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Full Length Research Paper

Antibiotic resistance profiles and relatedness of enteric bacterial pathogens isolated from HIV/AIDS patients with and without diarrhoea and their household drinking water in rural communities in Limpopo Province South Africa

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Antibiotic resistance profiles and the correlation of enteric bacterial pathogens from HIV positive individuals with and without diarrhoea and their household drinking water were determined using the Kirby Bauer disk diffusion and polymerase chain reaction methods respectively. The *sef* gene of *Salmonella enteritidis* was amplified with the primer pair *sefA*-1 and *sefA*-2. The fliC gene of *Salmonella typhimurium* was amplified with the primer pair *flicA-1 and flicA-2*. Heat-labile toxin (*LT*) primers (Lta and LTb) were used to amplify *Escherichia coli* isolates and VirA1 and VirA2 for the Vir A gene of *Shigella dysenteriae*. Results of antibiotic resistance profiles of enteric bacterial pathogens isolated from stool samples of HIV positive and negative individuals with and without diarrhea and their household drinking water showed very similar drug resistance patterns. Over 90% of all the organisms isolated from the various study cohorts showed resistance to penicillin, cloxacillin and amoxicillin. Conversely, almost all the organisms were sensitive to ciprofloxacin, gentamycin, meropenem and imipenem. About 50% of *E. coli* isolated from the various study cohorts showed multiple antibiotic resistance to penicillin, amoxicillin, ampicillin, erythromycin, tetracycline, doxycycline and cotri-moxazole (P^R, A^R, AP^R, E^R, T^R, DXT^R, and TS^R) whereas less than 10% resistance was consistently reported for ofloxacin, gentamycin, meropenem cefotaxime, cefuroxime and imipenem (OFX^S, GM^S, MEM^S, CTX^S, CXM^S and IMI^S). The majority of *Salmonella* and *Shigella* isolates from all the groups were sensitive to ciprofloxacin, gentamicin, amikacin, meropenem, imipenem, nalidixic acid, kanamycin, piperacillin-tazo bactam, cefuroxime, doxycyclin, cefepime and ceftazidime (CIP^S, GM^S, AK^S, MEM^S, IMI^S, NA^S, KN^S, DXT^S, CXM^S, CPM^S, CAZ^S and PTZ^S). For *Campylobacter*, over 30% of the isolates were resistant to erythromycin, ampicillin, tetracycline, cotrimoxazole and ceftazidime (E^R, AP^R TS^R and CAZ^R) whereas over 85% were susceptible to ciprofloxacin, ofloxacin, gentamycin, amikacin, mero-penem, and nalidixic acid (CIP^S, OFX^S, GM^S, AK^S, MEM^S and NA^S). In addition to penicillin, amoxicillin, ampicillin and erythromycin, *Aeromonas and Plesiomonas* spp were more resistant to chloramphenicol, but were susceptible to ciprofloxacin, gentamycin, amikacin, meropenem, imipenem and nalidixic acid (CIP^S, GM^S, AK^S, MEM^S, IMI^S and NAS). Polymerase Chain Reaction (PCR) experiments using targeted species genes of *S. enteritidis*, *S. typhimurium, E. coli, Sh. dysenteriae* showed that isolates from stool samples of HIV positive and HIV negative individuals with and without diarrhoea were also present in the household drinking water of the same study cohorts, suggesting that drinking water may have been the sources of the organisms in stool sample. Furthermore, by showing that the primers were able to amplify the genes in both clinical and environmental isolates, the link between the virulence of the pathogens was established.

Key words: Enteric, Bacteria, Pathogens, Antibiotics, HIV/AIDS, Household, Water, Diarrhoea.

INTRODUCTION

Diarrhea is one of the hallmarks of HIV/AIDS in developing countries and is also a cardinal clinical manifestation of water borne infections. Diarrhea is reportedly the cause of malabsorption, significant weight loss and extra-intestinal opportunistic infections in about 95% of individuals with HIV/AIDS in developing countries (Carcano et al., 2005). In Peru, diarrhea is frequently encountered among HIV/AIDS patients (Frisancho, 1991). In Lima, Peru, *Salmonella typhi, S. paratyphi and Clostridium* were more commonly isolated from HIV/AIDS patients than in control individuals with HIV infection (Gotuzzo, 1991). *Shigella spp, Campylobacter spp, E. coli, Aeromonas spp, and P.shigelliodes* have also been reported to occur more frequently in HIV infected persons than in control subjects without HIV infection in other parts of the world (Baer et al., 1991; Sorvillo et al*.,* 1991; Obi and Bessong, 2003; Awole et al., 2002; Gassama et al., 2001).

One of the major sources of diarrhoea in humans is polluted or contaminated water. Water meant for consumption should be free from pollution, safe and acceptable (Obi et al*.,* 2004). However, the microbial quality of some rural water sources in Limpopo province has been reported to be poor, unsafe and not acceptable for human consumption (Obi et al., 2002). Enteric bacterial pathogens, such as those already mentioned have been isolated from rural water sources. To compound the problem, majority of HIV/AIDS patients live in rural areas devoid of basic amenities and potable water supply. This poses a huge public health problem because HIV/AIDS patients require safe water for anti-retroviral (ARV) medications, drinking and the preparation of formula feeds for infected babies. Water quality is therefore critical for HIV/AIDS patients because of their susceptibility to opportunistic or low grade pathogens due to lowered immunocompetence.

Management of diarrhea due to bacteria require the use of antibiotics which shortens the duration of diarrhea, decrease frequency of stool output and abrogate complications (Black, 1993). Antibiotic susceptibility profile of microorganisms vary from country to country , province to province, town to town , and hospital to hospital in the same town as well as between private and public healthcare facilities in the same area (Sein et al., 2005). In addition, different pathologies may alter antibiotic sensitivity patterns. Consequently, periodic evaluation of antibiotic susceptibility is recommended to guide management of patients requiring antibiotic treatment. Because HIV disrupts the body's own disease-fighting immune system, antibiotics are critical for treating patients infected with HIV.

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Antibiotic resistance among enteric bacteria pathogens complicates the heavy diarrhea disease burden (Sinha et al., 2004; Obi et al., 1997, 1998). Antibiotic resistance among *Campylobacter* spp (Coker and Adefeso, 1994; Nachamkin et al., 2001; Steinbruckner et al., 2001), *Aeromonas* spp (Obi et al., 1998; Sinha et al., 2004) *Shigella* and *Salmonella* (Hoge et al., 1998; Sinha et al., 2004; Isenbarger et al., 2002; Awole et al*.,* 2002; Obi et al., 2004) and *E. coli* (Hart and Kariuki, 1998) have been noted. In a case control study in Senegal, enteric bacteria such as *Salmonella* and *Shi-gella* isolated from HIV/AIDS patients were found to be highly resistant to commonly used antibiotics (Gassama et al., 2001), while in Ethiopia *Salmonella*, *Shigella* and *Campylobacter* were reported to display a 100% suscep-tibility in a hospital-based study (Awole et al., 2002).

