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Original Article

Microbiology Section

A Comparative Study of Bacterial and Parasitic Intestinal Infections in India

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ABSTRACT

Background: Infectious diarrhea causes a major health problem in developing countries with significant morbidity and mortality. Very often, rehydration therapy alone does not suffice, mandating the use of antimicrobial agents. However, rapidly decreasing antimicrobial susceptibility is complicating the matters.

Materials and Methods: The study aimed to determine the prevalent bacterial and parasitic agents of diarrhea in India. A cross-sectional study was done at Maulana Azad Medical College and associated Lok Nayak Hospital, New Delhi, during 2012-14. Stool samples were received from patients of all age groups and processed for bacteriological and parasitological identification by microscopy, bacterial culture, biochemical identification, serotyping and antimicrobial susceptibility tests. The study also aimed to identify the recent papers (after year 2000) reporting aetiology of infectious diarrhea in India involving the general population as a whole and compare them with present findings.

Results: Out of 6527 samples, 581 (8.90%) were positive for bacterial pathogens. A total of 280 samples (of 3823 under-five year children) were positive for diarrheagenic *Escherichia coli*. Other organisms like *Vibrio cholera* were found in 159 (2.44%) cases, *Shigella* spp. in 126 (1.93%), *Salmonella Typhi* in 7 (0.11%), *Salmonella Typhimurium* in 6 (0.10%), *Aeromonas hydrophila* in 3 (0.05%) cases. Levels of resistance to nalidixic acid, amoxicillin and ciprofloxacin were alarmingly high. Third generation cephalosporins were seen to be moderately active except against *E. coli*.
Parasites were identified in 312 (4.78%) cases. *Giardia intestinalis, Ascaris lumbricoides* and *Entamoeba histolytica* were identified in 2.27%, 1.15% and 0.64% cases respectively.

Conclusion: Analysis of recent nationwide studies revealed *V. cholerae* was the most common bacterial/parasitic agent of diarrhea across all populations, being followed by diarrheagenic *E. coli* and *Giardia intestinalis*. Periodic laboratory monitoring of antimicrobial susceptibility pattern is essential, as is formulation of effective antibiotic use policy.

Keywords: Antimicrobial resistance, Bacteria, Diarrhea, Parasites

INTRODUCTION

Gastrointestinal infections are the leading cause of morbidity and mortality throughout the world. Children and young adults are the most commonly affected group [1]. Worldwide, 17% under-five mortality has been attributed to diarrhea [2]. In India, diarrhea is the cause of hospital admissions in one third paediatric patients and a cause of death in 17% of all indoor paediatric patients [3], however, the data in general population is sparse.

Diarrhea may be caused by bacteria, viruses and parasites. Bacterial organisms like diarrheagenic *Escherichia coli, Vibrio cholerae, Salmonella* spp., *Shigella* spp., *Aeromonas hydrophila, Plesiomonas shigelloides, Campylobacter* spp. are well-known causative agents for gastrointestinal diseases all over the world. Most infections caused by these organisms may be asymptomatic or can be effectively treated by fluid and electrolyte replacement, particularly in the case of viruses and some bacteria. The introduction of antibiotics in therapy may cut short the duration of diarrhea and also limit the shedding of the organisms which otherwise might continue to pollute the environment and pose further risk of transmission of infections. However, antimicrobial resistance is an important overgrowing problem. Intestinal parasites may be associated with serious clinical diseases and mortality and it may cause malnutrition leading to impairment of physical and mental development in children. Therefore, it is necessary to have an accurate description of the situation to target interventions in affected areas.

In the present study, we determined the prevalence of bacterial and parasitic agents of diarrhea in stools of individuals and the antimicrobial susceptibility of bacterial isolates. The results were then critically compared with previous studies to deduce the commonest enteric pathogens in India.

MATERIALS AND METHODS

Study Population

Six thousand five hundred and twenty seven stool samples from patients with diarrhea presenting in Lok Nayak Hospital, New Delhi were received over a period of three years (2012-14) in the Microbiology Laboratory, Maulana Azad Medical College, New Delhi, India. An episode of diarrhea was defined as three or more loose or watery stools per day. Stool samples were processed for parasitological and bacteriological identification and antimicrobial susceptibility of bacterial isolates.

