Scope and frequency of enteric bacterial pathogens isolated from HIV/AIDS patients and their household drinking water in Limpopo Province

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Abstract

Although HIV/AIDS and water-borne infections, exemplified by diarrhoea, are leading causes of morbidity and mortality in developing countries, their association has received only cursory attention. This study was therefore conducted to ascertain the scope and frequency of potential enteric bacterial pathogens isolated from stool samples of HIV-positive and -negative individuals with and without diarrhoea as well as household drinking water of the study groups in rural communities in Limpopo Province. A prospective study involving 330 HIV-positive individuals (200 with diarrhoea and 130 without diarrhoea) and 160 HIV-negative patients, (80 with diarrhoea and 80 without) was undertaken from August 2005 to January 2006. Stool and drinking water samples were analyzed for the presence of enteric bacterial pathogens using standard microbiological methods. Of the 330 HIV-positive individuals, 126 (38%) and 206 (62%) were males and females respectively. HIV prevalence was mostly common in the age group 21to 30 years. A potential enteric pathogen was isolated from all (100%) of the HIV-positive individuals with diarrhoea and 68 (52.3%) without diarrhoea (P = 0.0001). Bacteria that were significantly associated with diarrhoea among HIV-positive patients and their household drinking water were *Escherichia coli*, *Salmonella* spp., *Campylobacter* spp., *Shigella* spp. and *Aeromonas* spp. whereas *Plesiomonas shigelloides* was not. The same profiles of enteric bacterial pathogens were isolated from HIV-negative individuals but at lower frequencies (P = 0.0001). Enteric pathogens were distributed across gender and different age strata. A notable finding was the emergence of *Aeromonas* spp. and *Plesiomonas shigelloides* in HIV infected individuals with diarrhoea. This study provides the foremost baseline reference compendium on the scope and frequencies of enteric bacterial pathogens isolated from stool and household drinking water samples of HIV-positive and -negative individuals with and without diarrhoea in rural communities in the Limpopo Province.

Keywords: HIV/AIDS, household, drinking water, diarrhoea, enteric bacteria, pathogens

Introduction

Enteric bacterial pathogens are responsible for most diarrhoea episodes worldwide. Such pathogens incriminated in diarrhoeal cases among HIV/AIDS patients in Senegal were reportedly 19.6%, 7.6% and 4.4% for entero-aggregative *Escherichia coli, Shigella* spp. and *Salmonella enterica* respectively (Gassama et al., 2001). In Ethiopia the HIV/AIDS patient isolation rates were reportedly 8.1%, 4.0% and 13.1% respectively for *Salmonella, Shigella* and *Campylobacter* (Awole et al., 2002). In South Africa, rates of 20% for *Campylobacter* jejuni/coli, 16.6% for *Plesiomonas shigelloides*, 13.3% for *Aeromonas* spp. and 10% each for *Shigella, Salmonella* and *Escherichia coli* among HIV/AIDS patients have been reported (Obi and Bessong, 2002).

Although HIV/AIDS patients are susceptible to the same spectrum of enteric pathogens, which may cause diarrhoea in immuno-competent individuals, HIV/AIDS patients require more rigorous monitoring because of their increased susceptibility to opportunistic infections, many of which have a predilection for the gastro-intestinal tract (Fauci 1999; Lubeck et al., 1993). The linkage between HIV/AIDS and water may appear superficial but is indeed an in-depth association and water-borne bacterial pathogens are therefore strongly associated with HIV/AIDS (Obi et al., 2006). Despite this correlation, there are no available data on the scope and frequency of enteric bacterial pathogens isolated from HIV/AIDS patients and their household drinking water.

The aim of this study was to determine the scope and frequency of enteric bacterial pathogens isolated from HIV/AIDS patients with and without diarrhoea and their household drinking water in Limpopo Province.

Materials and methods

Study area

Areas for the collection of samples were stratified according to the 6 districts in Limpopo Province (Oni et al., 2002). Three districts were selected for the study based on familiarity with the area, HIV prevalence and other parameters. The selected districts comprised Vhembe, Waterberg, and Capricorn.

The sites in Vhembe such as Musina represent the northernmost part of Limpopo Province and are located in the Limpopo valley. It is the main gateway to South Africa from the north and Musina is also a border town linking South Africa and Zimbabwe.

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In the Waterberg district Bela-Bela area was chosen to represent the Bushveld region of the province. Bela-Bela is an important tourist centre and holiday resort town, with proximity to the Gauteng region. Waterberg district is also known to have a relatively higher HIV prevalence (26.6%) when compared to the provincial average (19.3%) (DOH, 2005). Mankweng area was selected to represent the Capricorn region or the central region of the Limpopo Province.

Prior to the commencement of the study, preliminary visits were undertaken to each of the chosen study areas by members of the research team. During these visits, the background, protocols, objectives and potential significance of the study including issues around confidentiality and consent were discussed with care-givers, support groups and non-governmental organisations and their support were sought before collection of specimens. The study team chose to work closely with support groups, NGOs and HIV care-givers because they provide a 'comfort zone' for HIV/AIDS patients, who in turn confide in the care-givers. Due to the stigmatisation of the disease, identifying HIV/AIDS patients is usually an uphill task in most South African communities. Health authorities and family members are usually hesitant to divulge information on HIV/AIDS status of individuals because of ethical issues and the pressure to maintain confidentiality. The support groups, care-givers and NGOs who work directly with HIV/ADIS patients provided the platform to reach out to the participants.

Study population

The present study was conducted between August 2005 to January 2006. Information on age, gender, diarrhoeal and HIV status was obtained.