The problem posed by antibiotic resistance among enteric bacterial pathogens necessitates the need to ascertain any association or linkage between enteric pathogens from clinical samples such as stool and environmental samples such as water which are common sources of diarrhoeal infections. Many methods such as polymerase chain reaction (PCR), restriction fragments length polymorphism (RFLP) and other molecular methods may be useful in determining such linkages.

In this study, antibiotic susceptibility profiles of enteric bacterial pathogens isolated from HIV/AIDS patients and their respective drinking water sources were ascertained in order to provide an updated reference data for effective empiric management of bacterial diarrhoea in HIV/AIDS patients. The linkage between enteric pathogens from stool and water samples of the study cohorts was determined using the polymerase chain reaction method.

MATERIALS AND METHODS

Study area

Areas for the collection of samples were stratified according to the six 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 compromised Vhembe, Waterberg, and Capricorn. In the Vhembe district, villages around Madombizha, Rathidili, Tshiozwi, Magau, Gogobole and Musina were selected.

The Musina area reflects the northernmost part of Limpopo Province and is located around Limpopo valley. It is the main gateway to South Africa from the north and is also a border town linking South Africa and Zimbabwe.

In 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 Gauteng region and with a high prevalence of HIV/AIDS. 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 stigmatization of the disease, identifying HIV/AIDS patients is usually an uphill task in most South African communities. Health authorities and family members are unusually 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 cohorts.

Study population

The present study was conducted prospectively from August 2005 to January 2006. Information on age, sex, diarrhoeal and HIV status was obtained by questionnaire administration.

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 diarrhoeic 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.

Ethical 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 Microbiology Laboratory, University of Venda, South Africa for bacterial analyses within 4 - 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 - 6h of collection (Obi et al., 2002).

Culture media

Isolation media commonly used for the isolation of enteric bacterial pathogens were employed in this study. They consisted of Mac-Conkay 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°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 (WHO, 1987; Sinha et al., 2004; Obi et al., 1995, 1998) and also confirmed using API 20E (Analytab product). For the isolation of *Campylobacters*, specimens were plated on Butzler's media and the inoculated plates were incubated under a microaerophilic atmosphere (Campy Pak, BBL, Microbiology Systems, Cockeysville, Md) at 42°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 hydrolyse indoxyl acetate (Prasad et al., 2002)

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 *Vibro* species, specimens were plated on thiocitrate bile salt sucrose medium and enriched with alkaline peptone water.

Data presentation

Graphs were used for data presentation to illustrate antibiotic resistance profile of each enteric pathogen isolated from the case and control groups. Figures were also used to depict results obtained from PCR experiments.

Antibiotic susceptibility testing

Antibiotic susceptibility of the isolates was determined using the Kirby Bauer disk-agar diffusion technique (Bauer et al., 1966). Briefly five pure colonies of each bacterial strain were inoculated into 2 ml of sterile Mueller Hinton broth in Bijou bottles and incubated at 37°C for 6 h. The turbidity was adjusted to match a 0.5 McFarland turbidity standard. Sterile cotton tipped swap was rotated against the wall of the tube above the liquid level to remove excess inoculums. The inoculums were swabbed on the entire surface of a Mueller-Hinton agar plate. The automatic disc dispenser, adjusted to dispense six antibiotic discs was applied on the surface of the agar and the plates were incubated at 37°C for 24 h. After incubation the organisms were classified as sensitive (S), and resistant (R) according to NCCLS (2003) guidelines. A total number of 25 antibiotics were tested.

The antibiotic containing disks were obtained from Oxoid and consisted of the following: penicillin (PG, 10 units), ciprofloxcain (CIP 5μg), vancomycin (VA, 30μg), erythromycin (E, 30 μg), tetracycline (T, 30 μg), fusidic acid (FC, 30 μg), cloxacillin (CL, 30 μg), rifampicin (RF, 30 μg), meropenem (MEM, 10 μg), imepenem (IMI, 10 μg), chloramphenicol (C, 10 μg), amoxacillin/clavulanic acid (AMC, 30 μg), nitrofurantoin (NI, 200 μg), gentamycin (GN, 10 μg), amikacin (AK10 μg), ampicillin (AMP, 10 μg), cefoxitin (FOX, 30 μg), nalidixic acid (NA, 30 μg), piperacillin/Tazobactam (PTZ, 110 μg), doxycycline (DOX, 30 μg), novobiocin (NO, 30 μg), cotrimoxazole (TS, 25 μg), cefotaxine (CTX, 30 μg), cefepime (CPM, 30 μg) and cefotrizone (CRO, 30 μg).

Reference strains

The following standard strains were included as controls; *E. coli* ATCC 35218 beta-lactamase producer and *K. pneumoniae* ATCC 700603 Extended spectrum β-lactamase (ESBL) producer. They were obtained from the Microbiology Department of the National Health Laboratory Services (NHLS), Johannesburg, South Africa. *E. coli* ATCC strain 25922, *S. typhimurium* ATCC strain 14028, *S. enteritidis* ATCC strain 13076, *Sh. dysenteriae* ATCC strain 29027 were obtained from the American Type Culture Collection, Rockville, MD.

Preparation of DNA templates for PCR

A direct lysis method was used for the isolation of DNA from bacteria. Briefly, overnight bacterial colonies were each suspended in 200 µl of sterile Milli-Q water and the bacteria were lysed by heating for 15 min at 100°C. Particulate material present after processing was removed by centrifugation at 13 000 x *g* for 2 min. The lysate supernatant was removed and 10 µl was used as the template in the PCR assays.

Primers and target gene regions

The *sef* gene of *S. enteritidis* was amplified with the primer pair *sefA*-1 and *sefA*-2. The fliC gene of *S. typhimurium* was amplified with the primer pair *flicA-1 and flicA-2* (Oliviera et al., 2002). Heat-labile toxin (*LT*) primers (Lta and LTb) were used to amplify *E. coli* isolates (Obi et al., 2004) and VirA1 and VirA2 for the Vir A gene of *Sh. dysenteriae* (Villabola and Torres, 1998). Primer details, target gene regions, expected product sizes and cycling conditions are shown in Table 1.

PCR amplification and electrophoretic detection of amplicons

The reaction mixtures were performed in a final volume of 50 µl containing 10 X SuperTherm GOLD Buffer with 1.5 mM $MgCl_2$ (Southern Cross Biotechnology), each deoxynucleotide triphosphate (Promega) at a concentration of 0.25 mM, 100 pmol of appropriate primers, and 1 U of SuperTherm *Taq* polymerase (Southern Cross Biotechnology); and 10 µl of DNA template. The standardized cycling conditions (Table 1) were carried out in an Eppendorf thermocycler (model AG22331) . The amplicons were resolved on a 2% (w/v) agarose gel in 1X - TAE buffer and visualized by UV translumination after staining with 0.5 µg of ethidium bromide per ml. A 100-bp ladder (Promega) was included to estimate the size of PCR products.

