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Microbiology

Microbiological Stydy of Diarrhoeal Patients Admitted in Tertiary Hospital of Telangana

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Original Research Article

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Abstract: Acute diarrhoeal illness is common in tropical regions during summer and monsoon seasons microbial agents causing diarrhea can be bacteria, virus, parasite or fungus. Our aim was to study the microbiological agents involved in causing diarrhoea in patients admitted at tertiary hospital Telanagana region.The study group included stool samples of 360 patients admitted in government general Hospital Nizamabad, Telangana suffering with acute diarrhea and stool samples of 50 non diarrhoeal patients as control. The samples were immediately transported to the laboratory and microscopy, culture was done .The identification of the organism was confirmed after biochemical reactions and agglutination tests with the polyvalent sera. Antibiotic susceptibility was done using Kirby Bauer disc diffusion method according to CLSI guidelines phage typing was done at National Institute of Cholera and Enteric disease Kolkata. Among 360 feacal samples, majority collected in summer season included males between 21-40 yrs of age, presented with watery diarrhea (162.) – 45%, (108) – 30%bloody diarrhea, (90) -25% with mucus the following organisms were isolated, Bacteria (303)-84.2%; parasite (102)-28.3 % and fungus (09)- 2.5% ; vibrio cholera 66(18.3%) was major isolate followed by E.coli 60 (18%), Campylobacter 12(3.3%), Shigella flexneri 10(2.7%), Staphylococcus 4(1.1%) Salmonella paratyphi 4 (1.1%). Antibiogram was done which showed 100% sensitivity to amikacin, gentamycin, ciprofloxacin, tetracycline, doxycycline, Cefotaxim and norfloxacin; 100% resistance to cotrimoxazole. Phage typing of vibriocholera done and found to be T-7, T-26.The study shows bacterial isolates were in majority including vibrio cholera ,males of age group 21-40 yrs more involved during summer season.
Keywords: Acute diarrhea, vibrio choler, antibiogram, phage typing.

INTRODUCTION

Water borne diseases are essentially biological caused by bacteria, viruses and parasites. There are various forms of manifestations due to infection of water borne pathogens most important being diarrhoea. Diarrhoea is defined as the passage of loose stools, liquid or watery stools (more than three times per day). It must be distinguished from pseudodiarrhoea (frequent passage of small volume of stools often associated with rectal surgery or irritable bowel syndrome) and faecal incontinence (involuntary discharge of rectal contents). Although it is more important in infants and children, diarrhoeal disease has an important impact on aults as well adults on an average, suffer one to two episodes of diarhoea yearly. This results in significant economic costs due to utilization of health care resources and lost productivity. Like the young, elderly especially those institutionalized in long term care facilities are at increased risk for serious consequences resulting from gastrointestinal infections.

Diarrhoeal illness accounts for 2.5 million deaths per year worldwide .Diarrhoea is usually a symptom of an infection in the intestinal tract , which can be caused by a variety of bacterial, viral, and parasitic organisms. Infection is spread through contaminated food or drinking water or from person to person as a result of poor hygeiene[1]. Cholera is an acute diarroeal disease that can kill within hours if left untreated. Researchers have estimated that each year there are 1.3 million to 4 million cases of cholera and 21000to 143000 deaths worldwide due to cholera [1]. In 2016 38 countries reported a total of 13121 cases including 2420 deaths resulting in an overall case fatality rate of 1.8%. In Asia, 12 countries reported a total of 18445 cases and 184 deaths during 2016. India reported 841 cases in 2016[2].

Bacteria and parasites continue to be recognized as important cause of diarrhea worldwide. The major cause of death of acute diarrhoeal disease is dehydration and circulatory collapse. An understanding of seasonal variation of enteric pathogen would contribute greatly in focusing health care initiatives in a climate of united resources to a cost effective reduction in disease mobidity and mortality .Objective of the study was to

identify the cause of acute diarrhea, seasonal variation among the isolated organism, antibiotic susceptibility pattern of isolated organism, changing profile of vibrio cholera.

MATERIALS AND METHODS

On approval from ethical committee 360 faecal sample from patients suffering from acute diarrhea admitted to Government General hospital nizamabad were processed ,50 faecal samples of non diarrhoeal cases were also included in the study. Fresh stool samples were collected, rectal swabs were used whenever required, samples were collected before antibiotic therapy, and collected samples were immediately transported to laboratory without any delay. .If any delay samples were transported in Cary Blair medium and brought to laboratory. Microscopic examination was done by preparing wet mounts with iodine and normal saline for ova and cyst; modified AFB staining was also done. Feacal sample were enriched on Selenite broth, alkaline peptone water for 6-8 hrs hanging drop preparation for motility then cultured on Blood agar, Maconkey agar, deoxycholate citrate agar, Thiosulphate Citrate Bile salt sucrose (TCBS) agar. Inoculated plates were examined for colony morphology, any pigmentation, metallic sheen and fermentation of lactose on media. Prelimnary test such as motility, Gram stain, Catalase test, oxidase test.and biochemical reactions such as indole test, methyl red test, Voges Proskauer test, citrate test urease test, triple sugar iron agar test, oxidation fermentation test, string test, nitrate reduction test, Aminoacid decarboxylase reaction, gelatin liquefaction test, coagulase test, germ tube test, sugar fermentation reactions were done for identification of bacteria up to species level[4]. Antibiotic sensitivity test of the organism isolated was determined as per Clinical and Laboratory Standards Institute (CLSI) guidelines by Kirby Bauer disc diffusion method on Muller Hinton Agar usng the following antimicrobial agents(ug/disc) such as ampicillin(10), tetacycline(30), gentamicin (10), Amikacin (30), cotrimoxazole(25), ciprofloxacin (5), furazolidone(50) [5]. Final identification by slide agglutination with polyvalent antisera and confirmed by monovalent antisera. Biotyping and phage typing of vibrio isolates were done by sending samples to NICED Kolkata.

