Original Research Article

# Diarrhoeagenic bacteria in HIV infected patients with chronic diarrhoea and their association with CD4 t-cell count and anti-retroviral therapy

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## Abstract

**Background:** Chronic bacterial diarrhoea is a major problem in HIV patients. The frequency of diarrhoeagenic bacteria in HIV varies at different immune level and from region to region. Against this background an attempt was made to identify the pattern of diarrhoeagenic bacteria in HIV positive patients with chronic diarrhoea and their association with CD4 counts and ART. **Materials and Methods:** A total 208 stool samples were collected and examined for diarrhoeagenic bacteria by using standard microbiological methods. All pathogenic isolates were tested for antibiotic susceptibility by using CLSI guidelines by modified Kirby - Bauer method. HIV testing was carried out as per NACO guidelines and CD4 T-cell counts were estimated by FACS count system. **Observations and Results:** Out of total 208 stool samples, *Salmonella* spp. (3.36%) was the commonest bacteria isolated followed by *Shigella* spp. (2.40%) and *Mycobacterium tuberculosis* (0.96%). Salmonella and shigella isolates tested for antibacterial susceptibility showed 100% susceptibility to cefotaxime followed by ciprofloxacin (83.33%) and amoxyclav (83.33%). More bacterial pathogens were identified in subjects with CD4 count < 200 cell/µl (7.69%) than with CD4 count > 200 cell/µl (5.34%) but this difference was not found to be statistically significant (p=0.5661). Bacterial pathogens were isolated in 6 (6.38%) subjects who were on ART and in 8 (7.01%) subjects who were not on ART. This difference was also not statistically significant (p=0.7742) in our study. **Conclusion:** Bacterial diarrhoea is still a problem in HIV infected patients irrespective of immune status and ART. Bacterial diarrhoea due to *Salmonella* spp. is the major problem in these patients in our area. Pattern of infections often guide the clinician about appropriate management of opportunistic infections and is as important as antiretroviral therapy (ART) in preventing mortality and morbidity among HIV-infected persons.

**Key Words:** HIV patients, Chronic diarrhoea, Diarrhoeagenic bacteria, CD4, ART.

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## INTRODUCTION

Acquired Immunodeficiency Syndrome (AIDS) caused by Human Immunodeficiency Virus (HIV) is the most important public health problem of 21st century. The total number of people living with HIV/AIDS in India is estimated at 21 lakh in 2012.[1] Since the beginning of the AIDS pandemic, opportunistic infections have been recognized as common complications of HIV infection. Among these, chronic diarrhoea is a major problem in patients with HIV, especially those with advanced disease. It has been estimated that 30-50% of patients with AIDS in USA and 63-93% in developing countries like India suffer from chronic diarrhoea.[2] Majority of the opportunistic infections are not observed until CD4 count falls below 200 cells/µl. Immune status of HIV infected patients improves with Antiretroviral Therapy (ART), they becomes less prone to opportunistic infections and infectious etiologies of diarrhoea are parallel to that of immunocompetent patients.[3] The causes of infectious diarrhea in HIV infected patients are extensive; which

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includes bacteria, parasites, viruses and fungi. Worldwide, the most common causes of diarrhoea in these patients are enteric bacteria including salmonella, shigella and campylobacter.[3] Other enteric bacterial pathogens responsible for chronic diarrhoea are *E. coli, Clostridium difficile, M. tuberculosis* and *Mycobacterium avium* complex.[4] Management of bacterial diarrhoea may require antibiotic therapy as antibiotics shorten the duration of diarrhoea and decrease stool output. Antibiotic susceptibility profiles of bacteria vary from time to time and from region to region. This creates a need for periodic updates on antibiotic susceptibility and prevalence of diarrhoeagenic bacteria in particular region. Against this background the present study was carried out to identify diarrhoeagenic bacteria in HIV positive patients presenting with chronic diarrhoea and their association with CD4 T cell count and ART.

