Diarrhoeagenic Bacterial Pathogens in HIV-positive Patients with Diarrhoea in Rural Communities of Limpopo Province, South Africa

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ABSTRACT

Potential enteric bacterial pathogens in 60 HIV-positive patients with chronic diarrhoea in rural communities of the Limpopo Province, South Africa, were identified using standard microbiological methods. The Kirby-Bauer disk-diffusion method was employed to determine antibiograms of isolated bacteria. Results revealed that diarrhoeagenic bacterial agents were isolated from 48 (80%) of the 60 HIV-positive patients with diarrhoea. Forty-four (73.3%) and 16 (26.7%) of the 60 patients were female and male respectively in the age range of 17-55 years with a mean of 34 years. Bacterial pathogens isolated comprised Campylobacter species (20.0%), Plesiomonas shigelloides (16.6%), Aeromonas species (13.3%), and Escherichia coli, Shigella and Salmonella species (10.0% each). No attempts were made to isolate parasites, fungi, or viruses. Antibiotic susceptibility profiles revealed resistance of the isolates to ampicillin, cephalothin, chloramphenicol, erythromycin, and streptomycin. However, all (100%) of P. shigelloides and Salmonella species were sensitive to nalidixic acid and ciprofloxacin. Most isolates were susceptible to nalidixic acid, ciprofloxacin, and gentamicin, indicating the usefulness of these drugs, although antibiograms may not always correlate with clinical usefulness.

Key words: Acquired immunodeficiency syndrome; Bacteria; Diarrhoea, Chronic; HIV; Antibiotics; Enteropathogens; South Africa

INTRODUCTION

The epidemic of HIV/AIDS in developing countries, particularly in sub-Saharan Africa, has reached a crisis point (1). In South Africa, the statistics are staggering: 1,700 new cases of HIV infection each day, seroprevalence of more than 30% among antenatal clinic attendees in the most severely-affected regions, 6-8 million people infected, and a burgeoning number of HIV/AIDS orphans who are more likely to drop out of school, have higher rates of illness and worst nutritional status when compared with children in intact families (1,2). Many HIV/AIDS patients in South Africa are residents of rural areas. Such rural areas are devoid of good sanitation, good hygienic practices, and adequate supply of potable water (3-5). Many rural households obtain water directly from streams, ponds, and rivers. These sources of water are contaminated, highly polluted, usually not treated, and thus, serve as sources for transmission of water-borne diseases exemplified by diarrhoea (6). Some rural residents are, therefore, prone to diarrhoea since the causative organism may be transmitted by poor quality of water and lack of good hygienic practices or sanitation (7).

Diarrhoea is characteristic of HIV/AIDS (8). Approximately, 90% of HIV/AIDS patients in Africa suffer from chronic diarrhoea (9). The substantial degree of morbidity and mortality due to diarrhoeal diseases in developing countries, particularly in Africa, is compounded by the epidemic of HIV/AIDS.
Management of bacterial diarrhoea and its complications may require antibiotic therapy because antibiotics can shorten the duration of diarrhoea, decrease stool output, and abrogate some complications (10). Antibiotic susceptibility profiles of bacteria vary from time to time and from region to region (11), and this creates the need for periodic updates of antibiotic susceptibility profiles and prevalence of the types of enteric bacterial pathogens. Such bacterial pathogens include species of Campylobacter, Aeromonas, Plesiomonas, Yersinia, Vibrio, Salmonella, and Shigella (10-12).

The Limpopo Province is predominantly rural and one of the poorest provinces in South Africa, with an estimated 13% prevalence rate of HIV/AIDS (13). Despite this, we are not aware of any study on isolation and antibiotic susceptibility of potential enteric bacterial pathogens in HIV-positive individuals with chronic diarrhoea in rural communities in the Limpopo Province. We are also not aware of any data or report indicating diarrhoea rates in HIV-infected individuals living in rural and urban areas. The focus of this study was on rural communities because of their peculiar conditions, such as lack of potable water supply, lack of good sanitation, debilitating poverty, and inadequate medical facilities.

This study documents the various enteric bacterial pathogens and their antibiotic susceptibility profiles in HIV-positive individuals with diarrhoea in rural communities in the Limpopo Province of South Africa.

