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Prevalence of Enteric Bacterial Pathogens among HIV Infected Versus Non-HIV Infected Patients with Diarrhoea in Northern India

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A B S T R A C T

Enteric microbial agents in HIV differ in several ways between developing and developed world, the awareness of which can often guide appropriate prevention and patient treatment when limitation of resources prevent laboratory diagnosis of exact etiological agent. The primary objective of this study was to define and compare enteric bacterial pathogens in HIV-1 infected with diarrheal symptoms and non-HIV infected controls with diarrheal symptoms at a tertiary care hospital in northern India. This prospective study was conducted between January 2014 and December 2015 of a tertiary care academic health organization of North India. Stool samples from 300 HIV seropositive cases with diarrhea (study group) and 600 HIV negative diarrhea cases (control group) were examined. Samples were inoculated onto standard culture media. All the isolates were tested for antimicrobial susceptibility. Out of 300 HIV seropositive cases with diarrhea, 114 (38%) were positive for bacterial pathogens whereas in 600 HIV seronegative with diarrhea controls 120 (20%) were positive for bacterial pathogens. 41 isolates of Diarrheagenic *Escherichia coli*, 24 *Campylobacter jejuni*, 10 *Aeromonas hydrophilia*, 16 *Shigella* spp., 9 *Salmonella* spp., and 14 *Yersinia enterocolitica* were recovered from the HIV infected cases. Most of the bacteria were resistant to nalidixic acid. There is underline need for epidemiological investigations to screen microbial etiological agents in HIV infected subjects with diarrhoea along with their antibiogram periodically for reduction of morbidity and mortality in these patients.

Introduction

HIV is a major problem in India with a total number of people living with HIV (PLHIV) estimated at 21.17 lakhs in 2015 (National Institute of Medical Statistics and National AIDS Control Organization, 2015). Patients with HIV are prone to developing a variety of opportunistic infections during their lifetime. Superimposed infections due to impaired immunity are a major health problem among HIV seropositive patients. Diarrhea one of the hallmarks of HIV/AIDS, is a significant cause of morbidity and mortality in people living with HIV especially in developing countries (Krones and Hogenauer, 2012). Diarrheal episodes may be acute and brief, intermittent or recurrent, or, in some cases, chronic and severe. Diarrhea has been associated with 50% of HIV/AIDS patients in the developed world

and in up to 90% of patients residing in developing countries (Feasey *et al.,* 2011). Chronic diarrhea associated with weight loss in association with a positive HIV-1 serology test is an AIDS-defining criterion, according to the World Health Organization classification (Gupta *et al.,* 2008).

With improvement of socioeconomic conditions etiology of diarrhea is changing from infectious causes to noninfectious causes like reactions to medication, intestinal disorders such as irritable bowel syndrome, or intestinal diseases, including ulcerative colitis, Crohn's disease, celiac disease and microscopic colitis (Humphries and Linscott, 2015). In these instances, laboratory tests for infectious etiologies, including a bacterial stool culture, are useful for diagnosis by either ruling out or ruling in a common infectious process. A wide variety of enteric pathogens have been isolated from AIDS patients with diarrhoea. *Shigella* spp, *Campylobacter* spp., *Escherichia coli*, *Aeromonas* spp. and *Plesiomonas shigelloides* have been reported to occur more frequently in HIV infected persons than in control subjects without HIV infection (Kownhar *et al.,* 2007; Prasad *et al.,* 2000; Obi *et al.,* 2007). Diarrhea caused by bacterial etiology requires the use of antibiotics which shortens the duration of diarrhea, decrease frequency of stool output and reduce complications. Nevertheless, this scenario becomes more relevant in HIV group, as majority of publications in it are on the enteroparasites infections (Rossit *et al.,* 2009). Antibiotic susceptibility profile vary from country to country and hospital to hospital in the same town as well as between private and public healthcare facilities in the same area (Rossit *et al.,* 2009; Sanchez *et al.,* 2005). Therefore, periodic evaluation of antibiotic susceptibility is recommended to guide management of patients requiring antibiotic treatment. The present study was undertaken as study of enteric bacterial pathogens in

patients with HIV infection and diarrhoea, and to determine whether there were significant differences in enteric infection when compared to HIV patients without diarrhoea.