RESULTS

Results of antibiotic resistance profiles of enteric pathogens isolated from stool samples of HIV positive individuals with diarrhea and their household drinking water are presented in Figures 1 to 5. The case and control groups showed very similar drug resistance patterns. Over 90% of all the organisms isolated from the various study cohorts showed resistance to penicillin, cloxacillin and amoxicillin. Conversely almost all the organisms were sensitive to ciprofloxacin, gentamycin, meropenem and imipenem.

Relative to the other organisms, 50% of *E. coli isolated* showed multiple antibiotic resistance to penicillin, amoxicillin, ampicillin, erythromycin, tetracycline, doxy-cycline and cotrimoxazole (P^R, A^R, AP^R, E^R, T^R, DXT^R, and TS^R). Among the *E. coli* isolates from all the groups, less than 10% resistance was consistently reported for ofloxacin, gentamycin, meropenem cefotaxime, cefuroxime and imipenem (OFX^S, GM^S, MEM^S, CTX^S, CXM^S and IMI^S) (Figure 1).

The majority of *Salmonella* isolates from all the groups were sensitive to ciprofloxacin, gentamicin, amikacin, meropenem, imipenem, nalidixic acid, Kanamycin, pipevacillin-tazo bactam, cefuroxime, doxycyclin, cefepime and ceftazidime (CIP^S, GM^S, AK^S, MEM^S, IMI^S, NA^S, KN^S, DXT^S, CXM^S, CPM^S, CAZ^S and PTZ^S). The high resistance of *Salmonella* isolates from stool samples of HIV negative individuals to tetracycline was also noted (Figure 2).

For *Campylobacter*, over 30% of the isolates were resistant to erythromycin, ampicillin, tetracycline, cotrimoxazole, and ceftazidime (E^R, AP^R TS^R and CAZ^R) whereas over 85% were susceptible to ciprofloxacin, ofloxacin, gentamycin, amikacin, meropenem, and nalidixic acid (CIP^S, OFX^S, GM^S, AK^S, MEM^S and NA^S) (Figure 3).

Majority of the *Shigella* isolates from HIV positive individuals with diarrhoea showed notable peaks in resistance to erythromycin, tetracycline, neomycin and doxycycline (E^R, T^R, Ne^R and DXT^R), but showed marked susceptibility to ciprofloxacin, norfloxacin, gentamycin, kanamycin, meropenem, imipenem, nalidixic acid, pipevacillin-tazo bactam, cefepime and ceftazidime (CIP^S, NOR^S, GM^S, KN^S, MEM^S, IMI^S, NA^S, PTZ^S, CPM^S, CAZ^S).

In addition to penicillin, amoxicillin, ampicillin and erythromycin, *Aeromonas* spp also showed marked resistance to chloramphenicol (Figure 4). *Aeromonas* spp from all the study cohorts were susceptible to ciprofloxacin, gentamycin, amikacin, meropenem, imipenem, naildixic acid and pipevacillin-tazo bactam (CIP^S, GM^S, AK^S, MEM^S, IMI^S, NA^S and PTZ^S).

P. shigelliodes isolates were markedly resistant to neomycin and chloramphenicol (Figure 5). Across the various study cohorts, *P. shigelliodes* was consistently sensitive to antibiotics such as ciprofloxacin, ofloxacin, amikacin, meropenem, imipenem, and cotrimoxazole (CIP^S, OFX^S, AK^S, MEM^S, IMI^S and $TS^{S\ 50\%}$)

Representative gel electrophoretic profiles of amplified products of the target genes are presented in Figures 6 to 22. Using primers for the target genes, as indicated for *Salmonella*, *Sh. dysentriae and E. coli*, electrophoretic profiles of stool and water samples for the various study cohorts were similar.

DISCUSSION

This study has revealed interesting findings concerning antimicrobial resistance among enteric bacterial pathogens isolated from HIV positive and negative patients with and without diarrhoea and household drinking water of study cohorts in rural communities in Limpopo Provin-

Table 1. Primers and cycling conditions used in the amplification of specific genes fragment for FliC-1 and FliC-2, SefA-1 and SefA-2, LT-1 and LT-2, VirA-1 and VirA-2 genes responsible for adhesion properties of *Salmonella typhimurium*, *Salmonella enteritidis*, *E. coli* and *Shigella dysenteriae*, respectively.

Target gene	Primer	Primer sequence (5'-3')	Product size	Cycling conditions	Reference
fliC	fliC-1 fliC-2	CGGTGTTGCCCAGGTTGGTAAT ACTCTTGCTGGCGGTGCGACTT	620 bp	94°C for 2 min, followed by 35 cycles of 94°C for 1 min, 62°C for 1 min, 72°C for 2.5 min, 72°C for 10 min final extension and hold 4˚C.	Kong et al., 2002; Oliviera et al., 2002
seFA	sefA-1 sefA-2	GATACTGCTGAACGTAGAAGG GCGTAAATCAGCATCTGCAGTAGC	488 bp	94°C for 2 min, followed by 35 cycles of 94°C for 1 min, 62°C for 1 min, 72°C for 2.5 min, 72°C for 10 min final extension and hold 4˚C.	Kong et al., 2002; Oliviera et al., 2002
Heat-labile toxin (*LT*)	LT a LT b	TCTCATTGTGCATACGGAGC CCATACTGATTGCCGCAAT	320 bp	95°C for 5 min, followed by 30 cycles of 95°C for 1 min, 60°C for 1 min, 72°C for 1 min, 72°C for 10 min final extension and hold at 4°C.	Matar et al., 2002
Vir A	Vir A-1 Vir A-2	CTGCATTCTGGCAATCTCTTCACATC TGATGAGCTAACTTCGTAAGCCCTCC	215 bp	94°C for 1 sec, followed by 35 cycles of 94°C for 45 sec, 65°C for 30 sec, 72°C for 30°C, 72°C for 1 sec final extension and hold at 22°C.	Villalobo and Torres, 1998