**MATERIALS AND METHODS**

**Study population**

The study group comprised 100 patients with chronic diarrhoea attending clinics in Makonde, Phophi, Tshakuma and Bela-Bela communities of the Limpopo Province, South Africa, during March 2001-February 2002. Chronic diarrhoea was defined as the passage of 3-4 watery stools per day for not less than 14 weeks (10). Samples from patients with chronic diarrhoea were investigated to isolate potential bacterial pathogens.

Ethical approval for this study was granted by the Research Department of the University of Venda, Thohoyandou, South Africa. Permission for the collection of samples was obtained from the Limpopo Provincial Department of Health, Polokwane. Informed consent was sought as previously reported (11).

**Testing for HIV**

Screening for HIV was determined using the OraQuick HIV1/2 (OraSure Technologies, USA) or Determine HIV1/2 (Abbott, USA) test kits as described by the manufacturers. Since the study cohort formed part of a related study on determination of HIV-1 subtypes, status of HIV was confirmed by nested reverse transcriptase-polymerase chain reaction (RT-PCR) and envelope heteroduplex mobility assay (HMA). Cycling conditions and HMA procedures were employed as previously reported (14,15). First round primers comprised ED5 (5'-ATG GGA TCA AAG CCT AAA GCC ATG TG-3') and ED12 (5'-AGT GCT TCC TGC TGC TCC CAA GAA CCC AAG-3'). Second round primers were ES7 (5'-tgt aaa ega cgg cca gta TGT TAA ATG GCA GTC TAG C-3') and ES8 (5'-cag gaa aca get gat gac cCA CTT CTC CAA TTG TCC CTC A-3'). Amplified product of approximately 700 base pairs, spanning the HIV-1 envelope V3-V5 region, was visualized with ultraviolet light after staining with ethidium bromide. A test sample was assigned the corresponding subtype of the reference strain with which it formed the fastest heteroduplex. Patients negative on the screening tests were not confirmed.

**Collection and transportation of stool specimens**

Stool specimens were collected in clean wide-mouth containers and immediately transported in cooler boxes to the base laboratory, Department of Microbiology, University of Venda, South Africa. Specimens were duly processed within 1-3 hour(s) of their collection.

**Isolation and identification of enteric bacterial pathogens**

For the isolation of Campylobacter jejuni from stools, Skirrow’s and Butzler’s media were employed as previously described (12,16,17). Briefly, the plates were incubated at 42 ºC under microaerophilic conditions for 72 hours. C. jejuni and C. coli were differentiated on the basis of hippurate and indoxyl acetate hydrolysis. Typically, C. jejuni is positive for the two tests, while C. coli is positive for indoxyl acetate hydrolysis only (8).

For the isolation of Aeromonas and Plesiomonas spp., a previously-described method was employed (18,19). For the isolation of other enteropathogens, the methods fully described by other researchers were used (11,16,20-22).

**Testing of antibiotic susceptibility**

Antibiograms of the bacterial isolates were determined by the disk-diffusion method (11,23) on Mueller-Hinton medium.
agar, employing ampicillin (10 µg), cephalothin (30 µg), chloramphenicol (30 µg), erythromycin (15 µg), gentamicin (10 µg), nalidixic acid (30 µg), ciprofloxacin (10 µg), tetracycline (30 µg), and streptomycin (10 µg). Some antibiotics, such as cephalothin, gentamicin, and streptomycin, which are not commonly used in the treatment of bacterial pathogens, were included in the study due to increasing resistance of pathogens to commonly-used antibiotics.

Student’s t-test/z-test for proportion was employed for analysis of data.

RESULTS

Of the 100 patients with chronic diarrhoea, 60 (60%) were HIV-positive. No discordant results were obtained. Of the 60 HIV-positive individuals, 44 (73.3%) were female, and 16 (26.7%) were male. The age range was 17-55 years with a mean of 34 years. The scope of potential enteric bacterial pathogens isolated is presented in Table 1.