Materials and Methods

This prospective study was conducted from January 2014 and December 2015 at a tertiary care academic health organization of North India. Three hundred HIV seropositive subjects diagnosed as per NACO guidelines with diarrhea were recruited for this study irrespective of their ART status (Singh, 1999). Only those HIV seropositive subjects with diarrhea were enrolled who had not received any specific antidiarrheal therapy in the last two weeks. Six hundred age and sex similar randomly selected HIV seronegative subjects with symptoms of diarrhea were also enrolled as control group who come for routine examinations of their stool samples to the clinical microbiology laboratory. Control subjects who had received any specific antidiarrheal therapy in the last two weeks and/or were not sure of their HIV status were excluded from the control group. Written, informed consent was obtained from all the study participants, or their legal representatives, before study enrolment. Each patient was interviewed using a questionnaire concerning socio-demographic characteristics, personal details, type of illnesses, and clinical symptoms. CD4 count done by Fluorescent Activated Cell Sorter (FACS) count system (Becton Dickenson Immunocytometry system, San Jose, CA) was recorded. A clean wide mouth screw capped disposable plastic container was used to collect samples and transported immediately to the clinical microbiology laboratory. Diarrhea was defined as the passage of three or more loose or watery bowel movements in a 24-hour period. Acute diarrhea is defined as three or more stools per day of decreased form from the normal, lasting for less than 14 days.

Those with diarrhea for >14 days, but <1 month were called persistent diarrhea. Those experiencing diarrhea for longer than 30 days were defined as chronic diarrhea (Humphries and Linscott, 2015).

Stool samples were cultured for enteric bacterial pathogens. All samples were cultured directly as well as after enrichment in Selenite F Broth and Alkaline peptone water onto Xylose Lysine Deoxycholate agar, Deoxycholate Citrate Agar and Bile Salt Agar respectively. The plates were incubated for 18-24 hours at 37ºC. Specialized selective media, readymade Campylobacter agar plates was used for isolation of *Campylobacter jejuni*. For isolation of *Aeromonas* spp. and *Yersinia enterocolitica*, stool samples were cultured on Aeromonas Isolation Medium Base with added with added Ampicillin supplement and Yersinia selective agar base with added Yersinia Selective Supplement (Cefsulodin, Triclosan, Novobiocin) respectively. The organisms were identified on the basis of their colony characteristics, biochemical tests, and serologically by slide agglutination test using commercially available specific antisera (Denka Seiken Co., Ltd., Tokyo, Japan). CD4 count of 290 HIV seropositive with diarrhea patients was available for comparison. The antimicrobial susceptibility testing was performed for all bacterial isolates by using the Kirby-Bauer's disk diffusion method for the following six antimicrobial agents- Nalidixic acid (30μg), Ampicillin (10μg), Co-Trimoxazole (25 μg), Amikacin (30 μg), Gentamicin (10 μg), Ciprofloxacin (5 μg), Cefotaxime (75 μg). Culture media and discs were commercially procured from HiMedia, Mumbai, India.

All data were stored in a common database and statistically analyzed using the SPSS version 20.0 software (SPSS Inc., Chicago, IL, USA). The significance of differences between cases and the controls were calculated by chi2 for background variables. To study the relation between the isolation rate of bacterial agents, HIV seropositivity status and CD4 count chi-square test and the Fisher's exact test were used. A p-value of $p < 0.05$ was considered statistically significant.

Results and Discussion

Out of the three hundred HIV seropositive subjects recruited 206 (68.67%) were males and 94 (31.33%) were females with a male to female ratio of approximately 2.1:1. Majority (69.33%) of the HIV seropositive cases had chronic diarrhea (Table 1) while most 486 (81%) of the HIV seronegative subjects had acute diarrhea. The proportion of males was higher for the HIV seropositive with diarrhea cases as well as in control group with diarrhea only. Maximum number of clients tested seropositive in the age group of 31-40 years (37.33%), followed by 17.67%, 15%, 15%, 9.67% and 5.33% in the age groups of 21-30 yrs, 11-20 years, 41-50 years, 2-10 years and above 51 years. The mean age of the participants in our study was 30.64 ± 13.47 years, and the age ranged between 1 and 65 years. In the control group, the mean age of patients was 34.74 ± 18.24 years, with 404 males and 196 females (Table 2).