Stool Sample Processing

Stool samples were examined grossly for color, consistency, presence or absence of blood, mucus and worms. Routine stool microscopic examination of saline and iodine preparation was done for red blood cells, pus cells, trophozoites and cysts of protozoa and ova of helminthes. Modified acid fast stain [4] was done to visualize oocysts of *Cryptosporidium* and *Isospora belli*.

Stool samples were cultured on several media for maximal yield. Sample was inoculated directly and after enrichment in selenite F broth and APW (Alkaline peptone water) onto MacConkey's agar, Xylose Lysine Desoxycholate agar, Deoxycholate Citrate Agar and Bile salt agar. The plates were incubated for 18-24 hours at 37°c. Suspected colonies were subjected to preliminary tests like Gram's staining, hanging drop for motility, catalase and oxidase tests. Their identity were established by a battery of biochemical tests like: fermentation of a variety of sugars, indole test, citrate utilization, urease production, MR and VP tests, production of H_2S on TSI and lysine and ornithine decarboxylase tests and arginine dihydrolase test.

All the organisms identified as *Shigella* spp., *Vibrio cholerae* or *Salmonella* on the basis of colony characteristics and biochemical reactions were confirmed by the slide agglutination test with specific antisera (Denka Seiken Co., Ltd., Tokyo, Japan). *E. coli* isolates from children below 5 years of age were also serotyped to identify diarrheagenic *E. coli* (Denka Seiken Co., Ltd., Tokyo, Japan).

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility testing was performed for all bacterial isolates by using the modified Stokes' disc diffusion method [5] for the following six antimicrobial agents- Nalidixic acid (30µg), Amoxicillin (10µg), Amikacin (30 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Cefotaxime (75 µg). These discs were either prepared in laboratory or commercially procured from HiMedia, Mumbai, India.

RESULTS

Out of 6527 enrolled patients, 3823 (58.57%) were under 5 y of age, 1505 (23.05%) were between 5 y and 12 y and 1199 (18.37%) were more than 12 y of age. On gross examination, 1209 (18.52%) samples were formed in consistency, 3215 (49.25%) were semi-formed and 2103 (32.22%) were liquid. Presence of mucus in stools was observed in 931 (14.26%) samples, while approximately 4.92% (321/6527) samples contained grossly visible blood.

Bacterial Pathogens

Bacterial pathogens were isolated from 581 (8.90%) of the total of 6527 stool samples. These included the diarrheagenic *E. coli*,that were identified by serotyping only in children who were below five years of age. Out of these 3823 samples, 194 (5.07%) yielded enteropathogenic *Escherichia coli* (EPEC). Enterotoxigenic *Escherichia coli* (ETEC), Entero aggregative *E. coli* (EAEC) and enteroinvasive Escherichia coli (EIEC) were isolated in considerably lesser numbers [Table/Fig-1]. The most common EPEC, ETEC, EAEC and EIEC serotypes isolated were O128, O147, O44 and O164, respectively.

Vibrio cholerae were found in 159 out of 6527 (2.44%) samples. All *Vibrio cholerae* were serotyped as O1 Ogawa. Other strains of *Vibrio cholerae* and *Vibrio parahemolyticus* were not isolated in any sample. A total of 126 (1.93%) *Shigella* spp. was isolated in cases with dysentery. *Shigella flexneri* was the most common isolated species and it was present in 0.96% cases, followed by *Shigella boydii, Shigella dysenteriae and Shigella sonnei. Salmonella Typhi* was isolated in 7 cases and *Salmonella Typhimurium* in 6 cases. *Aeromonas hydrophila* was found in 3 cases [Table/Fig-1].

