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A Study on Intestinal Pathogens in Diarrheal Patients

KEYWORDS: Diarrhea, Parasitic pathogens, Bacterial isolates, AST

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ABSTRACT *Diarrhea is one of the most common complaints in any hospital and continues to be a common cause for mortality and morbidity. The changing epidemiology and unpredictable resistance patterns of the infectious agents have made the stool examination more important than ever. In the present study, a total of 319 stool samples were observed for both parasitic and bacterial etiology. Parasites were found in 7.8% of the samples with nematodes predominating (75%). Bacterial pathogens were observed in 11.5% of the samples. Shigella was the most common bacterial isolate accounting to nearly half of the total bacterial isolates(48.6%) followed by Vibrio cholera (29.7%). Shigella was resistant to ampicillin (100%) and co-trimoxazole (83%) and ceftriaxone (44%). All the vibrio cholera isolates were resistant to co-trimoxazole (100%). Thus, the proper identification of etiology and their drug resistance patterns will help the clinician in selecting the appropriate treatment.*

INTRODUCTION:

Diarrhea is defined as having three or more loose stools per day. Dysentery is a term used to describe the condition in which diarrhea is accompanied by cramping abdominal pain andtenesmus. [1]Diarrheal diseases are one of the five leading causes of death worldwide. Four to six million children die each year of diarrheal diseases particular in developing countries.[2]The incidence of pathogens causing diarrhea varies from place to place and seasonal variation also seen within the same place. When compared to developed countries, in developing countries bacterial pathogens are recovered in significantly higher number of patients probably because of poor sanitation conditions. Antibiotic susceptibility patterns are often highly variable and increasingly resistant pathogens are being isolated nowadays.

Aim of our study was to identify the pathogenic organisms including both bacterial and parasitic agents from diarrheal patients and to determine the antimicrobial susceptibility patterns for the bacterial agents.

MATERIALS & METHODS

This study was conducted at SVS Medical College &Hospital, Department of Microbiology.A total of 319 stool samples were collected during the period of Sep 2013-Aug 2014 from the patients with complaints of diarrhea/dysentery.

The fecal samples are collected in in a clean, leak proof, screw capped container in accordance with standard routine procedures and transported to the laboratory as early as possible.

MACROSCOPIC EXAMINATION

The presence of blood, mucous, colour& consistency and any other abnormalities were observed.

MICROSCOPIC EXAMINATION

A direct saline wet mount and iodine mount was performed toobserve pus cells, RBC, ova,cyst,trophozoites and larval forms of parasites. Formal ether concentration method was done for all the samples.

IDENTIFICATION OF BACTERIA

A loop full of sample was streaked on blood agar, MacConkey agar and XLD agar plates. Enrichment in selenite F broth was also done for 6-8 hrs followed by subculture on the agar plates. All plates were incubated for 18-24 hrs at 37^{0}C and observed for bacterial growth. Cultures were further identified by doing biochemical tests and serotyping. Antibiotic susceptibility testing was carried out using the disc diffusion method, according to Clinical and Laboratory Standards Institute (CLSI) guidelines.[3] E.coli ATCC 25922 was used as control.

RESULT

A total of 319 diarrheal stool samples were obtained from all age groups visiting SVS Medical College and Hospitals.

Out of 319 cases, parasites were found in 25 (7.8%) samples. Among these 25 samples, 22 were single and the remaining 3 were mixed parasitic infections. Thus, a total of 28 parasites were observed from 25 patients. Helminthic parasites were found predominantly 21(75%) followed by protozoal parasites 7(25%).

Among 21 helminthic parasites, 14 (66.7%) were Ascaris lumbricoides, 4(19%) strongyloides stercoralis and 3(14.2%) anchylostoma duodenale. Among 7 protozoal parasites 5(71.4%) were giardia intestinalis and 2(28.6%) E. histolytica.

In the three patients with mixed infections, two were observed with giardia intestinalis cysts and strongyloides stercoralis larvae and the other being E.histolytica cysts with A.duodenale ova. Majority of the parasitic infections were observed among males (16/25) followed by females (9/25). And the predominant age group was 46-60 years (44%) followed by pediatric age below 15 years(40%).

Out of 319 samples, pathogenic bacteria were isolated in 37 samples(11.5%). Among this shigella flexneri was the predominantly isolated accounting to 18 (48.6%), followed

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by vibrio cholera 29.7% and plesiomonas shigelloides 10.8%. The remaining organisms were three salmonella typhi (8.1%) and one aeromonas hydrophila. (2.7%)

Distribution of the bacterial pathogens:

Organism	Number (%)
Shigella flexneri	18 (48.6%)
Vibrio cholera	11 (29.7%)
Pleisomonas shigelloides	4 (10.8%)
Salmonella typhi	3 (8.1%)
Aeromonas hydrophila	1 (2.7%)

Shigella isolates were highly resistant to ampicillin (100%), amoxyclav and cotrimoxazole (83% each) and ciprofloxacin (67%). Majority of the isolates were sensitive to cefaperazone/sulbactum(72%), Ceftriaxone (56%) and all the isolates were sensitive to imipenem and tigecycline.