HIV-positive and HIV-negative individuals were enlisted in this study. The study group consisted of 330 HIV-positive individuals made up of 200 HIV-positive individuals with diarrhoea and 130 HIV-positive individuals without diarrhoea. Similarly, a total of 160 HIV-negative individuals consisting of 80 HIV-negative individuals with diarrhoea and another 80 HIV-negative individuals without diarrhoea were also included as unmatched controls

A diarrheic stool in this study was regarded as the passage of loose or watery stools. Stool samples from HIV-positive and -negative individuals with and without diarrhoea were analysed for the presence of potential bacterial pathogens.

Ethics approval

The Health, Safety and Ethics Committee of the University of Venda, Thohoyadou, South Africa granted ethical approval for this study. Informed consent was obtained from study subjects before collection of stool and water samples. Issues of confidentiality and anonymity were also maintained.

HIV testing

Screening for HIV sero-status was preformed using the OraQuick HIV1 and HIV2 (Ora Sure Technology, USA) test kit as described by the manufacturers and as previously reported (Obi and Bessong, 2002).

Collection and transportation of stool and water samples

Stool specimens were collected in clean, sterile wide-mouth containers and transported in cooler boxes to the laboratory for bacterial analyses within 4 to 6 h of collection. Water samples were collected in sterile containers from the same households where stool specimens were collected and transported in cooler boxes to the same laboratory for bacteriological analyses within 4 to 6 h of collection.

Culture media

Isolation media commonly used for the isolation of enteric bacterial pathogens were employed in this study. They consisted of MacConkay agar (MCA), Shigella-Salmonella agar (S-S agar), Xylose deoxycholate citrate agar (XDCA), Thiocitrate bile salt (TCBS) agar, Kleigler's iron agar (KIA), enrichment broths and alkaline peptone water.

Isolation of bacterial enteric pathogens

All cases and controls had stool and water specimens collected and processed in the same manner. For the isolation of *Aeromonas* and *Plesiomonas species*, incubation of seeded XDCA agar plates was at 37^{O}C for 24 h. Colonies were screened for oxidase production and oxidase positive colonies were identified as belonging to the genera *Aeromonas* and *Plesiomonas* using a battery of biochemical tests (Sinha et al., 2004; Obi et al., 1995; 1998) and also confirmed using API 20E (Analytab product). For the isolation of *Campylobacter*, specimens were plated on Butzler's media and the inoculated plates were incubated under a micro-aerophilic atmosphere (Campy Pak, BBL, Microbiology Systems, Cockeysville, Md) at 42^{O}C for 72 h. One typical colony was selected and identified by testing for Gram-stain reaction, microscopic cell morphology, catalase and oxidase production. *Campylobacter jejuni* and *coli* were separated based on hydrolysis of hippurate and indoxyl acetate. *C. jejuni* is positive for both tests whereas *C. coli* only hydrolyses indoxyl acetate (Prasad et al., 1994)

Schemes for the isolation of *Salmonella* and *Shigella* species included primary isolation on DCA or S-S agar and subculturing of suspected colonies on KIA and testing for motility urea hydrolyses and indole production. Selenite F broth was used to enhance recovery of *Salmonella* and *Shigella* (Farmer, 1995). For the detection of *Vibrio* species, specimens were plated on thiocitrate bile salt sucrose medium and enriched with alkaline peptone water.

Statistical analysis

Graphs and tables were used for data presentation. Statistical differences between the proportions of enteric pathogen isolates in the case and control groups were established with chi-square test. Spearman's rho correlation was used to assess the strength of association between enteric pathogen isolates from respective stool and water samples of the various case and control groups.

Results

Demographic characteristics of study participants

A total of 330 HIV-positive individuals comprising 200 HIV-positive individuals with diarrhoea and 130 HIV-positive individuals without diarrhoea were enrolled in the study. Similarly, 160 HIV-negative individuals comprising 80 each of HIV-negative individuals with and without diarrhoea respectively were also enrolled. The age and gender distribution of the study participants are presented in Table 1.

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TABLE 1
Gender and age strata of HIV-positive and -negative individuals with and without diarrhoea

	Gender		Age group					
	Male	**Female**	**0-10**	**11-20**	**21-30**	**31-40**	**41-50**	**>50**
HIV-positive Individuals (n = 330)								
HIV positive with diarrhoea (n = 200)	86 (43.0%)	114 (57.0%)	8 (4.0%)	38 (19.0%)	60 (30.0%)	39 (19.5%)	29 (14.5%)	26 (13.0%)
HIV positive without diarrhoea (n = 130)	40 (31.0%)	90 (69.0%)	4 (3.0%)	26 (20.0%)	44 (33.9%)	24 (18.5%)	23 (17.7%)	9 (6.9%)
Total	**126 (38.0%)**	**204 (62.0%)**	**12 (3.6%)**	**64 (19.4%)**	**104 (31.5%)**	**63 (19.1%)**	**52 (15.8%)**	**35 (10.6%)**
HIV-negative individuals (n = 160)								
HIV negative with diarrhoea (n = 80)	36 (45.0%)	44 (55.0%)	5 (6.3%)	19 (23.8%)	20 (25.0%)	14 (17.5%)	12 (15.0%)	10 (12.5%)
HIV Negative without diarrhoea (n = 80)	33 (41.3%)	47 (58.7%)	4 (5%)	21 (26.3%)	16 (20%)	19 (23.8%)	9 (11.3%)	11 (13.8%)
Total	**69 (43.0%)**	**91 (57.0%)**	**9 (5.6%)**	**40 (25.0%)**	**36 (22.5%)**	**33 (20.6%)**	**21 (13.1%)**	**21 (13.1%)**

From the 330 HIV-positive individuals, 126 (38%) and 206 (62%) were males and females respectively. HIV infection was predominant in the age group 21 to 30 (31.5%) and in the age brackets 11 to 20 years and 31 to 40 years (Table 1).