RESULTS

Majority of patients presented with watery diarrhea and the average hospital stay was less than three days. Mortality was nil due to mild dehydration. Males were affected predominantly .Majority of cases were reported between 21-40 yrs of age. Maximum samples were processed during summer and monsoon period. Out of 360 samples 162 (45%) cases presented with acute watery diarrhea, 108(30%) presented with blood in the stool samples, 90 (25%) presented with mucus in the stool samples.

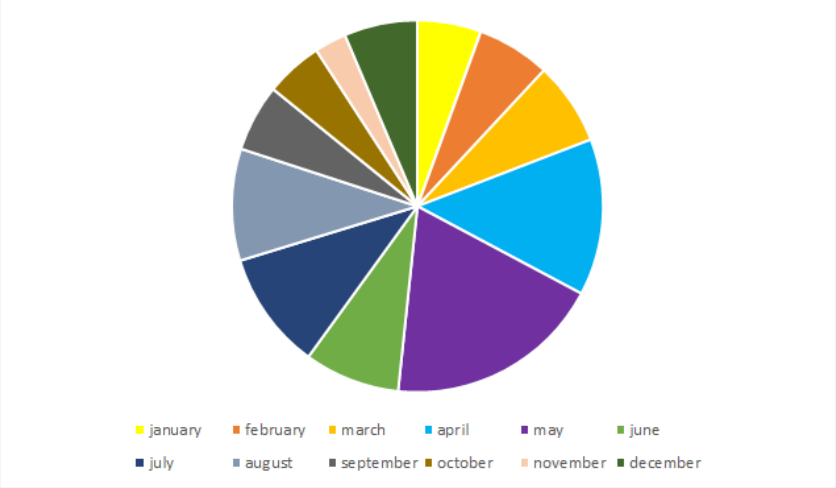

Chart-1: Month wise distribution of stool samples

Table-1: Analysis of stool samples

Total no. of samples	Pathogens					
	Bacteria		Parasites		Fungus	
	No.	%	No.	%	No.	%
360	303	84.2	102	28.3	09	2.5

There was no growth in 48 samples (13.3%).

Table-2: Incidence of organism isolated from 360 diarrhoeal and 50 non diarrhoeal (control)

Organisms	Diarrhoeal (study group)		Nondiarrhoea (control group)	
Bacteria	No. of cases	%	No.	%
Vibrio cholerae	66	18.3	…..	…..
Escherichia coli	60	18	24	48
Campylobacter spcies	12	3.3	….	….
Shigella flexneri	10	2.7	…..	…..
Staphyloccus aureus	04	1.1	…..	…..
Salmonella paratyphi A	04	1.1	…..	…..
Klebsiella	36	10	10	20
Pseudomonas	45	12.5	07	14
Proteus	27	7.5	04	8
Others(coagulase negative staphyloccus, enterococci, Citrobacter)	39	10.8	05	10

Other agents identified as candida albicans 09 (2.5%) on culture, parasites as Entamoeba histolytica cyst 89 (24.7%), Ascaris ova 08 (2.2 %) Giardia cyst 03 (0.83%) were identified on wet mount.

Table-3: Seasonal Variation

Months	Pathogens				
	Vibrio cholerae	E.coli	Campylobacter species	S. flexneri	S. paratyphi A
April – May	19	40	02	07	02
June, July , august	36	28	10	…	02
September – October	10	22	…	03	…