Table 1. Potential enteric bacterial pathogens isolated from HIV-positive patients with chronic diarrhoea in rural communities of the Limpopo Province, South Africa

<table>
<thead>
<tr>
<th>Bacteria (n=60)</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter jejuni</td>
<td>8</td>
<td>13.3</td>
</tr>
<tr>
<td>Campylobacter coli</td>
<td>4</td>
<td>6.7</td>
</tr>
<tr>
<td>Plesiomonas shigelloides</td>
<td>10</td>
<td>16.6</td>
</tr>
<tr>
<td>Aeromonas species</td>
<td>8</td>
<td>13.3</td>
</tr>
<tr>
<td>Shigella species</td>
<td>6</td>
<td>10.0</td>
</tr>
<tr>
<td>Salmonella species</td>
<td>6</td>
<td>10.0</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>6</td>
<td>10.0</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Vibrio cholerae</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
<td>80.0</td>
</tr>
</tbody>
</table>

Potential diarrhoeagenic bacterial agents were isolated from 48 (80%) of the 60 HIV-positive patients. The predominant isolates were C. jejuni/coli (n=12). This was followed by Plesiomonas shigelloides (16.6%), Aeromonas species (13.3%), Shigella, Escherichia coli, and Salmonella species (10.0% each). Vibrio cholerae and Yersinia enterocolitica were not isolated. No attempts were made to isolate parasites, fungi, or viruses.

Antibiotic susceptibility patterns of the isolates are presented in Table 2. Antibiograms revealed resistance of the isolates to ampicillin, cephalothin, chloramphenicol, erythromycin, streptomycin, and tetracycline. However,
all (100%) of \textit{P. shigelloides} and \textit{Salmonella} species were sensitive to nalidixic acid and ciprofloxacin, and all (100%) of \textit{Shigella} isolates were sensitive to gentamicin. About 92% of the \textit{Campylobacter} isolates were sensitive to ciprofloxacin and gentamicin. Most bacterial isolates were susceptible to gentamicin, nalidixic acid, and ciprofloxacin.

**DISCUSSION**

Although diarrhoea is characteristic of HIV infection, there is a paucity of data on the association of enteric bacterial pathogens and HIV infection in rural communities of the Limpopo Province of South Africa despite the recognition of HIV/AIDS as a major health problem (13). The problem of diarrhoea as a common complication of HIV infection is further compounded by lack of a potable water supply and lack of good hygienic practices in most rural communities of the Limpopo Province. Villagers in rural communities in the study areas collect their drinking-water from rivers, ponds, wells, and streams (3). Such water sources are faecally contaminated and devoid of treatment, and thus, serve as important vehicles for the transmission of water-borne diseases exemplified by diarrhoea (3,7). Diarrhoea may be experienced by about 30-90% of HIV/AIDS patients, and the frequency of isolation of aetiologic agents varies with time and geographical areas reportedly from 40% to 83% (24).

In this study, seropositivity for HIV was more often encountered in females (73.3%) than males (26.7%), and this difference was statistically significant (p<0.05). Globally, more women are infected than men (25). Most women contract HIV via sexual contact (26), and this may be associated with unequal power relations between men and women, rape cases involving women as victims, and the difficulty encountered by women in resisting unprotected sex by their male partners. The higher prevalence in females is critical since it affects maternal morbidity and mortality and because the major responsibility of childbearing and rearing rests mostly on females (27-30). Potential enteric bacterial agents were identified in 48 (80.0%) of the study cohort. The most frequently-isolated pathogens were \textit{Campylobacter} species (20.0%), whereas \textit{P. shigelloides} and \textit{Aeromonas} species accounted for 16.6% and 13.3% of the isolates respectively. \textit{V. cholerae} and \textit{Y. enterocolitica} were sought for, but no isolates were obtained. There were no attempts to isolate viruses, fungi, or parasites because the primary focus was on bacteria. Another limitation of this study was the paucity of samples investigated due mainly to the silent chain that encircles HIV and the stigmatization of infected individuals.

Determination of antibiograms revealed that the majority of all the enteric bacterial isolates were susceptible to nalidixic acid, gentamicin, and ciprofloxacin but were resistant to ampicillin, tetracycline, chloramphenicol, streptomycin, and cephalothin. Varying degrees of susceptibility and resistance of enteric bacterial pathogens have been documented (10,11). The susceptibility of the isolates to gentamicin, nalidixic acid, and ciprofloxacin may be of great value in the empiric management of diarrhoea cases requiring antibiotic therapy in HIV patients with chronic diarrhoea. This study only examined in-vitro susceptibility of the potential pathogens to antibiotics, and such susceptibility patterns may not necessarily correlate with clinical usefulness.

In conclusion, this study provided preliminary data on the scope of enteric bacterial pathogens and their in-vitro susceptibility patterns in HIV infection among rural residents in the Limpopo Province of South Africa.

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**REFERENCES**

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