Prevalence of enteric pathogens across study groups

Figure 1 shows the prevalence rates of potential bacterial diarrhoea agents from HIV-positive and -negative individuals with and without diarrhoea. A potential enteric bacterial pathogen was isolated from all (100%) of HIV-positive individuals with diarrhoea and 68 controls without diarrhoea (52.3%) (P = 0.0001)

Bacteria that were significantly associated with diarrhoea were *Escherichia coli*, *Salmonella* spp., *Campylobacter* spp., *Shigella* and *Aeromonas* spp. whereas *P. shigelloides* was not. *Yersinia* spp. and *Vibrio* spp. were not isolated from HIV-positive individuals with diarrhoea.

The same type of enteric bacterial pathogens was also isolated from HIV-negative individuals with and without diarrhoea but at lower frequencies (P = 0.0001).

Shigella spp. (26.5%) and *Aeromonas* (20%) were more prevalent among HIV-negative individuals (Fig. 2). Profiles of enteric pathogens from household drinking water of HIV-positive study participants with and without diarrhoea also showed that *Escherichia coli* (EPEC), *Salmonella* spp., *Campylobacter* spp., *Shigella* and *Aeromonas* spp. were isolated at comparable frequencies as in the respective study groups (Fig. 3).

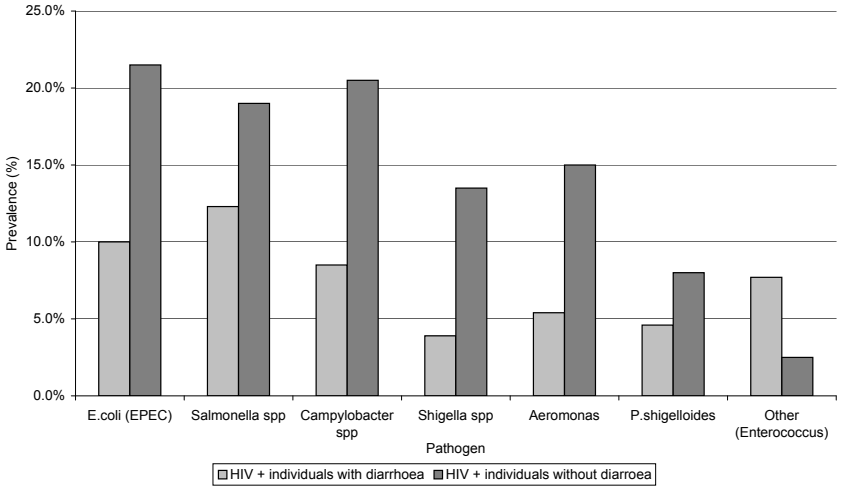

Figure 1
Isolation of potential bacterial diarrhoea agent from HIV-positive individuals with and without diarrhoea

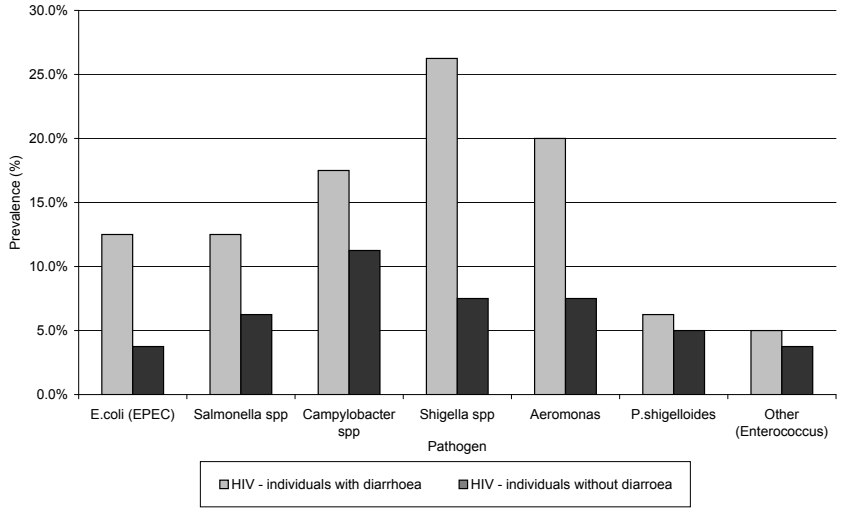

Figure 2
Isolation of potential bacterial diarrhoea agents from HIV-negative individuals with and without diarrhoea

Data on enteric bacterial pathogens from HIV-positive and -negative individuals with diarrhoea accordingly to gender showed a significant association with diarrhoea for all pathogens except *Yersinia* and *Vibrio* spp. Age distribution showed that enteropathogens were isolated from virtually all age groups (Table 2). For the control group (without diarrhoea) isolation according to gender and age groups was similar but with lower frequencies (Table 3).