The HIV infected group presented a total number of 114 (38%) bacterial isolates whereas the non-HIV infected group yielded only 120 (20%) bacterial isolates. 24 *Campylobacter jejuni*, 16 *Shigella* sp., 41 Diarrheagenic *E. coli*, 14 *Yersinia enterocolitica*, 10 *Aeromonas hydrophila,* 6 *Salmonella typhi* and 3 *Salmonella typhimurium* isolates were recovered from the HIV infected cases. In HIV seronegative individuals without diarrhea, *Escherischia coli* again was the most predominant bacteria identified in stool samples (9%) followed by *Campylobacter* spp. in 4.33 % cases (Table 3) The occurrence of *C. jejuni, Shigella* spp.,

Salmonella spp., *Yersinia* spp., *A. hydrophila* and Diarrheagenic *E. coli* in HIV diarrhoeal specimens were higher than the non-HIV diarrhoeal cases. The profile of enteric bacterial isolates encountered in the investigation is shown in Table 3. Multiple infections were seen in 3 HIV infected cases. Concurrent *Shigella* and *Yersinia* spp. infection was noticed in the 2 cases while *Shigella* and *C. jejuni* infection was noticed in the 1 case. In non-HIV infected cases no bacterial co-infection was seen.

In HIV seropositive patients, Diarrheagenic *E. coli* was strongly associated with chronic and persistent diarrhea in HIV. Among *Shigella* spp., *Shigella flexneri* (9 cases) was the most common causative agent followed by *Shigella dysenteriae* (5 cases) and *Shigella boydii* (2 cases). Enteric salmonellosis was exclusively seen in patients in the advanced stages of HIV infection with CD4 count less than 200 cells/µL. Among fungal pathogens, the Candida species was identified in 16.33 % and 3.56% of the cases and control group respectively.

Susceptibility levels of bacterial pathogens to various antimicrobial agents are shown in Table 4. Most of the bacterial isolates were highly sensitive to Amikacin, Gentamicin and Cefotaxime and poorly sensitive to Nalidixic acid. Among Diarrheagenic *E. coli* maximum sensitivity was for Amikacin (85.36%), Gentamicin (68.29%) and cefotaxime (65.85%). *Shigella dysenteriae* was completely resistant to Nalidixic acid. The majority of *Salmonella* isolates were highly susceptible to Amikacin, Gentamicin, and Cefotaxime while *Shigella* isolates were highly susceptible for Amikacin, Cotrimoxazole, Gentamycin and Cefotaxime.

CD4 count of 290 HIV seropositive cases was available. CD4 counts less than 200 cells/µL had a higher rate of infection with certain bacteria like *Campylobacter jejuni*, *Yersinia enterocolitica*, *Shigella* spp. and Diarrheagenic *E. coli* which was statistically significant as shown in Table 5. In *Salmonella typhimurium*, *Yersinia enterocolitica, Diarrheagenic E. coli* and *Aeromonas hydrophila* CD4 count between isolates <200 cells/µL and CD4 ≥ 200 cells/µL was statically not significant (p = 0.0780, 0.2731, 0.1753 and 0.3845) respectively.

Diarrheal diseases continue to be a major cause of morbidity and mortality in India and other developing countries of the tropical zone (Kumar and Subita, 2012). Thus, there is a constant need to keep the knowledge updated with regards to etiological agents 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. In addition, there is lack of recent published data on the diarrheal pathogens isolated in HIV cases in relation to CD4 T lymphocyte counts.

In our study most of the patients were in age group 31-40 years old, 21-30 years old, and 11-20 years old with 37.33%, 17.67%, and 15% of the cases, respectively. This is similar to NACO nation-level statistics with an estimated adult (15-49 age group) HIV prevalence of 0.27% in 2011 (Annual Report, 2013-14) The male preponderance observed in present study might have been due to fact that females face more stigma and discrimination in a variety of settings including households, the community and workplaces so do not seek medical care fearing ostracism, loss of family support and financial constraint (Goel *et al.,* 2016). Laborers constituted 32% of patients while 14% were truck drivers; 12% were housewife. Studies in the India have found high HIV (2% - 14%) among long distance truck drivers (Pandey *et al.,* 2012).