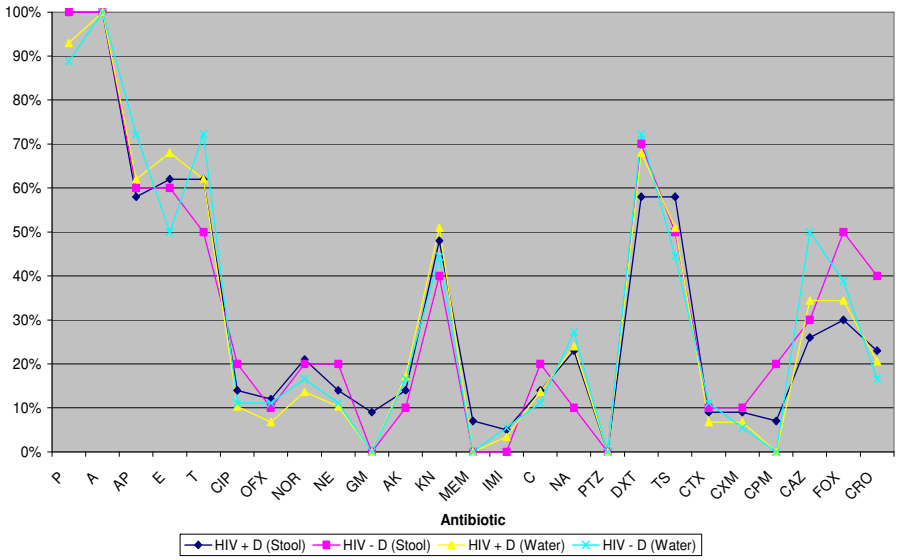

Figure 1. Antibiotic resistance profile of *Escherichia coli* isolated from stool and household drinking water sampleS of HIV positive and negative individuals with diarrhoea.

ce, South Africa. For example, virtually all the enteric bacterial pathogens isolated from HIV positive cases (70-100%) showed multiple resistances to penicillin, amoxicillin and cloxacillin. About 15-70% of enteric bacterial isolated from HIV positive individuals with diarrhoea displayed multiple resistance profiles to erythomycin, tetracycline, doxycycline, chloramphenicol, cefotaxime, cefuroxime, ceftazidime, cefoxitin, ceftriaxone, ofloxacin and norfloxacin. A similar trend in resistance patterns was also noted among HIV positive individuals without diarrhoea as well as HIV negative individuals with and without diarrhoea including enteric pathogens isolated from household drinking water of study cohorts.

This study has therefore unravelled multiple antibiotic resistance patterns of enteric bacteria isolated from clinical and environmental sources in the era of HIV/ADIS. Our results are consistent with reports of other investigators (Gassama et al., 2001; Awole et al., 2002; Obi et al., 2004). It is speculated that the widespread use of antibiotics may create pressure that encourages the selection of multi-drug resistance among bacteria (Hoge et al., 1998; Pratts et al., 2000; Sack et al., 1997).

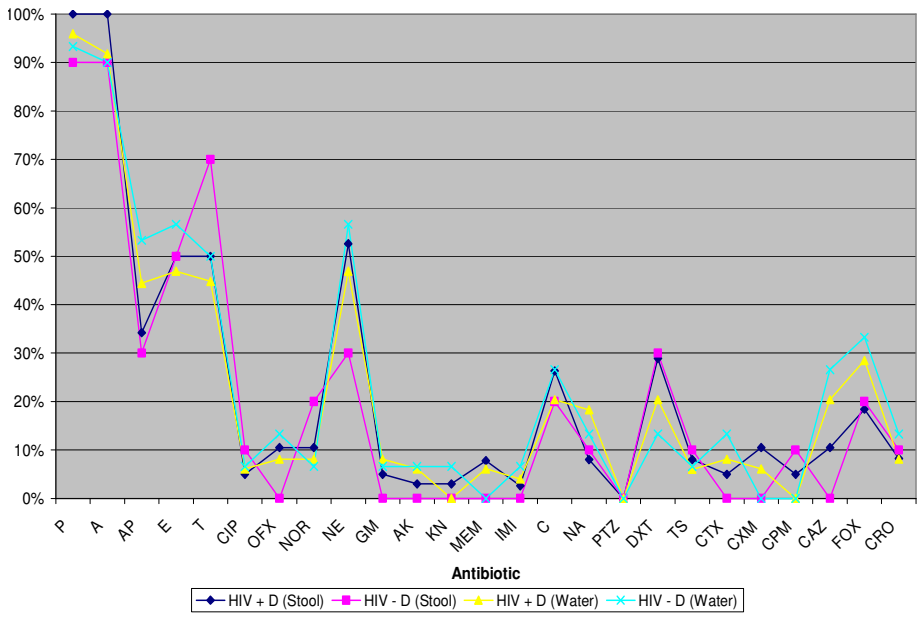

Figure 2. Antibiotic resistance profile of *salmonella* species isolated from stool and household drinking water samples of HIV positive and negative individuals with dairrhoea

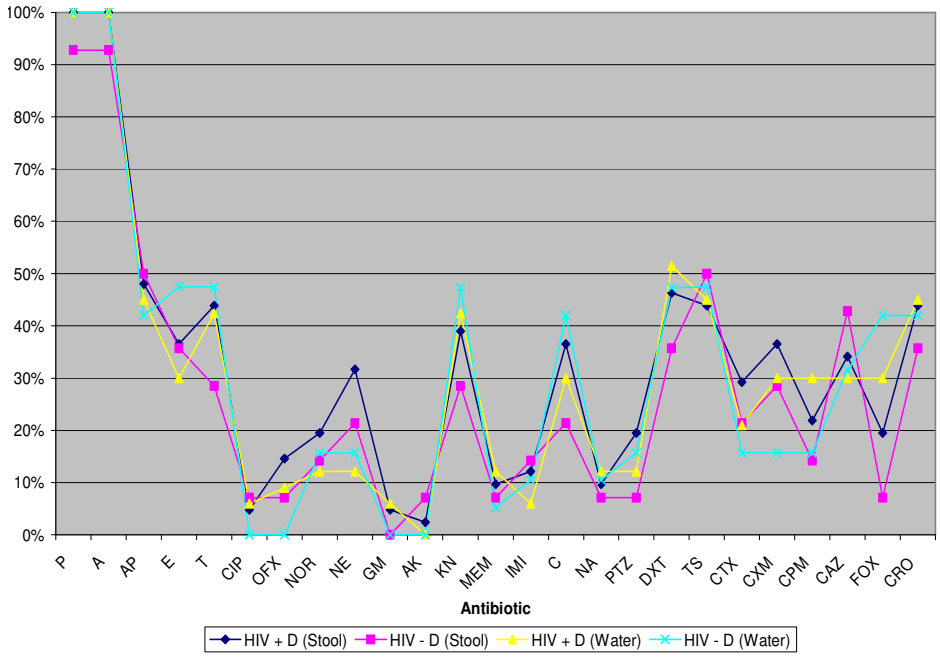

Figure 3. Antibiotic resistance profile of *campylobacter* species isolated from stool and household drinking water samples of HIV positive and negative individuals with dairrhoea

The multiple antibiotic resistances among bacterial isolates from the various study groups is frightening because such organisms can become endemic within the environment and pose serious public health threats. Multiple antibiotic resistances is becoming increasingly widespread and therefore, antimicrobial agents are becoming less and less effective. Consequently, majority of the older antibiotics (penicillin, tetracycline, ampicillin, cloxacillin) have been rendered ineffective whilst the efficacy of the newer antibiotics is being increasingly negated. The rip-ple effects of these developments connotes that for certain strains, there are few or no effective antibio-