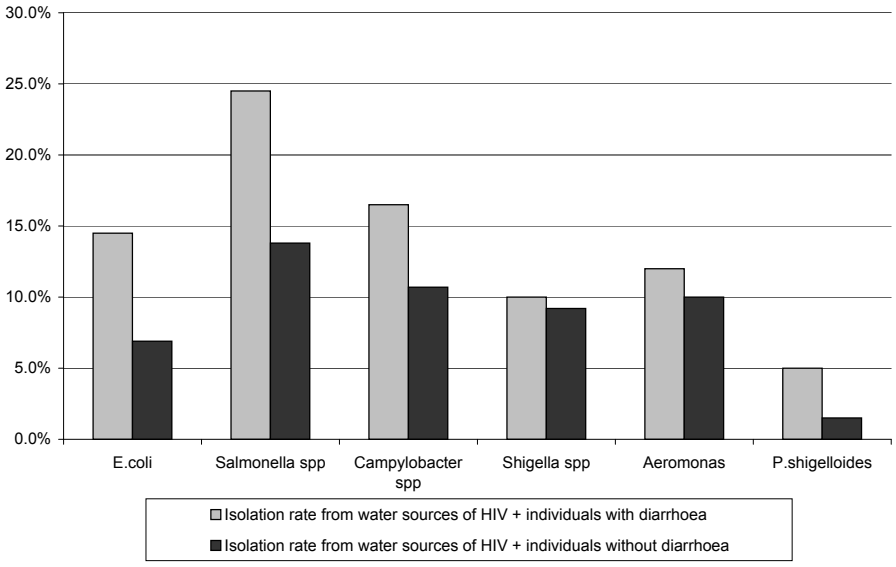

Figure 3
Profiles of enteric bacterial pathogens isolated from household drinking water of HIV-positive individuals with and without diarrhoea

TABLE 2
Gender and age distribution of enteric bacterial pathogens isolated from HIV-positive and HIV-negative individuals with diarrhoea

Bacterial pathogens	Gender		Age group					
	Male	Female	0-10	11-20	21-30	31-40	41-50	>50
A) HIV-positive individual								
Escherichia coli (n = 43)	15 (34.9%)	28(65.1%)	2 (4.7%)	8 (18.6%)	14 (32.6%)	9 (20.9%)	7 (16.3%)	3 (7.0%)
Salmonella (n = 38)	16 (42.1%)	22 (57.9%)	1 (2.6%)	3 (7.9%)	12 (31.6%)	11 (29%)	6 (15.8%)	5 (13.2%)
Campylobacter (n = 41)	20 (48.8%)	21 (51.2%)	3 (7.3%)	16 (39.0%)	11 (26.8%)	7 (17.1%)	4 (9.8%)	0 (0.0%)
Shigella (n = 27)	15 (55.6%)	12 (44.4%)	0 (0.0%)	9 (33.3%)	9 (33.3%)	5 (18.5%)	3 (11.1%)	1 (3.7%)
Aeromonas hydrophila (n = 30)	13 (43.3%)	17 (56.7%)	0 (0.0%)	11 (36.7%)	9 (30.0%)	7 (23.3%)	3 (10.0%)	0 (0.0%)
Plesiomonas (n =16)	6 (37.5%)	10 (62.5%)	0 (0.0%)	4 (25.0%)	4 (25.0%)	3 (18.8%)	3 (18.8%)	2 (12.5%)
Enterococcus (n = 5)	3 (60.0%)	2 (40.0%)	0 (0.0%)	4 (80.0%)	1 (20.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Yersinia spp. (n = 0)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Vibrio spp. (n = 0)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
B) HIV-negative individuals								
Escherichia coli (n =10)	6 (60.0%)	4 (40.0%)	0 (0.0%)	3 (30.0%)	4 (40.0%)	0 (0.0%)	3 (30.0%)	0 (0.0%)
Salmonella (n =10)	3 (30.0%)	7 (70.0%)	4 (40.0%)	6 (60.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Campylobacter (n =14)	5 (35.7%)	9 (64.3%)	0 (0.0%)	8 (57.1%)	4 (28.6%)	1 (7.1%)	1 (7.1%)	0 (0.0%)
Shigella (n =21)	9 (42.9%)	12 (57.1%)	0 (0.0%)	6 (28.6%)	2 (9.5%)	6 (28.6%)	3 (14.3%)	4 (19.1%)
Aeromonas hydrophila (n =16)	7 (43.8%)	9 (56.3%)	0 (0.0%)	7 (43.8%)	0 (0.0%)	3 (18.8%)	4 (25.0%)	2 (12.5%)
Plesiomonas (n =5)	2 (40.0%)	3 (60.0%)	0 (0.0%)	5 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Enterococcus: (n =4)	3 (75.0%)	1 (25.0%)	2 (50.0%)	0 (0.0%)	2 (50.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Yersinia spp. (n = 0)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Vibrio spp. (n = 0)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Comparison of proportions of enteric pathogens isolated from stool and water samples of study participants

Figures 5 and 6 show the prevalence of enteric pathogens isolated from stool and water samples respectively for HIV-positive and HIV-negative individuals with diarrhoea. *Shigella* spp. (26.25%) were the most prevalent enteric pathogens isolated from the stool samples of HIV-negative individuals with diarrhoea followed by *Aeromonas* spp. (20.00%) and *Campylobacter* (17.50%). Among HIV-positive individuals with diarrhoea, *E. coli* (21.50%) was most prevalent followed by *Campylobacter* (20.50%). *P. shigelloides* was the least commonly isolated enteric pathogen in both HIV-positive (8.0%) and HIV-negative individuals (6.25%). The relative risk (which is the ratio of HIV infected individuals with diarrhoea to HIV-negative individuals with diarrhoea) was highest with *E. coli* (1.7) followed by *Salmonella* (1.5), *P. shigelloides* (1.3) and *Campylobacte*r (1.2).

