%	777.8%	3100%	1100%	2062.5%

TABLE 3: SEX DISTRIBUTION WITH RELATION TO BACTERIAL PATHOGEN TYPE

Group	Bacterial pathogens isolated				Total (n=32)
	Shigella (n=19)	Salmonella (n=9)	Aeromonas (n=3)	V. cholerae (n=1)	
Males	1052.6%	222.2%	133.3%	1100%	1443.8%
Females	947.4%	777.8%	266.6%	-	1856.2%

TABLE 4: RELATIONSHIP OF BACTERIAL ISOLATES TO MICROSCOPIC FINDINGS OF STOOL SAMPLES

Bacterial Pathogens	Number of Strains	Presence of Pus cells >10 / hpf	Presence of red blood cells / hpf
Shigella	19	13 (68.4%)	8 (42.1%)
Salmonella	9	5 (55.6%)	1 (11.1%)
Aeromonas	3	3 (100%)	1 (33.3%)
Vibrio cholerae	1	-	-
Total	32	21 (65.6%)	10 (31.3%)

TABLE 5: ANTIMICROBIAL SENSITIVITY PATTERN OF BACTERIAL ISOLATES (PERCENTAGE)

Antimicrobial	Shigella	Salmonella	Aeromonas	Vibrio cholerae
Ampicillin	14.3	44.4	-	0
Amoxycillin +Clavulanic acid	50	100	0	0
Cefoperazone	61.1	77.7	0	-
Ceftriaxone	50	77.7	0	0
Cefixime	22.2	77.7	0	-
Cefipime	57.9	77.7	0	0
Nalidixic acid	0	77.7	-	-
Ciprofloxacin	10.5	88.8	33.3	100
Ofloxacin	10.5	88.8	33.3	100
Levofloxacin	10.5	88.8	66.6	100
Cotrimoxazole	5.3	66.6	0	0
Chloramphenicol	-	100	33.3	-
Amikacin	-	-	100	0
Gentamicin	-	-	66.6	0
Tetracycline	-	0	0	100
Doxycycline	-	-	-	100

TABLE 6: COMPARISON OF ISOLATION RATE AND CONVENTIONAL PATHOGENS ENCOUNTERED IN 2006 AND 2015 (MAY TO DECEMBER)

Year	Percentage isolation	Number of Bacterial pathogens isolated			
		Shigella	Salmonella	Aeromonas	V. cholerae
2006	6.9	13	2	5	12
2015	5.9	19	5	3	1

REFERENCE

1. Sur D, Dutta P, Nair GB, Bhattacharya SK. Severe cholera outbreak following floods in a northern district of West Bengal. Indian J Med Res. 2004; 112:178-182. 2. Carlos CC, Saniel MC. Etiology and epidemiology of diarrhea. Phil J Microbial Infect Dis. 1990; 19(2):51-53. 3. Mathur M, De A, Saraswathi K, Varaiya A, Athalye. Vibrionaceae from cases of acute diarrhea and their antimicrobial sensitivity pattern - A five year prospective study. Ind J Med Microbiol. 2003; 21(3):199-200. 4. Haque R, Mondal D, Kirkpatrick BD, Akther S, Farr BM, Sack RB et al. Epidemiologic and clinical characterization of acute diarrhea with emphasis on Entamoeba histolytica infections in preschool children in an urban slum of Dhaka, Bangladesh. Am J Trop Med Hyg. 2003; 69(4):398-405. 5. Collee JG, Fraser AG, Marmion BP, Simmons A. Mackie and McCartney Practical Medical Microbiology. 14th Ed. Edinburg: Churchill Livingstone; 1996. 6. Das S, Saha R, Singal S. Enteric pathogens in north Indian patients with diarrhea. Ind J Comm Med. 2007; 32(1):27-31. 7. Mishra M, Mohammed F, Katkar VJ. Reemergence of El Tor Vibrio in outbreak of Cholera in and around Nagpur. Indian J Med Res. 2004; 120:478-488. 8. Taneja N, Kaur J, Sharma K. A recent outbreak of Cholera due to Vibrio cholerae O1 Ogawa in and around Chandigarh, North India. Indian J Med Res. 2003; 117:243-245. 9. Jesudason MV. Shigella isolation in Vellore, South India (1997-2001). Indian J Med Res. 2002; 115:11-13. 10. Saxena M, Aggarwal S, Gupta V, Sharma S. Shigella carriage in children. Indian J Med Microbiol. 1995; 13(3):131-133. 11. Khanna S, Aggarwal A, Gulati V, Manjari M. Bacteriological profile of acute diarrhea in Amritsar. Indian J Med Microbiol. 1996; 14(1):33-35. 12. Echeverria P, Sethabutr O, Pitarangsi C. Microbiology and diagnosis of infections with Shigella and Enteroinvasive Escherichia coli. Review of Infect Dis.1991: 13(4):220-225.