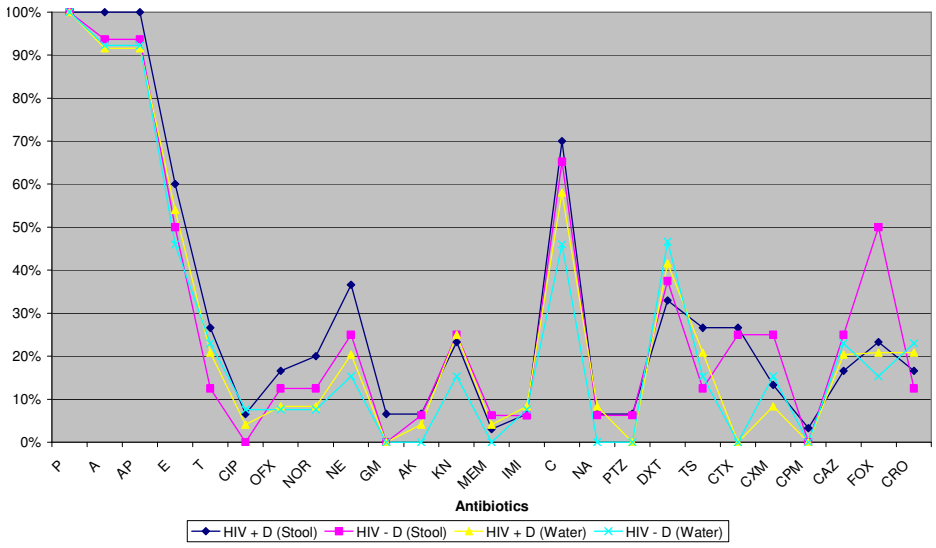

Figure 4. Antibiotic resistance profile of *aeromonas* species isolated from stool and household drinking water samples of HIV positive and negative individuals with dairrhoea

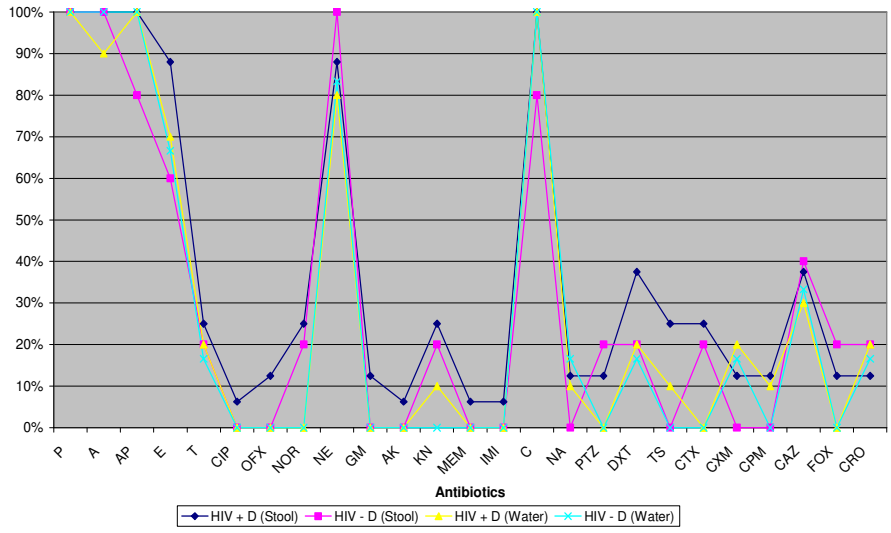

Figure 5. Antibiotic resistance profile of *plesimonas* species isolated from stool and household drinking water samples of HIV positive and negative individuals with dairrhoea

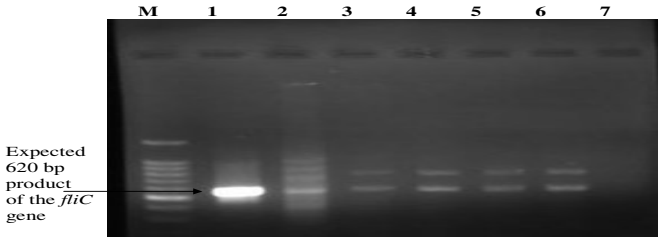

Figure 6. Sample - S1 - S5 HIV + H_2O (tested for *Salmonella typhimurium*). Lane M: Marker, Lane 1: *S. typhimurium* (Positive control), Lane 2: S1, Lane 3: S2, Lane 4: S3, Lane 5: S4, lane 6: S5, Lane 7: Negative control.

tics.

Most cases of gastroenteritis are self-limiting and in healthy individuals, antibiotic therapy may not be indicated. However, in infants, elderly people and immunocompromised patients, such as HIV/AIDS patients with enteritis, antibiotic therapy is a fundament for illness control (Pratts et al., 2000). In such cases, antibiotics become important adjuvants for therapy and may result in a dramatic decrease in stool output and decreased length of illness. Pathogenic *E. coli* showed multiple resistances including resistance to drugs of choice, such as the fluoroquinolones. The resistance to fluoroquinolones in

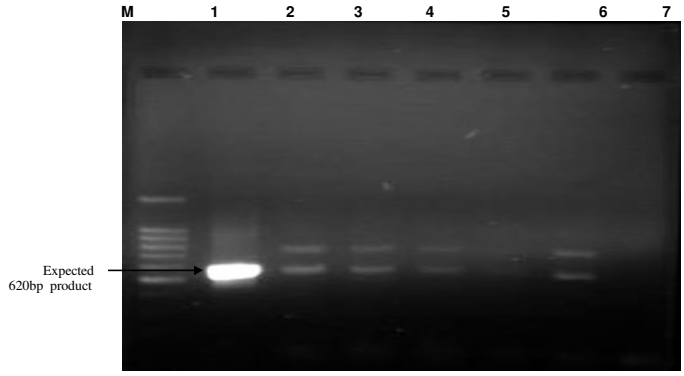

Figure 7. Sample S1 – S5 HIV– H_2O (tested for *Salmonella typhimurium*). Lane M: Marker, Lane 1: *S. typhimurium* (Positive control), Lane 2: S1, Lane 3: S2, Lane 4: S3, Lane 5: S4, lane 6: S5, Lane 7: Negative control.