Higher percentages of enteric pathogens were isolated from household drinking water of HIV-negative individuals with diarrhoea when compared to percentages isolated from household drinking water of HIV-positive individuals. Among HIV-negative individuals *Salmonella* (37.50%) was the most prevalent isolate followed by *Shigella* (25.0%). Similarly, *Salmonella*

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TABLE 3
Gender and age distribution of enteric bacterial pathogens isolated from HIV-positive and HIV-negative individuals without diarrhoea

Bacterial pathogens	Gender		Age group					
	Male	Female	0-10	11-20	21-30	31-40	41-50	>50
A) HIV-positive individual								
Escherichia coli (n = 13)	5 (38.5%)	8 (61.5%)	0 (0.00%)	3 (23.1%)	7 (53.9%)	3 (23.1%)	0 (0.0%)	0 (0.0%)
Salmonella (n = 16)	7 (43.8%)	9 (56.3%)	2 (12.5%)	5 (31.3%)	6 (37.5%)	0 (0.0%)	3 (18.8%)	0 (0.0%)
Campylobacter (n = 11)	6 (54.6%)	5 (45.5%)	0 (0.0%)	4 (36.4%)	5 (45.5%)	0 (0.0%)	0 (0.0%)	2 (18.2%)
Shigella (n = 5)	2 (40%)	3 (60.0%)	0 (0.0%)	1 (20.0%)	3 (60.0%)	0 (0.0%)	1 (20.0%)	0 (0.0%)
Aeromonas hydrophila (n = 7)	4 (57.1%)	3 (42.9%)	0 (0.0%)	2 (28.6%)	3 (42.9%)	0 (0.0%)	0 (0.0%)	2 (28.6%)
Plesiomonas (n =6)	2 (33.3%)	4 (66.7%)	0 (0.0%)	2 (33.3%)	0 (0.0%)	0 (0.0%)	4 (66.7%)	0 (0.0%)
Enterococcus: (n = 10)	4 (40.0%)	6 (60.0%)	1 (10.0%)	6 (60.0%)	0 (0.0%)	3 (30.0%)	0 (0.0%)	0 (0.0%)
Yersinia spp. (n = 0)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Vibrio spp. (n = 0)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
B) HIV-negative individuals								
Escherichia coli (n =3)	2 (66.7%)	1 (33.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Salmonella (n =5)	2 (40.0%)	3 (60.0%)	0 (0.0%)	3 (60.0%)	2 (40.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Campylobacter (n =9)	4 (44.4%)	5 (55.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Shigella : HIV-WD (n =6)	2 (33.3%)	4 (66.7%)	1 (16.7%)	2 (33.3%)	3 (50.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Aeromonas hydrophila (n =6)	3 (50.0%)	3 (50.0%)	0 (0.0%)	4 (66.7%)	0 (0.0%)	2 (33.3%)	0 (0.0%)	0 (0.0%)
Plesiomonas (n =4)	2 (50.0%)	2 (50.0%)	0 (0.0%)	3 (75.0%)	2 (50.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Enterococcus (n = 3)	3 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Yersinia spp. (n = 0)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Vibrio spp. (n = 0)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Figure 4
Profiles of enteric bacterial pathogens isolated from household drinking water of HIV-negative individuals with and without diarrhoea

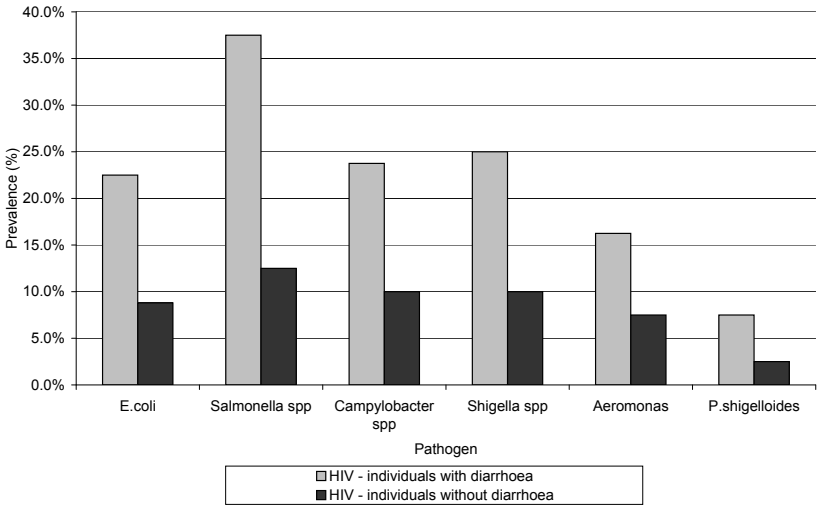
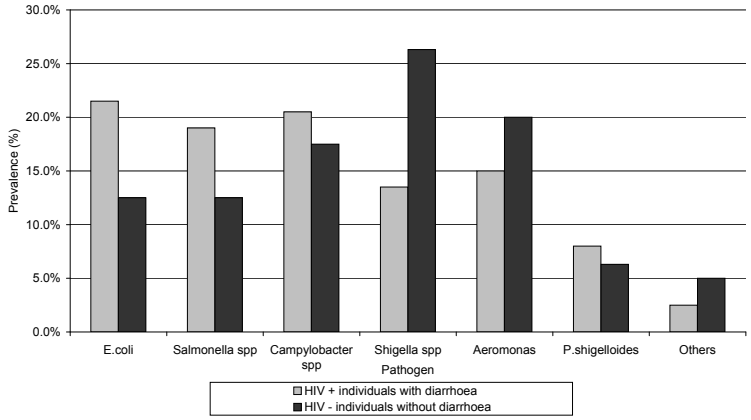


Figure 5: Profiles of enteric bacterial pathogens isolated from HIV-positive and -negative individuals with diarrhoea

Figure 5
Profiles of enteric bacterial pathogens isolated from HIV-positive and -negative individuals with diarrhoea