## MATERIAL AND METHODS

The study was carried out in the department of microbiology and ART centre at tertiary care hospital after taking approval from institutional ethics committee. A total 208 HIV seropositive patients with chronic diarrhoea (three or more liquid stools daily) for more than 14 days were included in this study. After taking detailed history and written informed consent, Blood and stool samples were collected from each patient. HIV testing was carried out using commercially available kits (HIV Comb, Parikshak, and Tridot Kits) by HIV testing strategy II B as per NACO guidelines.[5] CD4 T-cell count was performed by automated flow cytometry analyzer (FACS Calibur, Becton Dickinson) and the study subjects were categorized in three categories (< 200 cells/µl, 200-499 cells/µl, ≥ 500 cells/µl) depending on their immune status. A single fresh stool sample was collected in leakproof, labeled and sterile plastic container. After macroscopic and microscopic examination, stool specimens were inoculated directly on Blood agar, MacConkey agar and on Deoxycholate citrate agar[6] (DCA) directly as well as after enrichment in selenite F broth for 6 hrs. Plates were incubated for 18-24 hrs.[6,7] Two sets of Lowenstein Jensen (LJ) media were inoculated after decontamination with 3% oxalic acid and incubated for up to 12 weeks with regular examination of cultures.[8] Bacterial isolates were identified by standard microbiological methods.[9] Antimicrobial susceptibility testing was performed for all diarrhoeagenic bacterial isolates as per CLSI guidelines by modified Kirby - Bauer method.[10,11] The statistical analysis was performed to test the significance of association between pathogenic bacteria and CD4 T-cell count and ART by using chi-square test and software Graph pad prism 5. Values were considered to be statistically significant when the *P* value was less or equal to 0.05.

## OBSERVATIONS AND RESULTS

Out of total 208 patients, 136 (65.39%) were males and 72 (34.61%) were females. The Majority (46.16%) of the patients belongs to age group 31-40 years. Different bacteria isolated from stool specimens of 208 HIV seropositive patients with chronic diarrhoea are shown in Table 1. *E. coli, K. pneumoniae* and *E. fecalis* were considered as commensals. *Salmonella* spp., *Shigella* spp., and *Mycobacterium tuberculosis* were considered as enteric pathogens. Mixed growth of *E. coli* and *Salmonella* spp. was found in 6 stool specimens, *E. coli* and *Shigella* spp. in 4, *E. coli* and *Enterococcus fecalis* in 5, *E. coli* and *K. pneumoniae* in 4 stool specimens, *E. coli, K. pneumoniae* and *Salmonella* spp. was found in one stool specimen. *E. coli, Shigella* spp. and *Enterococcus fecalis* was found in one stool specimen. All isolated pathogens were tested for antibacterial susceptibility pattern (Table 2). CD4 T-cell count of the study population is shown in figure 1. Association of bacterial pathogens with CD4 T-cell count and ART was assessed and shown in table 3 and 4 respectively.

**Table 1:** Bacteria isolated from stool specimens (n=208)

| Bacteria | No. of isolates |
|---|---|
| *E. coli* | 201 (96.63%) |
| *Klebsiella pneumonia* | 12 (5.76%) |
| *Enterococcus fecalis* | 06 (2.88%) |
| *Salmonella* ser. Typhimurium | 05 (2.40%) |
| *Salmonella* ser. Enteritidis | 02 (0.96%) |
| *Shigella dysentriae* | 03 (1.44%) |
| *Shigella flexnerii* | 02 (0.96%) |
| *Mycobacterium tuberculosis* | 02 (0.96%) |

**Table 2:** Antibiotic susceptibility pattern of bacterial enteropathogens

| Isolates (no. tested) | Antibiotic susceptibility of bacterial pathogens | | | | | | |
|---|---|---|---|---|---|---|---|
| | A | Ac | Ce | Co | C | Cf | NA |
| *Salmonella* Typhimurium (05) | 03 (60%) | 04 (80%) | 05 (100%) | 04 (80%) | 04 (80%) | 04 (80%) | 04 (80%) |
| *Salmonella* Enteritidis (02) | 01 (50%) | 02 (100%) | 02 (100%) | 01 (50%) | 01 (50%) | 02 (100%) | 02 (100%) |
| *Shigella dysentriae* (03) | 01 (33.33%) | 02 (66.6%) | 03 (100%) | 02 (66.6%) | 02 (100%) | 02 (66.66%) | 02 (66.66%) |
| *Shigella flexneri* (02) | 01 (50%) | 02 (100%) | 02 (100%) | 01 (50%) | 01 (50%) | 02 (100%) | 02 (100%) |
| **Total (12)** | **06 (50%)** | **10 (83.33%)** | **12 (100%)** | **08 (66.66%)** | **08 (66.66%)** | **10 (83.33%)** | **10 (83.33%)** |