Table.1 Duration of diarrhea in study and control subjects

Duration	HIV seropositive no. (%) (n = 300)	Control group no. (%) (n = 600)	p value
Acute diarrhea	41 (13.67)	486 (81)	0.0001
Persistent diarrhea	51 (17.00)	90 (15)	0.4379
Chronic diarrhea	208(69.33)	24 (4)	0.0001

Table.2 Socio-demographic data for HIV seropositive with diarrhea cases (n = 300) and diarrheal only controls (n = 600)

	HIV seropositive no. (%) (n = 300)	Control group no. (%) (n = 600)
Sex:		
Male	206(68.67)	404 (67.33)
Female	94(31.33)	196(32.67)
Age groups: (yrs)		
< 10	29 (9.67)	58 (9.67)
11-20	45 (15)	80 (13.33)
21-30	53 (17.67)	115(19.17)
31-40	112(37.33)	145 (24.17)
41-50	45(15)	92(15.33)
>51	16(5.33)	110(18.33)
Educational level:		
Primary school	113(37.67)	275(45.83)
Secondary school	82(27.33)	152(25.33)
Upper secondary school	60(20)	110(18.33)
University college	45(15)	63(10.50)
Occupation		
Laborer	96(32)	150 (25)
Housewife	36 (12)	164 (27.33)
Driver	42 (14)	32(5.33)
Professional	36 (11)	66 (11)
Others	90 (30)	188 (31.33)

Table.3 Enteric microbial pathogens in stool samples of study and control subjects

Bacterial isolates	Microbial isolates	
	HIV with diarrhoea (n=300)	**Non-HIV with diarrhoea (n=600)**
Campylobacter jejuni	24(8)	26(4.33)
Shigella flexneri	9(3)	5(0.83)
Shigella boydii	5(1.67)	4(0.67)
Shigella dysenteriae	2(0.67)	1(0.17)
Salmonella typhi	6(2)	4(0.67)
Salmonella typhimurium	3(1)	3(0.50)
Yersinia enterocolitica	14(4.67)	12(2)
Diarrheagenic *E. coli*	41(13.67)	54(9)
Plesiomonas shigelloides	0(0)	2(0.33)
Aeromonas hydrophila	10(3.33)	9(1.5)
Candida species	49(16.33)	18(3)
Total	163(54.33)	138(23)

Note: figure in parentheses indicate percentage of isolates

Table.4 Antimicrobial resistance of bacterial isolates from HIV seropositive diarrheal stool

Organisms	No.	NA	Cip	Am	Cot	AK	G	CF
Campylobacter jejuni	24	4(16.67)	5(20.83)	10(41.67)	10(41.67)	15(62.5)	12(50)	17(70.83)
Shigella flexneri	9	1(11.11)	6(66.67)	6 (66.67)	7((77.78)	8(88.89)	8(88.89)	7 (77.78)
Shigella boydii	5	0 (0)	3 (60)	2 (40)	4(80)	5 (100)	3(60)	4 (80)
Shigella dysenteriae	2	0(0)	0(0)	1 (50)	2(100)	2 (100)	2 (100)	2 (100)
Yersinia enterocolitica	14	9(64.28)	9(64.28)	10(71.43)	9(64.28)	13(92.86)	12(85.71)	12(85.71)
Salmonella typhi	6	1(16.67)	4(66.67)	3(50)	4(66.67)	6 (100)	5 (83.33)	6 (100)
Salmonella typhimurium	3	0(0)	2(66.67)	1 (33.33)	2(66.67)	3 (100)	3(100)	3(100)
Escherichia coli	41	7(17.07)	24 (58.53)	21(51.22)	15(36.58)	35(85.36)	28(68.29)	27(65.85)
Aeromonas hydrophila	10	0 (0)	9 (90)	7 (70)	6(60)	10 (100)	10 (100)	9 (90)

NA- Nalidixic acid, Cip- Ciprofloxacin, Am- Ampicillin, Cot- trimethoprim-sulfamethoxazole, AK- Amikacin, G-Gentamicin, CF- Cefotaxime
Note: figure in parentheses indicate percentage sensitivity, No.-Number of isolates