Antibiotic resistance of shigella:

Antibiotic	Resistance %
Ampicillin	100 %
Amoxyclav	83 %
Co-trimoxazole	83 %
Ciprofloxacin	67 %
Ceftriaxone	44 %
Amikacin	44 %
Piperacillin-tazobactum	33 %
Cefaperazone/sulbactum	28 %
Imipenem	0 %
Tigecycline	0 %

All the vibrio cholera strains isolated were of El tor biotype and Ogawa serotype.All of them were resistant to Co-trimoxazole (100%). Resistant to ampicillin (45%) and azithromycin (27%) was also seen.

Highest sensitivity was observed against chloramphenicol followed by ceftriaxone(91%). Ciprofloxacin and doxycycline resistance was also considerable low(18% each).

Antibiotic resistance of vibrio cholera:

Antibiotic	Resistance %
Azithromycin	27 %
Ceftriaxone	9 %
Ampicillin	45 %
Doxycycline	18 %
Chloramphenicol	0 %
Ciprofloxacin	18 %
Co-trimoxazole	100 %

Other organisms like plesiomonas spp, salmonella typhi and aeromonas spp have shown variable resistance pattern. Ciprofloxacin resistance was seen in 50% of the plesiomonas isolates where as 33% of salmonella typhi isolates were resistant to it. All the pleisomonas isolates were sensitive to ceftriaxone where as 33% of the salmonella isolates were resistant to it. Co-trimoxazole resistance was considerably high in both the isolates with 75% of plesiomonas isolates and 67% of the salmonella isolates being resistant to it.

DISCUSSION:

Majority of the diarrheal diseases are self-limiting, antibiotics are often prescribed in order to decrease the severity of the infection and thus decreasing the mortality and morbidity. In this context, choosing the appropriate drug is very important which in turn depend on the etiological agents and their susceptibility patterns. In parasitic diarrhea, proper investigation of the parasitic etiology leads to prompt and effective management.

In the present study, out of 319 stool samples observed, parasites were found in 25 (7.8%) samples. In Chandrasekhar et al[4] study the prevalence rate of parasitic infection was 7.9% which is very much similar to our study. In our study, the predominant age group for the parasitic infections was 46-60yrs (44%). Similarly in Chandrasekhar et al [4] study, the predominant age group was 45-58yrs where nearly half of the total parasitic infections was observed, but in their study protozoan parasites were detected predominantly,with 185 out of 187. Entamoeba histolytica was the most common organism observed accounting for 110 out of 187 parasites observed. But in our study, protozoan parasites accounted for only 25% and helminthic parasites75%.

In another study by Narayan srihari et al [5], the prevalence of parasitic infections was 24.78% with the majority of the stool samples showing E.histolytica as the predominant parasite (43.86%). In our study, E.histolytica accounted only for 9.5%, giardia 23.8% of the total parasites.

Though, nematodes are not very common diarrheagenic pathogens, in our study they accounted predominantly to an account of 75%, with ascaris (66.7%) followed by stronglyoides (19%). There is a chance for the accidental discovery of these parasites in patients with other underlying cause for diarrhea, these agents were considered significant because majority of the ascaris were identified in malnourished pediatric patients and also no other bacterial pathogen was isolated in theses samples. All the strogyloides larvae were observed in the adults where two of the four patients were HIV positive and one patient was having underlying malignancy. In Shailesh Sutariya et al[6] study conducted in pediatrics population with diarrhea, 2/3rds of the infections were of helminthic parasites with ascaris predominating followed by hookworm which is similar to our results.

11.5% of the total samples pathogenic bacteria were isolated. This is similar to Kansakar et al[7] and Santos et al [8] studies where the bacterial pathogens were isolated to an account of 16.8% and 13.1% of the total samples respectively. In our study, shigella flexneri(48.6%) was the most common bacterial pathogen, followed by vibrio cholera (29.7%). In Santos et al[8] study shigella was the most common isolate accounting for 54.3% of the bacterial isolates where as in Kansakar et al[7] study the predominant organism was vibrio cholera 66.2% of the total bacterial isolates

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followed by shigella 27.7%. While salmonella was the second most common isolate in Santos et al [8] study accounting to 38.4% of the isolates, in our study salmonella constituted only 8.1% of the total isolates.

In our study plesiomonas accounted for 10.8% of the pathogenic bacterial isolates. Though, plesiomonas was not isolate routinely by many authors, in a very huge study by Escobar et al[9] plesiomonas accounted for 8.6% of the total samples.