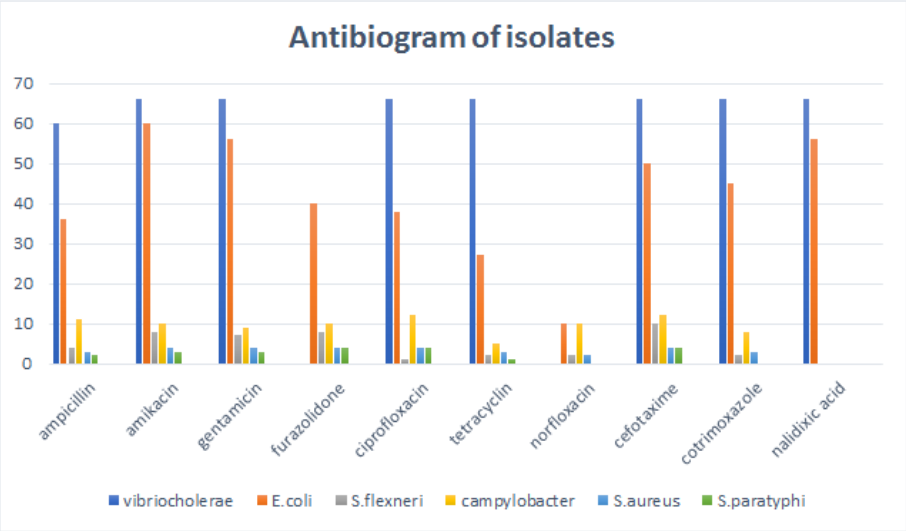

Chart-2: Antibiogram of different isolates

Out of 66 isolated Vibriocholerae samples Ogawa 13(19.7%) and Inaba 53(80.3%) isolated. Phage typing was done At NICED Kolkata phage type T7, T26 were isolated.

DISCUSSION

Infectious diarrhoea is a major public health concern .the diagnosis of infecting microbe is important for the management of severe cases as also for the prevention and control of epidemics. The present study was undertaken to study the bacterial, fungal, parasitic agents causing diarrhea and to study the changing serotype pattern and antibiotic susceptibility pattern of vibrio cholerae. The symptomatology in diarrhea was similar irrespective of the isolates. Males and adults accounted for 55% and 73 % respectively where cases were repoted between age group 21-40. Same was observed by ChihYu Chi *et al*.[6]. Major isolates were of bacterial origin (84.2%) followed by parasites (28.3%) then fungal etiology (2.5 %) virus isolation was not done in our study. The findings were comparable with Iruka N. Okeke *et al*. study [7].

The isolation rate of vibrio cholerae O1 ElTor reported by M.Alam et al in Pakistan (33.8%); 43.1% was reported by NC Sharma in Delhi [2007], 31 % by Sudheendhra Kulkarni *et al*. in Bidar, Karnatka, 6 out of 30 cases (20%) by Prakash Hindurao Waghmare *et al*. in solapur, Maharashtra, studies were carried out during outbreaks. Ajanta sharma *et al*. reported 3.93 % in Assam [8-12]. In our study isolation rate is 18.3 %as collection of samples were done from sporadic cases. Among 66 (18.3%) Inaba serotype were 53 (80.3 %) and 13 were Ogawa strain, O139 was not reported during our period. All were sensitive to Ampicillin, Amikacin, gentamicin, ciprofloxacin, tetracycline, Norfloxacin, Cefotaxime, and resistance to furazolidone, cotrimoxazole. N. C. Sharma reported vibrio cholerae O1 resistant to cotrimoxazole and furazolidone similar findings were found in our study. Multi drug Resistance was not reported in our study. Majority of E.coli isolates in our study were reported during the months of April and May as explained by Quadri *et al*. Schultsz reported 10.7 % [13, 14]. Daniel reported resistant to Doxycycline (49%) 9% cotrimoxazole 35% to ampicillin but sensitive to Norfloxacin [15]. Chakraborty reported resistant to Nalidixic acid and fluroquinolones. In our studye coli isolates reported resistant to ampicillin, tetracycline, cotrimoxazole, ciprofloxacin, and sensitive to amikacin, gentamicin, and cefotaxime [16]. As reported by Barbara *et al*. shigella and Entamoeba histolytica were found in cases complaining of bloody diarrhea .similar findings were noted in our study, the incidence of Shigellosis is 2.7 % and Entamoeba histolytica 28.3% in our study. The studies of Irika N. Okeke showed shigella isolates 7% and Entamoba histolytica 35 % out of 113 samples in south western Nigeria. Komathi A.G, Anantham .S in 1998 reported 3.5% of Salmonella gastroenteritis, in there study Salmonella Typhi A was maximal isolate. Irika N. Okeke also isolated salmonella tyhi 1%, in our study we isolated 1.1% of salmonella paatyphi A. Isolation rate of Pseudomonas, Klebsiella , proteus and others (CONS, enterococci, Citrobacter) was equal both in control and diarrhoeic samples..Carole Tremblay *et al.* reported campylobacter species (20%) with tetracycline resistance 34% .in our study campylobacter species 3.3% were reported with58% tetracycline resistance. Speciation of the isolate could not be done due to constraints, Staphylococcus aureus isolation is 1.1% from diarrheal samples, etiology being food poisonng. Isolation of candida albicans 9(2.5%) may be due to immunocompromised condition. 13.3% cases reported suppressed normal flora the reason could be previous antibiotic intake.

CONCLUSION

Samples were collected sporadically bacterial, parasites; fungal agents are involved in causing Diarrhoea. Vibrio cholerae O1 ElTor serotype Inaba was isolated in maximum in our study, O139 was not isolated. Adults with age group 21-40 yrs are affected most isolation rate of Vibrio cholerae is maximum during premonsoon period and E.coli during summer season .this shows that the changing seasonality pattern plays a major part in the etiology of diarrhoeal disease.

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