Parasitic Pathogens

Parasites were identified in 312 (4.78%) patients as the aetiological agent of diarrhea. *Giardia intestinalis, Ascaris lumbricoides and Entamoeba histolytica* comprised the major parasitic pathogens (2.27%, 1.15% and 0.64% respectively) [Table/Fig-1]. *Cryptosporidium* and *Isospora* were identified in 10 (0.15%) and 9 (0.13%) cases respectively and all of these patients were retrospectively found to be HIV positive.

Antimicrobial Susceptibility Testing

Susceptibility levels of bacterial pathogens to various antimicrobial agents are shown in [Table/Fig-2]. All bacterial isolates were highly sensitive to amikacin and poorly sensitive to nalidixic acid. *E. coli* and *Vibrio cholerae* showed 89.64% and 96.22% sensitivity to amikacin respectively. Some pathogens like *Shigella boydii* and *Shigella dysenteriae* were completely resistant to nalidixic acid. *Shigella dysenteriae* was completely resistant to amoxicillin. Activity of ciprofloxacin was seen to fall against *E. coli* and *Shigella* spp. High resistance to third generation cephalosporins was seen among diarrheagenic *E. coli* and *Shigella* spp.

	Present Study	Das et al., [6]	Nair GB et al., [7]	Tejashree A et al., [8]	Total
Period of study	**2012-14**	**2002-04**	**Nov 2007 – Oct 2009**	**Jan 2012 – July 2013**	
Sample size	6527	2534	2519	1584	13164
Region	Delhi	Delhi	West Bengal	Mysore	
Bacterial agents:					
EPEC	194 (5.07)*	38 (1.49)	45 (1.8)	229 (14.45)	865 (8.27)#
ETEC	49 (1.28)*		114 (4.5)		
EAEC	28 (0.73)*		159 (6.3)		
EIEC	9 (0.23)*		-		
Vibrio cholerae	159 (2.44)	330 (13.02)	711 (28.3)	116 (7.32)	1316 (9.99)
V. parahemolyticus	-	-	74 (2.9)	-	74 (0.56)
V. fluvialis	-	-	55 (2.2)	-	55 (0.42)
Shigella flexneri	63 (0.96)	20 (0.79)	154 (6.1)	17 (1.07)	331 (2.51)
Shigella boydii	36 (0.55)	-		-	
Shigella dysenteriae	14 (0.21)	6 (0.24)		1 (0.06)	
Shigella sonnei	13 (0.19)	-		7 (0.44)	
Salmonella Typhi	7 (0.11)	3 (0.12)	23 (0.9)	5 (0.32)	66 (0.50)
Salmonella Typhimurium	6 (0.10)	11 (0.43)		9 (0.57)	
Aeromonas hydrophila	3 (0.05)	-	25 (1.0)	2 (0.12)	30 (0.23)
Campylobacter spp.	-	-	140 (5.6)	-	140 (1.06)
Parasitic agents:					
Entamoeba histolytica	42 (0.64)	28 (1.10)	82 (3.3)	-	152 (1.15)
Giardia intestinalis	148 (2.27)	37 (1.45)	281 (11.2)	4 (0.23)	470 (3.57)
Cryptosporidium	10 (0.15)	8 (0.32)	158 (6.3)	-	176 (1.34)
Isospora belli	9 (0.13)	-	-	-	9 (0.07)
Blastocystis hominis	4 (0.06)	-	11 (0.4)	2 (0.12)	17 (0.13)
Ascaris lumbricoides	75 (1.15)	25 (0.99)	-	-	100 (0.76)
Hymenolepis nana	15 (0.23)	-	-	-	15 (0.11)
Hookworm	2 (0.03)	21 (0.83)	-	1 (0.06)	24 (0.18)
Trichuris trichura	2 (0.03)	9 (0.36)	-	-	11 (0.08)
Taenia spp.	3 (0.04)	7 (0.28)	-	-	10 (0.07)
Enterobius vermicularis	1 (0.01)	-	-	-	1 (0.01)
Strongyloides stercoralis	1 (0.01)	-	-	-	1 (0.01)

[Table/Fig-1]: Aetiological agents of diarrhea (number of isolates)

*Calculated out of n=3823 (cases under 5 years of age)
#calculated out of n=10460
Note: figure in parentheses indicate percentage

DISCUSSION

Our hospital is a centrally located, 1676 bedded tertiary care hospital with thickly populated catchment areas. Most of the patients fall in the low to middle socio-economic groups. This explains the large sample load that we received over a period of two years.