**Table 3:** Association of bacterial pathogens with CD4 T cell count

| Bacteria | No. of subjects with CD4 T cell count | | | Total (n=208) |
|---|---|---|---|---|
| | < 200 cells/µl (n=143) | 200-499 cells/µl (n=56) | ≥ 500 cells/µl (n=9) | |
| *S.* Typhimurium | 04 (2.80%) | 01 (1.78%) | - | 05 (2.40%) |
| *S.* Enteritidis | 02 (1.40%) | - | - | 02 (0.96 ) |
| *S. dysentriae* | 02 (1.40%) | 01 (1.78%) | - | 03 (1.44%) |
| *S. flexnerii* | 01 (0.70%) | 01 (1.78%) | - | 02 (0.96%) |
| *M. tuberculosis* | 02 (1.40%) | - | - | 02 (0.96%) |
| **Total** | **11 (7.69%)** | **03 (5.34%)** | **-** | **14 (6.72%)** |

**Table 4:** Distribution of bacterial pathogens in subjects taking ART and not taking ART

| Bacterial pathogens | No. of subjects taking ART (n=94) | No. of subjects not taking ART (n=114) | Total (n=208) |
|---|---|---|---|
| *Salmonella* spp. | 03 (3.19%) | 04 (3.51%) | 07 (3.37%) |
| *Shigella* spp. | 02 (2.13%) | 03 (2.63%) | 05 (2.40%) |
| *M. tuberculosis* | 01 (1.06%) | 01 (0.87%) | 02 (0.96%) |
| **Total** | **06 (6.38%)** | **08 (7.01%)** | **14 (6.73%)** |

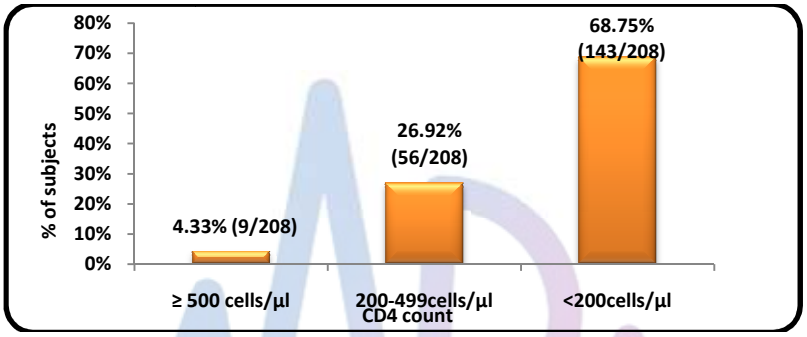


**Figure 1:** CD4 T-cell count of the study population

## DISCUSSION

In HIV, the gastrointestinal tract is major target organ and diarrhoea is among the most common problems accounting for 30-60% of AIDS patients in industrialized countries and up-to 90% in underdeveloped world.[12] Chronic diarrhoea is a very frequent and frustrating problem in peoples living with HIV/AIDS (PLHA); at least 50% of these experience it sometime during the progression of the disease.[4] The etiology for such diarrhoea could be parasitic, bacterial, fungal, enteric viruses, or HIV itself may contribute to diarrhoea. In resource limited countries like India, enteric infections are common in the general population and in HIV infected peoples with geographic differences. There are many reports regarding the frequency of various pathogens causing diarrhoea from different parts of India.[2,12,13] Out of a total 208 stool specimens, bacterial pathogens were isolated in 14 (6.73%) stool specimens in our study which is similar to study by Prasad *et al*[2] who reported 7.6% bacterial pathogens. Awole *et al*[14] reported 25% bacterial pathogens while Gassama *et al*[15] reported 42.40% bacterial pathogens in HIV positive patients with diarrhoea. Becker *et al*[16] in 2007 reported 2.8% bacterial pathogens. Most common bacterial gastrointestinal pathogens associated with HIV/AIDS diarrhoea are salmonella, shigella and campylobacter. Though enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC), enteroinvasive *E. coli* (EIEC), and enterohemorragic *E. coli* (EHEC) are primary intestinal pathogens, their identification in most of the patients is not necessary[42] or is done only in special situations like diarrhoeal outbreaks where no other recognized enteropathogen has been identified and also it is restricted to the reference laboratories.[17] As *E. coli* strains are predominant among the aerobic commensal bacteria in healthy human intestine[17] and also phenotypic assay or serotyping of *E. coli* for diagnosis of diarrhoeagenic strains is not routinely performed in the clinical laboratory.[18] *K. pneumoniae* also is commonly isolated from healthy human faeces and *E. fecalis* occur as a commensal in the mouth and intestine in man and animals.[17] *E. coli, K. pneumoniae,* and *E. fecalis* were considered as commensals in gastrointestinal tract. Among the enteric bacterial pathogens, *Salmonella* spp. can be considered an important pathogen for the HIV-infected group as these enterobacteria are 20 to 100 times more frequently isolated among HIV individuals compared to the immunocompetent population. The frequencies of salmonella isolation varies from 2.5% to