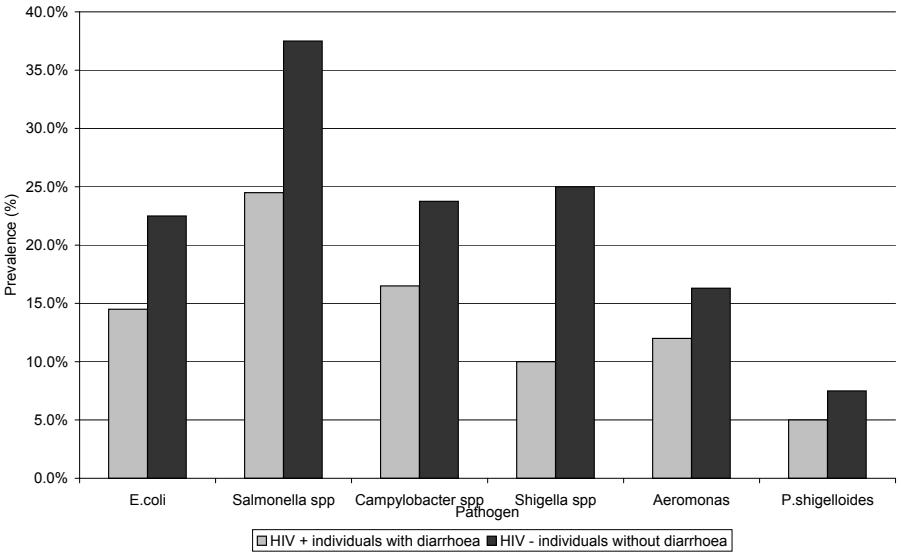


Figure 6
Profiles of enteric bacterial pathogens isolated from water samples of HIV-positive and -negative individuals with diarrhoea

(24.50%) was the most prevalent enteric pathogens isolated from household drinking water of HIV-positive individuals. *P. shigelloides* was the least isolated organism from household water sources of both groups.

Further to the above figures, the chi-square analyses below try to establish whether the differences in proportion of enteric pathogens isolated from stool and water samples of the case and control groups differed significantly.

E. coli, Salmonella, Campylobacter and *P. shigelloides* isolated from the stool samples exhibited very similar patterns (Tables 4 to 7). In both comparisons (HIV-negative individuals without diarrhoea vs. HIV-positive individuals without diarrhoea; and HIV-negative with diarrhoea vs. HIV-positive individuals with diarrhoea), higher proportions of the enteric pathogens were isolated from stool samples of HIV-positive individuals with and without diarrhoea. However, only the comparison of prevalence of *E. coli* across HIV-positive and -negative individuals without diarrhoea reached statistical significance ($p = .018$) (Table 4). In this instance, *E. coli* was significantly more common among HIV-positive individuals without diarrhoea.

Shigella and *Aeromonas* (Tables 8 and 9) were, however, more prevalent in the stool samples of HIV-negative individuals (with or without diarrhoea) when compared to the HIV-positive individuals (with or without diarrhoea), but these were not statistically significant except for the comparison of the prevalence of *Shigella* species across HIV-negative with diarrhoea vs. HIV-positive individuals with diarrhoea ($p = 0.011$) (Table 9). In this comparison, *Shigella* species were significantly more prevalent among HIV-negative individuals with diarrhoea.

For the water samples, HIV-negative individuals had higher percentages of all 6 enteric pathogens (*E. coli, Salmonella, Campylobacter, Shigella, Aeromonas* and *P. shigelloides*) in their household drinking water when compared with percentages isolated from water samples from households of HIV-positive individuals (Tables 4 to 9). In these comparisons ((1) HIV

TABLE 4
Chi-square analysis of *E. coli* isolated from stool and household drinking water by HIV status and presence or absence of diarrhoea

	***E. coli* in stool**		***E. coli* in household drinking water**	
Health status	**Count (percentage)**	**Significance**	**Count (percentage)**	**Significance**
HIV-negative individuals without diarrhoea	3 (3.8%)	.018*	7 (8.8%)	.628
HIV-positive individuals without diarrhoea	18 (13.8%)		9 (6.9%)	
HIV-negative individuals with diarrhoea	10 (12.5)	.083	18 (22.5%)	.106
HIV-positive individuals with diarrhoea	43 (21.5%)		29 (14.5%)	

** Significant at the 0.05 level (2-sided)*

TABLE 5
Chi-square analysis of *Salmonella* species isolated from stool and household drinking water by HIV status and presence or absence of diarrhoea

	***Salmonella* spp. in stool**		***Salmonella* spp. in household drinking water**	
Health status	**Count (percentage)**	**Significance**	**Count (percentage)**	**Significance**
HIV-negative individuals without diarrhoea	5 (6.3%)	.155	10 (12.5%)	.780
HIV-positive individuals without diarrhoea	16 (12.3%)		18 (13.3%)	
HIV-negative individuals with diarrhoea	11 (13.8%)	.296	30 (37.5%)	.029*
HIV- positive individuals with diarrhoea	38 (19.0%)		49 (24.5%)	

** Significant at the 0.05 level (2-sided)*

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TABLE 6
Chi-square analysis of *Campylobacter* species isolated from stool and household drinking water by HIV status and presence or absence of diarrhoea

	***Campylobacter* spp. in stool**		***Campylobacter* spp. in household drinking water**	
Health status	**Count (percentage)**	**Significance**	**Count (percentage)**	**Significance**
HIV- negative individuals without diarrhoea	9 (11.3%)	.504	8 (10%)	.860
HIV- positive individuals without diarrhoea	11 (8.5%)		14 (10.8%)	
HIV- negative individuals with diarrhoea	14 (17.5%)	.568	19 (23.8%)	.159
HIV- positive individuals with diarrhoea	41 (20.5%)		33 (16.5%)	

** Significant at the 0.05 level (2-sided)*

TABLE 7
Chi-square analysis of *Plesiomonas* species isolated from stool and household drinking water by HIV status and presence or absence of diarrhoea