Diarrhea is a major cause of morbidity and mortality in India and other developing countries of the tropical zone [9-12]. Thus, there is a constant need to keep the knowledge updated with regards to pathogens associated with diarrheal illness and their antimicrobial sensitivity pattern for providing effective health care facilities. The empiric antimicrobial treatment, if required should be directed against locally prevalent pathogens.

Organisms	NA	CP	AX	AK	G	CF
Escherichia coli (n=280)	13 (4.64)	47 (16.78)	54 (19.28)	251 (89.64)	170 (60.71)	65 (23.21)
Vibrio cholerae (n=159)	7 (4.40)	124 (82.66)	19 (11.94)	153 (96.22)	144 (90.56)	140 (88.05)
Shigella flexneri (n=63)	3 (4.76)	46 (73.01)	5 (7.93)	61 (96.84)	51 (80.95)	53 (84.12)
Shigella boydii (n=36)	0 (0)	19 (52.77)	3 (8.33)	36 (100)	25 (69.44)	25 (69.44)
Shigella dysenteriae (n=14)	0 (0)	8 (57.14)	0 (0)	13 (92.85)	14 (100)	10 (71.42)
Shigella sonnei (n=13)	1 (7.69)	7 (53.84)	3 (23.07)	13 (100)	7 (53.84)	9 (69.23)
Salmonella Typhi (n=7)	3 (42.85)	6 (85.71)	3 (42.85)	7 (100)	6 (85.71)	6 (85.71)
Salmonella Typhimurium (n=6)	2 (33.33)	3 (50)	2 (33.33)	6 (100)	6 (100)	5 (83.33)
Aeromonas hydrophila (n=3)	2 (66.67)	3 (100)	0 (0)	3 (100)	3 (100)	3 (100)

[Table/Fig-2]: Antimicrobial Sensitivity pattern of isolated bacterial pathogens
NA- Nalidixic acid, CP- Ciprofloxacin, AX- Amoxycillin, AK- Amikacin, G- Getamicin, CF- Cefotaxime
Note: figure in parentheses indicate percentage sensitivity

One of the objectives of the study was to identify the recent papers (after year 2000) reporting aetiology of infectious diarrhea in India involving the general population as a whole and compare them with present findings. Thus, we searched the PubMed/Medline, Google scholar and various popular journals to look for various combination of following terms: 'diarrhea', 'gastroenteritis', 'enteropathogens', 'aetiology', 'epidemiology', 'India', etc. Studies enrolling only patients with clinical signs of dysentery, i.e. blood in the stool, studies conducted in special populations such as infants, children, travellers, patients hospitalized for other specific reasons, or only HIV positive persons were excluded.

Majority of the studies conducted in India, since the year 2000, have restricted themselves to narrow groups (such as infants, children, HIV positive patients, etc.). Despite thorough search, we were able to find very few studies meeting the exclusion and inclusion criteria [Table/Fig-1]. Paucity of similar studies has also been reported by Walker CLF et al., [11] on a global scale. They were able to identify only 22 studies worldwide, meeting exclusion and inclusion criteria which were similar to ours.

The data from various Indian studies was then compiled to make an attempt to identify the most common bacterial or parasitic agent responsible for diarrhea in India. The compiled sample size for all the national studies was 13164 [Table/Fig-1]. *Vibrio cholerae* was found to be the most common bacterial agent in Indian studies, responsible for 9.99% cases. This was followed by diarrheagenic *E. coli* (8.27%) and *Shigella* spp (2.51%) as the second and third most frequent bacterial agents. The findings of the present study differed slightly; showing that in our region diarrheagenic *E. coli* (7.31%) is most common, followed by *Vibrio cholerae* (2.44%) and *Shigella* spp (1.91%).