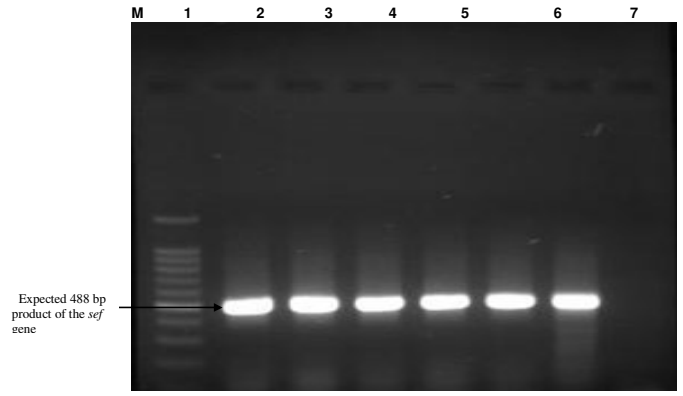

Figure 8. Sample - S1 – S5 HIV+ H_2O (tested for *Samonalla enteritidis*). Lane M: Marker, Lane 1: *S. enteritidis* (Positive control), Lane 2: S1, Lane 3: S2, Lane 4: S3, Lane 5: S4, lane 6: S5, Lane 7: Negative control.

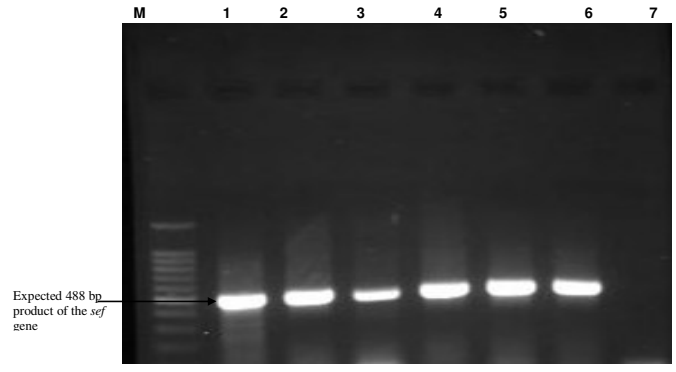

Figure 9. Sample S1 – S5 HIV- H_2O (Tested for *Salmonella enteritidis).* Lane M: Marker, Lane 1: *S. enteritidis* (Positive control), Lane 2: S1, Lane 3: S2, Lane 4: S3, Lane 5: S4, lane 6: S5, Lane 7: Negative control.

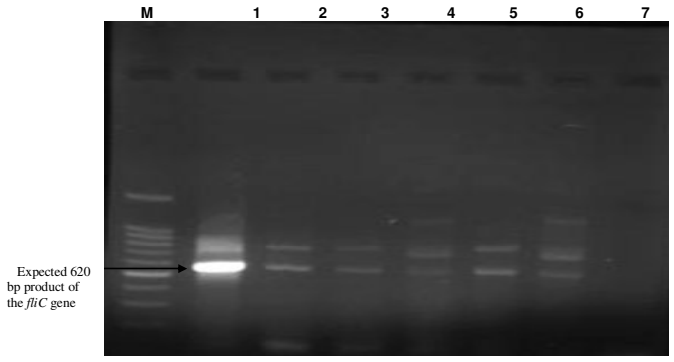

Figure 10. Sample S1 – S5 HIV+ stool (Tested for *Salmonella typhimurium).* Lane M: Marker, Lane 1: *S. typhimurium* (Positive control), Lane 2: S1, Lane 3: S2, Lane 4: S3, Lane 5: S4, lane 6: S5, Lane 7: Negative control.

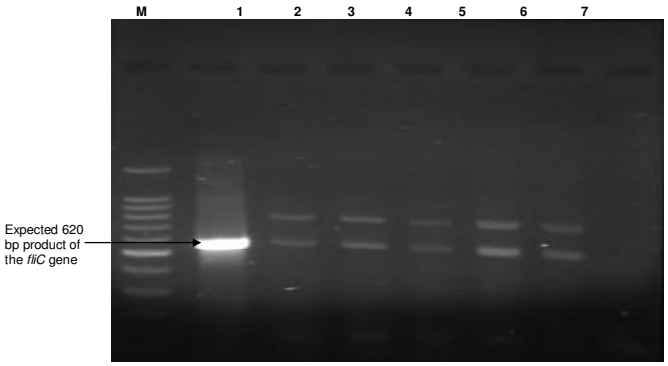

Figure 11. Sample S1 – S5 HIV- stool (Tested for *Salmonella typhimurium*). Lane M: Marker, Lane 1: *S. typhimurium* (Positive control), Lane 2: S1, Lane 3: S2, Lane 4: S3, Lane 5: S4, lane 6: S5, Lane 7: Negative control.

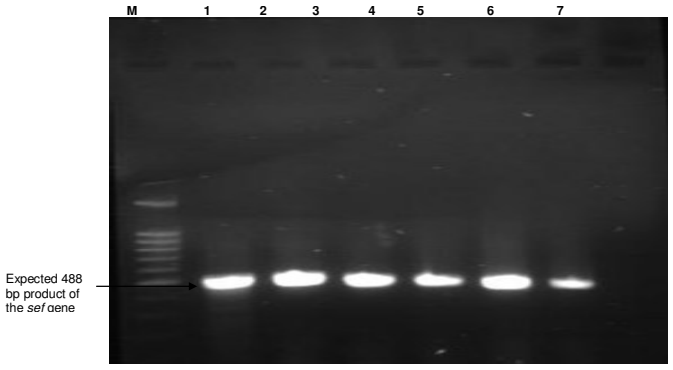

Figure 12. Sample- S1 – S5 HIV+ stool (tested for *Salmonella enteritidis).* Lane M: Marker, Lane 1: *S. enteritidis* (Positive control), Lane 2: S1, Lane 3: S2, Lane 4: S3, Lane 5: S4, lane 6: S5, Lane 7: Negative control.

E. coli is mainly associated with mutation in the chromosonal genes for DNA gyrase (gyrA) or topoisomerase IV (topo IV), and these are usually targets of action by the quinolone class (Hooper, 2000; Vila et al., 2000). Although resistance to nalidixic acid, ciprofloxacin and chlora-

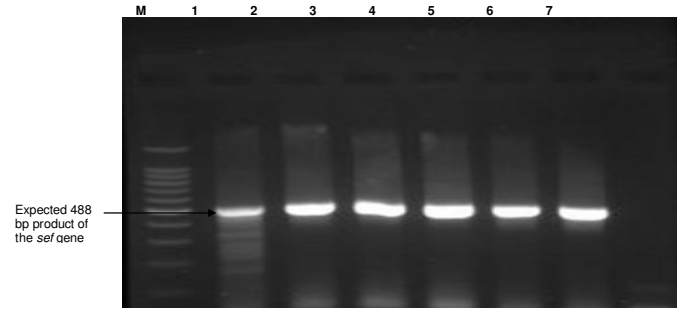

Figure 13. Sample S1 – S5 HIV- stool (Tested for *Salmonella enteritidis).* Lane M: Marker, Lane 1: *S. enteritidis* (Positive control), Lane 2: S1, Lane 3: S2, Lane 4: S3, Lane 5: S4, Lane 6: S5, Lane 7: Negative control.