Some of the E.coli like EPEC, EHEC, EIEC are considered as potential pathogenic agents and E.coli has been isolated either as predominant or one of the most common diarrhea genic bacterial pathogen by many authors, we have not included it under pathogens because it needs a battery of tests including antisera for the correct identification of these pathogenic subtypes which is not routinely practiced. In our study, E.coli accounted for 70% of the total isolates. In Santos et al[8] study EPEC accounted for 7.3% of the total isolates which were identified by using antisera.

In the present study, shigella isolates were highly resistant to ampicillin (100%), amoxyclav and co-trimoxazole (83% each). Ciprofloxacin resistance was also considerably high with 67%. Ceftriaxone resistance was observed in 44% of the isolates. Our findings correlate well with Bhattacharya et al[10] study where ampicillin resistance was seen in 100% of isolates ciprofloxacin resistance in 82% and co-trimoxazole resistance in 80% of the shigella isolates. But the resistance to amoxyclav and ceftriaxone was relatively less (12% each) when compared to our results.

In our study vibrio cholera was highly resistant to co-trimoxazole (100%) and ampicillin (45%). Resistance to azithromycin was also high 27%. Ciprofloxacin and doxycycline resistance accounted for 18% each. The most effective antibiotics against vibrio were ceftriaxone and chloramphenicol with 91% and 100% sensitivity respectively. Our findings correlate with the findings of Shukla das et al[11] and Kansakar et al[7] studies. In Shukla das et al[11] study, all the vibrio isolates were resistant to ampicillin and co-trimoxazole(100%). Ciprofloxacin and doxycycline resistance was observed in 30% and 20% of the isolates respectively. In Kansakar et al[7] study also co-trimoxazole and nalidixic acid resistance was 100% whereas tetracycline and ciprofloxacin were 100% sensitive.

In our study salmonella was highly resistant to ampicillin(100%) and co-trimoxazole(67%) while ceftriaxone and ciprofloxacin resistance was seen in 33% of the isolates. These findings correlate with Kansakar et al[7] study where ampicillin resistance was 71.4%, co-trimoxazole 66% and ciprofloxacin resistance 23.8% of the salmonella isolates.

Though our study consists of few samples, it gives an idea about the distribution of the pathogens among diarrhea samples and resistance patterns of various bacterial isolates in our region which helps practitioners in choosing the appropriate treatment and thus decreases the mortality and morbidity.

REFERENCE

1. Ansari S, Sherchand J B et al Pattern of Acute Parasitic Diarrhea in Children under Five Years of Age in Kathmandu, Nepal. Open Journal of Medical Microbiology 2012;2: 95-100 | 2. World Health Organization, "Diarrheal Disease," 2009. http/whqlibdoc.who,int(pdf) | 3. Clinical and Laboratory Standards Institute (2010) Performance standards for antimicrobial susceptibility testing. 20th Informational Supplement Document M100-S20, CLSI, Wayne. | 4. ChandrashekarV ,et al. Detection and Enumeration of the Commonest Stool Parasites Seen in a Tertiary Care Center in South India. ISRN Tropical Medicine 2013;1-6 | 5. Narayan Shrihari, KumudiniT.SD.Bact, Mariraj. J, Krishna.S .The Prevalence of Intestinal Parasitic Infections in a Tertiary Care Hospital-a retrospective study. JPBMS2011;12(13):1-4 | 6. ShaileshSutariya, NitibenTalsania, Chintul Shah. Study of prevalence of diarrhoeal diseases amongst under five population.National journal of community medicine 2011;2(1):96-99 | 7. P Kansakar, PankajBaral, SaralaMalla, Gokarna Raj Ghimire. Antimicrobial susceptibilities of enteric bacterial pathogens isolated in Kathmandu, Nepal, during 2002-2004. J Infect DevCtries 2011; 5(3):163-168 | 8. Daniel R. Diniz-Santos, José S. Santana, Junaura R. Barretto1, MariaGoreth M. Andrade, Luciana R. Silva. Epidemiological and Microbiological Aspects of Acute Bacterial Diarrhea inChildren from Salvador, Bahia, Brazil. BJID 2005; 9:77-83 | 9. Juan C. Escobar, Darlene Bhavnani, Gabriel Trueba, Karina Ponce, William Cevallos, et al. Plesiomonasshigelloides Infection, Ecuador, 2004–2008. Emerging Infectious Diseases • www.cdc.gov/eid 2012;18(2):322-324 | 10. Bhattacharya D, Sugunan A.P, HaimantiBhattacharjee, R. Thamizhmani, Sayi D.S. , et alAntimicrobial resistance in Shigella- rapid increase & widening of spectrum in Andaman Islands, India. Indian J Med Res 2012;135:365-370 | 11. Shukla Das, RumpaSaha,Iqbal R. Kaur. Trend of antibiotic resistance of Vibrio cholerae strains from East Delhi. Indian J Med Res 2008;127:478-482 |