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CONVENTIONAL BACTERIAL PATHOGENS IN PATIENTS WITH DIARRHEA ATTENDING A TERTIARY CARE HOSPITAL IN GOA

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Background: Diarrheal disease remains one of the largest health problems in many parts of the world. To evaluate bacterial etiological agents in cases of diarrhea and

m>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.

Diarrhea, Etiological agents, Sanitation

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, Desoxy- cholate 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 37°C. 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.

2-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 typhoid) - IJSR - INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH 43

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3-Research Paper

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

مقدمة

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*, *Campylobacter 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. 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 aids 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.

1-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

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.

استنتاج

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.

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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).

4-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 al¹¹ 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



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