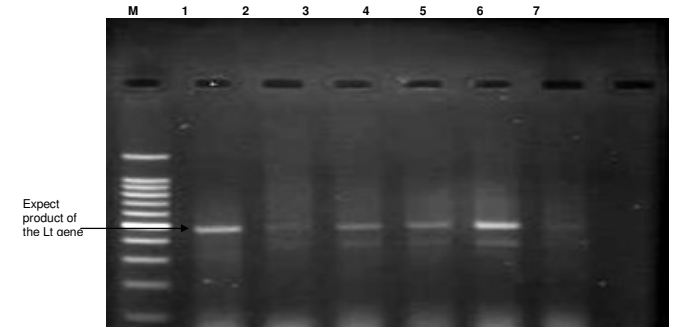

Figure 14. Sample 1 – 5 HIV+ H_2O (Tested for *Escherichia coli* strain 20). Lane M: Marker, Lane 1: *Esc20* (Positive control), Lane 2: 1, Lane 3: 2, Lane 4: 3, Lane 5: 4, lane 6: 5, Lane 7: Negative control.

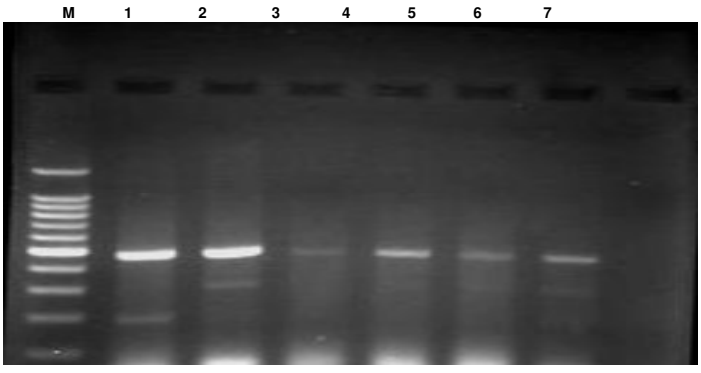

Figure 15. Sample 1 – 5 HIV- H_2O (Tested for *Escherichia coli* strain 20). Lane M: Marker, Lane 1: *Esc20* (Positive control), Lane 2: 1, Lane 3: 2, Lane 4: 3, Lane 5: 4, lane 6: 5, Lane 7: Negative control.

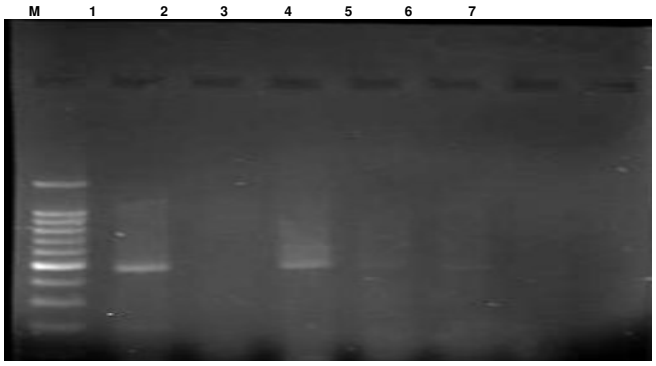

Figure 16. Sample 1 – 5 HIV+ Stool (Tested for *Escherichia coli* strain 20). Lane M: Marker, Lane 1: *Esc20* (Positive control), Lane 2: 1, Lane 3: 2, Lane 4: 3, Lane 5: 4, lane 6: 5, Lane 7: Negative control.

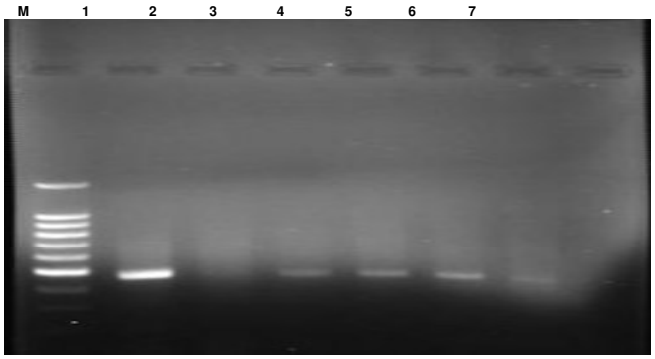

Figure 17 Sample 1 – 5 HIV- Stool (Tested for *Escherichia coli* strain 20). Lane M: Marker, Lane 1: *Esc20* (Positive control), Lane 2: 1, Lane 3: 2, Lane 4: 3, Lane 5: 4, lane 6: 5, Lane 7: Negative control.

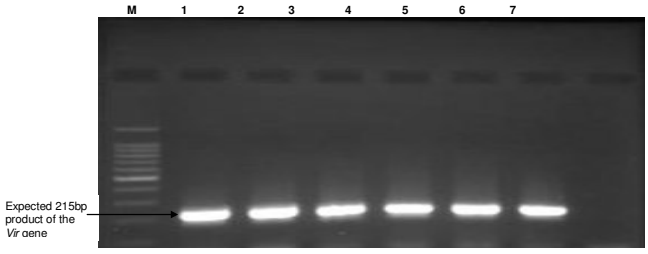

Figure 18. Sample - SD1 – SD5 HIV+ H_2O. Lane M: Marker, Lane 1: *Shigella dysenteriae* (Positive control), Lane 2: SD1, Lane 3: SD2, Lane 4: SD3, Lane 5: SD4, lane 6: SD5, Lane 7: Negative control.

mphenicol was reportedly low (2 - 4%) among strains of *E. coli* (Isenbarger et al., 2002), this study noted an increased resistance profiles in most of the study cohorts.

Resistance may be due to mutations in the genes encoding ribosomal proteins but has also been reported-ly associated with decreased permeability of the cell envelop in enteric bacteria, including plasmid mediated mechanisms (Isenbarger et al., 2002). Cross-resistance due to decreased permeability or other factors have been noted among antibiotics and it is thought that a common plasmid-mediated mechanism may increase the likelihood of horizontal spread.

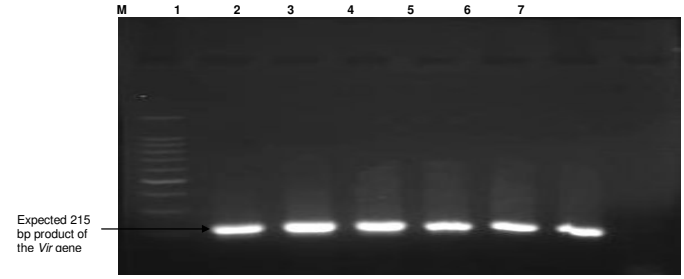

Figure 19. Sample SD1 – SD5 HIV- H_2O. Lane M: Marker, Lane 1: *Shigella dysenteriae* (Positive control), Lane 2: SD1, Lane 3: SD2, Lane 4: SD3, Lane 5: SD4, lane 6: SD5, Lane 7: Negative control.