In this study, serotyping for diarrheagenic *E. coli* was performed only for children under 5 years of age, keeping in view the fact that *E. coli* causes infection mostly in children and also due to limited availability of antisera. Out of 3823 under-5 year cases, 280 (7.31%) cases were infected with diarrheagenic *Escherichia coli*. This finding is in accordance with several studies from other developing countries as well [6,10,13-15]. A systematic review concluded that rotavirus, calicivirus, enteropathogenic and enterotoxigenic *E. coli* cause more than half of all diarrheal deaths in children <5 y in the world, thus emphasizing the role of *E. coli* in childhood diarrhea [16]. There were no major differences between the susceptibility profiles of EPEC, ETEC, EAEC and EIEC [Table/Fig-2].

Vibrio cholerae is endemic in India and is characterized by yearly and seasonal outbreaks. In our study, *V. cholerae* was found to be the second most common cause of diarrhea, with almost three-fourth of strains being isolated during the months of July to September. All of the isolated *Vibrio cholerae* strains were of the Serogroup O1, serotype Ogawa. However, Das et al., found 56 of 96 strains isolated in 2004 were of serotype Inaba [6]. These strains were largely sensitive to ciprofloxacin, amikacin, gentamicin and cefotaxime and resistant to nalidixic acid and amoxicillin [Table/Fig-2], in agreement with previous studies from Delhi [6] Chandigarh [17] and Calcutta [18].

In industrialized countries, shigellosis occurs mostly due to *Shigella sonnei* while in the developing countries, most cases are due to *Shigella flexneri*, *Shigella dysenteriae* and *Shigella boydii* [19]. In the present study, *Shigella flexneri* was the most common causative agent followed by *Shigella boydii, Shigella dysenteriae* and *Shigella sonnei*. Poor sensitivity to nalidixic acid and amoxicillin and rising resistance to ciprofloxacin and third generation cephalosporins raises concerns and demands more restricted use of these antibiotics to control further increase in resistance [Table/Fig-2].

Compiled data from recent national studies [Table/Fig-1] suggested that among the protozoans, *Giardia intestinalis* (3.57%), *Cryptosporidium* spp. (1.34%) and *Entamoeba histolytica* (1.15%) were most frequent offenders. Helminthic infections such as *Ascaris lumbricoides* (0.76%), Hookworm (0.18%) and *Trichuris trichura* (0.08%) were less common findings. Findings of the study at our centre were slightly different with respect to the incidence of various parasitic infections.

Cryptosporidium and *Isospora* are infrequent finding in India accounting for 1.34% and 0.07% cases of diarrhea [Table/Fig-1]. Another study found that 70 (2.7%) of 2,579 (2.7%) children were found be positive by microscopic methods, and *Cryptosporidium hominis* was the most frequent species as determined by genotypic methods [20]. In the current study, these were identified in 0.15% and 0.13% cases, respectively. All these patients were HIV positive, thus suggesting that HIV positive individuals should be examined for these parasites.

LIMITATIONS

This study was limited by the fact that there is very little recent published data from India documenting the exact prevalence of bacterial and parasitic diarrhea causing agents that met our inclusion and exclusion criteria, encompassing the general population and not restricting to specific groups. Moreover, use of different methods and use of special media for specific organisms such as *Campylobacter* spp. may have resulted in non-uniform reporting of results. Nevertheless, inclusion of large compiled sample size of 13164 specimens compensates for the limited number of studies available to a certain extent.

CONCLUSION

Analysis of recent nationwide studies revealed *V. cholerae* was the most common bacterial/parasitic agent of diarrhea across all populations, being followed by diarrheagenic *E. coli* and *Giardia intestinalis*.

This study also documents the antimicrobial susceptibility of bacterial agents to different antibiotic agents. Amikacin and third generation cephalosporins retain their activity as an anti-diarrheal

agent. However, their indiscriminate use may cause development of resistance to these therapeutic agents in the near future. Antimicrobials should be resorted to only in severe cases, who do not improve despite rehydration therapy. Proper maintenance of sanitation and hygiene is also useful to check transmission of enteric pathogens.

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