	***Plesiomonas* spp. in stool**		***Plesiomonas* spp. in household drinking water**	
Health status	**Count (percentage)**	**Significance**	**Count (percentage)**	**Significance**
HIV-negative individuals without diarrhoea	4 (5.0%)	.903	2 (2.5%)	.621
HIV-positive individuals without diarrhoea	7 (5.4%)		2 (1.5%)	
HIV-negative individuals with diarrhoea	5 (6.3%)	.615	6 (7.5%)	.416
HIV-positive individuals with diarrhoea	16 (8.0%)		10 (5.0%)	

** Significant at the 0.05 level (2-sided)*

TABLE 8
Chi-square analysis of *Shigella* species isolated from stool and household drinking water by HIV status and presence or absence of diarrhoea

	***Shigella* spp. in stool**		***Shigella* spp. in household drinking water**	
Health status	**Count (percentage)**	**Significance**	**Count (percentage)**	**Significance**
HIV-negative individuals without diarrhoea	6 (7.5%)	.248	8 (10.0%)	.845
HIV-positive individuals without diarrhoea	5 (3.8%)		12 (9.2%)	
HIV-negative individuals with diarrhoea	21 (26.3%)	.011*	20 (25.0%)	.001*
HIV-positive individuals with diarrhoea	27 (13.5%)		20 (10.0%)	

** Significant at the 0.05 level (2-sided)*

TABLE 9
Chi-square analysis of *Aeromonas* species isolated from stool and household drinking water by]HIV status and presence or absence of diarrhoea

	***Aeromonas* spp. in stool**		***Aeromonas* spp. in household drinking water**	
Health status	**Count (percentage)**	**Significance**	**Count (percentage)**	**Significance**
HIV-negative individuals without diarrhoea	6 (7.5%)	537	6 (7.5%)	.540
HIV-positive individuals without diarrhoea	7 (5.4%)		13 (10.0%)	
HIV-negative individuals with diarrhoea	16 (20.0%)	.308	19 (23.8%)	.014*
HIV-positive individuals with diarrhoea	30 (15.0%)		24 (12.0%)	

** Significant at the 0.05 level (2-sided)*

negative without diarrhoea vs. HIV-positive individuals without diarrhoea and (2) HIV negative with diarrhoea vs. HIV-positive individuals with diarrhoea), HIV-negative individuals with diarrhoea had significantly higher proportions of *Salmonella* (p = .029) (Table 5), *Shigella* (p = .001) (Table 8) and *Aeromonas* (p = .014) (Table 9) in their water sources when compared with proportions isolated from their respective HIV-positive individuals with diarrhoea cohorts.

The Spearman's correlation analysis below established a strong correlation between the proportions of enteric pathogens isolated from water samples with proportions isolated from stool samples. For all the organisms and cohort groups the level of significance was at 0.000. The strength of association exceeded 0.7 in most of the cases but varied with organism and cohort group with no consistent pattern (Table 10)

Discussion

This study provides the first baseline reference data on the scope and frequency of enteric bacterial pathogens isolated from the stool samples and household drinking water of HIV-positive and -negative individuals with and without diarrhoea in rural com-

TABLE 10
Spearman's rho correlation of respective enteric pathogen isolated in stool and household drinking water by HIV status and presence or absence of diarrhoea

Organism		HIV-negative individuals without diarrhoea	HIV-positive individuals without diarrhoea	HIV-negative individuals with diarrhoea	HIV-positive individuals with diarrhoea
E. coli	**Correlation coefficient**	.637(**)	.680(**)	.701(**)	.787(**)
	Significance	.000	.000	.000	.000
Salmonella	**Correlation coefficient**	.683(**)	.935(**)	.515(**)	.850(**)
	Significance	.000	.000	.000	.000
Campylobacter	**Correlation coefficient**	.936(**)	.875(**)	.825(**)	.875(**)
	Significance	.000	.000	.000	.000
Shigella	**Correlation coefficient**	.854(**)	.627(**)	.968(**)	.844(**)
	Significance	.000	.000	.000	.000
Aeromonas	**Correlation coefficient**	1.000(**)	.716(**)	.896(**)	.879(**)
	Significance	.	.000	.000	.000
Plesiomonas spp.	**Correlation coefficient**	.698(**)	.524(**)	.907(**)	.778(**)
	Significance	.000	.000	.000	.000
Total number		80	130	80	200

** Significant at the 0.05 level (2-sided)*

munities in Limpopo Province. Inclusion of controls has made it possible to ascertain any epidemiological linkage of pathogens studied with diarrhoea, water sources and HIV/AIDS.

Gender distribution among HIV-positive patients, consisting of 62% infection rate in women as opposed to 38% in males (P = 0.0001) confirms previous reports on feminisation of the epidemic (UNAIDS, 2006) Feminisation has been attributed to unequal power relations between men and women, and increasing vulnerability of women due to unemployment and poverty, which drives them to prostitution as a means of survival. Age distribution of HIV-positive individuals with and without diarrhoea indicated that infection burden was common to all age groups studied but mostly in the 21 to 30 years age group (31.5%), followed by the age groups 11 to 20 (19.4%) and 31 to 40 (19.1%). The pooled affected age group (11 to 40 years) constitutes the most productive range of any economy. The ripple effects of these observations on the various sectors of the economy including water resource management will be increasingly devastating.

The high frequency of isolating *Escherichia coli*, *Salmonella* species, *Campylobacter jejuni/coli* and *Aeromonas* species from HIV-positive individuals with diarrhoea in contrast with the low frequency of isolation from those without diarrhoea strongly incriminates the pathogens in diarrhoeal cases of HIV-positive individuals. These findings agree with the reports of Weiss et al. (2005) from Lima, Peru. They identified one or more enteric pathogens in 53% of case subjects and 21% of control subjects. In a related study on enteric pathogens in Southern Indian HIV infected patients with and without diarrhoea, enteric pathogens were detected from stool samples in 57.4% of diarrhoeal patients compared to 40% of those without diarrhoea (P>0.05) (Mukhopadhya et al., 1999). Specifically, bacterial pathogens were isolated more commonly from patients with diarrhoea (12/61) compared to patients without diarrhoea (2 /50) (P < 0.05).