Table.5 Frequency of bacterial enteropathogens in relation to CD4 counts in HIV seropositive cases

Organisms	Number	CD4 < 200 cells/μL (n=50)	CD4 $\geq$ 200 cells/μL (n=250)	P value
Campylobacter jejuni	24	9	15	0.0087
Shigella spp.	16	7	9	0.0099
Salmonella typhi	6	4	2	0.0091
Salmonella typhimurium	3	2	1	0.0780
Yersinia enterocolitica	14	4	10	0.2731
Escherichia coli	41	10	31	0.1753
Aeromonas hydrophila	10	3	7	0.3845

Note: CD4 count of only 290 isolates were available

Chronic diarrhea was statically significantly more (69.33%; p = 0.0001) in the HIV positives than the HIV negatives similar to other studies (Pandey *et al.,* 2012; Tuli *et al.,* 2008)

In present study diarrheagenic *E. coli* (13.67%) was most common bacterial isolate followed by *Campylobacter jejuni* (8%) and *Yersinia enterocolitica* (4.67%). Diarrheagenic *E. coli*, *Shigella* spp., *Vibrio cholera* have been found to be frequent bacterial agents in HIV seropositive with diarrhea in many Indian studies (Kownhar *et al.,* 2007; Prasad *et al.,* 2000; Pandey *et al.,* 2012; Tuli *et al.,* 2008) In South Asia and African countries, *C. jejuni* detection varied between 3.8% and 13.1%, respectively, in diarrheic feces from HIV seropositive individuals (Rossit *et al.,* 2009). However, in one study from Delhi among bacterial isolates only *Escherichia coli,*

C. difficile and *Shigella* species were isolated from stool specimens of HIV-positive individuals with diarrhea (Kumar *et al.,* 2002). These differences may be explained on the basis of geographical variations.

In developed 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* (Jha *et al.,* 2012; Dekker *et al.,* 2015). In the present study, *Shigella flexneri* (9 cases) was the most common causative agent followed by *Shigella dysenteriae* (5 cases) and *Shigella boydii* (2 cases). In HIV seropositive patients, Diarrheagenic *E. coli* was strongly associated with chronic and persistent diarrhea in HIV in present study. However, in one study similar frequencies of colonizing *E. coli* were found in diarrheic and non-diarrheic children in HIV positive population (Rossit *et al.,* 2009). In present study enteric salmonellosis was also more frequently seen in patients in the advanced stages of HIV infection with CD4 count < 200 cells/μL implying need for a prompt and accurate diagnosis and treatment because of possible haematogenous spread and relapses in remain untreated.

One of the limitations of this study was the fact that there is paucity of data published from India documenting the exact prevalence of bacterial diarrhea causing agents in HIV seropositive patients. Moreover, use of different methods and use of special media for specific organisms such as *Campylobacter* spp., *Aeromonas* spp. and *Yersinia enterocolitica* in this study may have resulted in non-uniform reporting of results. Nevertheless, inclusion of large compiled sample size of 300 samples exclusively of HIV seropositive with diarrhea compensates for the limited number of studies available to a certain extent.

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. There is significant resistance to ampicillin and ciprofloxacin in *Shigella* isolates. A recent report show an increase in resistance to ciprofloxacin from 0% in 2004 to 44% in 2010 (Jha *et al.,* 2012). *Salmonella* isolates tested were susceptible to ciprofloxacin but resistant to nalidixic acid in vitro, a pattern associated with fluoroquinolone therapeutic failures. In a study from India, over a 5-year period, the numbers of nalidixic acid-resistant *Salmonella typhi* (NARST) increased from 51 to 93% and instances of ciprofloxacin-resistant enteric fever further increased from 0.6 to 15.2% (Niyogi, 2005) In a study from Peru high frequency of antimicrobial resistance of diarrheagenic *E. coli* to commonly used antibiotics such as ampicillin (85%) and cotrimoxazole (79%) (Capoor *et al.,* 2007) as in present study of 48.78 % and 63.42%. Understanding the burden of pathogen specific diarrheal disease and the variation by region is important for planning effective control programs for the overall reduction of diarrhea disease.

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