18%.[19] *Salmonella* spp. were isolated in 3.36% of stool specimens in our study (Table 4) which is comparable to study by Uppal *et al*[12] in 2009. An approximately twenty-fold increase in risk of developing gastroenteritis has been described in AIDS patients carrying shigella compared to the general population.[19] *Shigella* spp. were isolated in 2.40% of stool specimens in our study similar to the other studies by Uppal *et al*[12] and Mwachari *et al*.[20] The presentation of tuberculosis in AIDS is well recognized to be different and often extrapulmonary. Autopsy studies suggest that the gastrointestinal tract is commonly involved in disseminated tuberculosis and such involvement can result in gastrointestinal symptoms including diarrhoea.[21] In the present study, *Mycobacterium* spp. were identified in 0.96% of stool samples obtained from HIV infected patients with chronic diarrhoea. We were unable to test for *Clostridium difficile* and *Campylobacter* spp. due to limited resources at our setup.

Uppal *et al*[12] in 2009 reported *Salmonella* species in 4% and *Shigella* species in 2% of stool samples. Another study by Awole *et al*[14] in 2002 isolated *Salmonella* spp. in 8.1%, *Shigella* spp. in 4% and *Mycobacterium* species were identified in 3% and Mwachari *et al*[20] in 1998 isolated *Salmonella* spp. in 6%, *Shigella* spp. in 3%, and *Mycobacterium* species were identified in 13% of stool samples. Detection of enteric pathogens in HIV positive patients with diarrhoea is important as a number of efficacious antimicrobial therapies are available. All salmonella and shigella isolates were tested for antibiotic susceptibility. Most of the opportunistic infections are not observed until the CD4 count falls below 200 cells/µl. Bacterial enterocolitis can occur at any immunodeficiency level.[3] There seems to be no differences related to the immune condition and the commonest isolated species of the *Shigella* genus, although an approximately twenty-fold increase in risk to develop gastroenteritis has been described in AIDS patients carrying this bacterium compared to general population.[19] Enteric salmonellosis was more frequently seen in patients with advanced stages of HIV infection[19] while tuberculosis can manifest with less advanced immune deficiency, and CD4 cell counts may exceed 200 cells/µl.[3] In our study, 7.69% (11/143) bacterial pathogens were isolated in subjects who had CD4 count < 200 cell/µl and 4.61% (3/65) bacterial pathogens were isolated in subjects who had CD4 count > 200 cell/µl (Table 3). Bacterial pathogens were found to be more in subjects with CD4 count < 200 cell/µl than subjects with CD4 count > 200 cell/µl but this difference was not found to be statistically significant (p=0.5661). Appropriate management of opportunistic infections is as important as antiretroviral therapy (ART) in preventing mortality and morbidity among HIV-infected persons.[22] Use of Highly Active Anti-Retroviral Therapy (HAART) as primary therapy of opportunistic infection represent a new model for management which is effective in raising the CD4 count and lowering HIV RNA levels, OIs may completely remit with HAART alone.[3] In the West, the incidence of OIs has markedly declined because of the widespread availability of highly active antiretroviral therapy (HAART).[22] In our study, bacterial pathogens were isolated in 6.38% subjects taking ART and 7.01% subjects who were not taking ART (Table 4). This finding shows that bacterial diarrhoea is still a problem in HIV infected patients irrespective of use ART.

## CONCLUSION

Bacterial diarrhoea is still a problem in HIV infected patients irrespective of immune status and ART. Bacterial diarrhoea due to *Salmonella* spp. is the major problem in these patients in our area. Pattern of infections and their susceptibility patterns often guide the clinician about appropriate management of opportunistic infections and is as important as antiretroviral therapy (ART) in preventing mortality and morbidity among HIV-infected persons. Various studies from different parts of the world show marked geographical variations with different antibiotic susceptibility patterns. This emphasizes the need for thorough investigations of these patients to identify pathogens and their susceptibility patterns for proper management in particular region. This will not only prolong the life of HIV infected individuals but also improve quality of life.

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