Another interesting aspect of the present study was the observation on the differences in main bacterial aetiologies of diarrhoea according to HIV status of patients. In immuno - suppressed HIV-positive individuals with diarrhoea, the leading bacterial aetiologies were enteropathogenic *E. coli* (21.5%), *Campylobacter jejuni/ coli* (20.5%), *Salmonella* species (19%) and *Aeromonas* species (15%). In immuno-competent individuals, *Campylobacter* (17.5%), and *Shigella* species (26%) were the main pathogens although Gassama et al., (2001) reported *Shigella* and *Salmonella* for immuno-competent individuals, and *E.coli*, *Shigella* and S*almonella* for immuno-compromised individuals. HIV-infected patients are usually susceptible to the same spectrum of clinical manifestations and enteric bacterial pathogens implicated in diarrhoeal cases in HIV-negative individuals (Weber et al., 1999; Wilcox, 2000) and this was variously observed in this study. *Salmonella* gastro-enteritis usually manifests as watery diarrhoea, abdominal pain, fever, nausea, vomiting or as an enteric fever. *Shigella* and *Campylobacter* usually show as dysentery marked by muco-purulent bloody diarrhoea and fever (Wilcox, 2000). In addition to stool culture, routine blood tests may reveal the severity of the diarrhoea, such as the state of dehydration and electrolyte profiles. A highly elevated leukocyte count may be suggestive of bacterial colitis or a complication such as perforation or intra-abdominal abscess formation.

The present investigation has revealed the frequent association of *Aeromonas* spp. in cases of diarrhoea in HIV patients. However, the role of *Aeromonas* spp. as a diarrhoeagenic bacterial pathogen is still regarded by some investigators as controversial (Albert et al., 1999). *Aeromonas* spp. have been associated with diarrhoea in some studies (Pazzaglia et al., 1991; Obi et al., 1995). Albert et al. (1999) asserted that no well-described epidemiologically linked outbreaks of *Aeromonas*-induced diarrhoea have been reported. *Plesiomonas shigelloides* was not strongly associated with diarrhoea in HIV-positive individuals studied despite its incrimination in several case reports of diarrhoea and numerous diarrhoea cases linked to the organism (Obi et al., 1998; Brenden et al., 1988).

Diarrhoea has assumed an elevated status among HIV-positive patients because of the chronicity of diarrhoea in HIV-infected patients. Chronic diarrhoea results in substantial morbidity and mortality, reduced quality of life and increased health-care burden (Lubeck et al., 1993). Although CD4 counts were not included in this study, previous studies showed that CD4 counts were lower in patients with diarrhoea, corroborating the assertions that diarrhoea is a debilitating medical condition

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and that effective management can prevent immuno suppression (Gassama et al., 2001).

Another observation in this study was the infection with multiple pathogens, indicating that pathogens may act in synergy to induce diarrhoea. Reports on the isolation of mixed pathogens from diarrheic stool samples abound in the literature (Faruque et al., 1994; Albert et al., 1999). Interestingly, *Yersinia* spp. and *Vibrio cholerae* were not isolated from HIV-positive and -negative individuals with and without diarrhoea in the present study. It may therefore be concluded that both pathogens may be rare aetiological agents of diarrhoea in rural communities in Limpopo Province. Schemes for the isolation of *Clostridium difficile*, a notable cause of antibiotic associated diarrhoea and pseudo-membranous colitis (Albert et al., 1999) were not included in this study. No attempts were made to isolate viral and parasitic agents.

The scope and frequencies of enteric bacterial pathogens isolated from household drinking water of HIV-positive individuals were not significantly different from those isolated from their stool samples. The cankerworm of HIV and water-borne infections is bound to be rapidly debilitating and fatal.

This is compounded by hygiene and sanitation issues. The majority of the households studied had no proper water storage containers. Water storage containers had no lids, were dirty and not regularly washed. General personal and environmental hygiene was poor, toilet facilities were buzzing with flies and hand-washing after using toilet facilities was not common. In some cases, faeces were not properly disposed of as they were discharged into gutters or into surrounding veld. Drainage systems were poor in the majority of the study areas.

Mitigating factors should include unrelenting efforts to destigmatise HIV infections, continuous public awareness and educational campaigns on the relationship between HIV/AIDS and water quality and the various methods for household treatment and storage of water, including sanitation and hygiene. Educational campaigns should also target caregivers, lay counsellors and household family members.

Management of diarrhoeal cases may be predicated on the use of antibiotics because antibiotics have been reported to shorten the duration of diarrhoea, decrease stool output and abrogate some complications. Determination of antibiotic susceptibility profiles of isolated pathogens is therefore crucial and is indeed the focus of an extended investigation. Another interesting focus of an extended study is to undertake genetic studies to unravel any relatedness between enteric bacterial pathogens from water sources and stool samples.

In conclusion, the present study has revealed E. *coli, Salmonella, Campylobacter* and *Aeromona*s as the major pathogens incriminated in diarrhoeal cases among HIV-positive individuals and their household drinking water. Other findings include low prevalence of *P. shigelloides* and lack of isolation of *Yersinia* spp. and *Vibrio cholerae* from diarrheic cases and the seeming synergistic action of the pathogens in the induction of diarrhoea. A notable finding was the emergence of *Aeromonas* spp. in diarrhoeal cases of HIV-positive individuals and their household drinking water. This study is the first report on the scope and frequency of enteric bacterial pathogens isolated from HIV/AIDS patients and their household drinking water in Limpopo Province, South Africa.

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