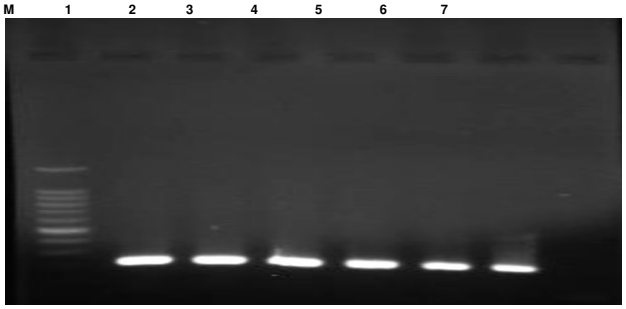

Figure 20. Sample SD1 – SD5 HIV- Stool. Lane M: Marker, Lane 1: *Shigella dysenteriae* (Positive control), Lane 2: SD1, Lane 3: SD2, Lane 4: SD3, Lane 5: SD4, lane 6: SD5, Lane 7: Negative control.

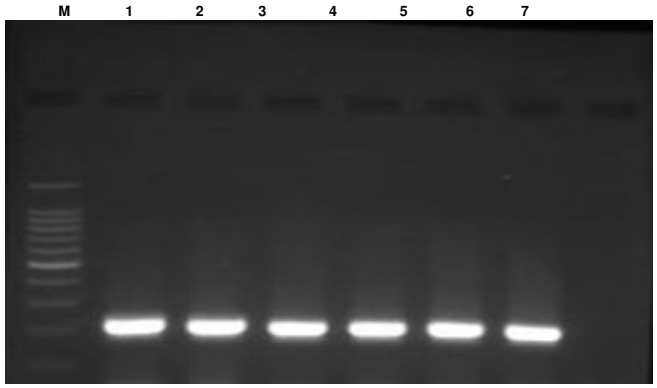

Figure 21. Sample SD1 – SD5 HIV+ Stool. Lane M: Marker, Lane 1: *Shigella dysenteraie* (Positive control), Lane 2: SD1, Lane 3: SD2, Lane 4: SD3, Lane 5: SD4, lane 6: SD5, Lane 7: Negative control.

Among *Campylobacters,* multiple antibiotic resistances were also noted. Erythromycin and gentamicin are usually recommended in bacteremic patients and fluoroquinolones are also indicated in the treatment of Campylobacteriosis. In some countries (Thailand and Spain) with a high frequency of resistance to fluoroquinolones, the recommendation is that when antibiotic therapy for Campylobacteriosis is indicated, the drug of choice should be erythromycin. In this study, about (30 - 50%) of *Campylobacter* isolates from all the study cohorts were resistant to erythromycin whereas about (10 - 18%) were resistant to norfloxacin and ofloxacin. In 1984, 82% of *Campylobacter* isolates from Lagos, Nigeria were sensitive to erythromycin; 10 years later, only 20.8% were sensitive (Coker and Adefeso, 1994). In Thailand, ciprofloxacin resistance among *Campylobacter* species increased from zero before 1991 to 84% in 1995 and recent data have indicated an increase in resistance to quinolones (Hoge et al., 1998; Nachamkin et al., 2000; Steinbrukner et al*.,* 2001). Some risk factors involved in the acquisition of fluoroquinolone resistance among *Campylobacter* species include the extensive use of quinolones in veterinary medicine. This includes the use of enrofloxacin for chicken farming in the first and third weeks of life to reduce vaccination problems and to combat respiratory problems due to *E. coli* (Piddok, 1995). Majority of *Campylobacter* isolates (over 90%) in this study were sensitive to gentamicin, amikacin, meropenem and ciprofloxacin.

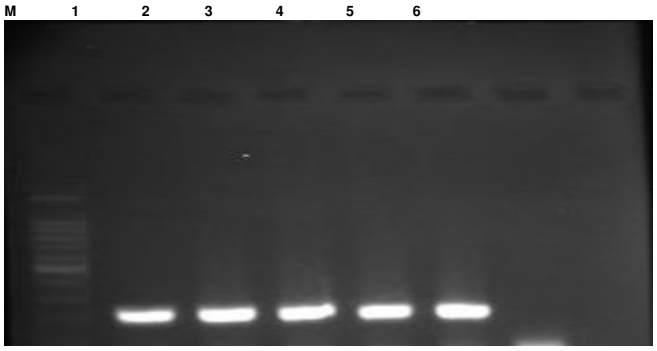

Figure 22. Sample SD1 – SD5 HIV+ H_2O. Lane M: Marker, Lane 1: *Shigella dysenteriae* (Positive control), Lane 2: SD6 HIV+ H2O, Lane 3: SD6 HIV- Stool, Lane 4: SD6 HIV+ Stool, Lane 5: SD6 HIV- H_2O, Lane 6: Negative control. SD : Shigella dysenteriae ; HIV+ : HIV positive ; H_2O : water

Salmonella and *Shigella* species also showed resistance to widely used and inexpensive drugs such as tetracycline, ampicillin, cotrimoxazole, nalidixic acid and chloramphenicol, some of which had been used as first line antibiotics in many parts of the world. Although resistance among *Salmonella* and *Shigella* species to quinolones and nalidlxlc acid was low in this study (0 - 10%), a study in Barcelona revealed that 56 *Shigella* isolates from patients with enteritis between 1995 and 1998 showed no resistance to fluoroquinolones (Pratts et al*.,* 2000) whereas nalidixic acid resistance was found in 1 - 2% of *Shigella* species in Thailand (Hoge et al., 1998). It should be noted that epidemologic investigation have revealed that the use of antimicrobial agents in livestock is the major cause of the emergence and spread of

antibiotic resistant strains of non-typhiodal salmonellae (Villar et al., 1999).

Aeromonas species and *P. shigelloides* were markedly resistant to penicillin, ampicillin and cloxacillin but were susceptible to gentamicin, amikacin, meropenem, ciprofloxacin, piperacillin-tazobactam, ciprofloxacin, norfloxacin and nalidixic acid. These findings are consistent with previous reports (Obi et al., 1998; Carcamo et al*.,* 2005; Sinha et al*.,* 2005). Furthermore, our data suggest that ampicillin, tetracycline, cotrimoxazole, chloramphenicol may not be appropriate in the emperic treatment of diarrhoea or dysentery in the study locales.

The multi-resistance documented could reflect overall resistance among human isolates and this is consistent with a previous finding (Isenbarger et al., 2002). The problem of antibiotic resistance in bacterial enteropathogens typifies the growing concern among health care workers on the continued effectiveness of antibiotics in the emperic management of infections. At this juncture, it is critical to recall that the essence of monitoring antibiotic resistance profiles among enteric pathogens is to provide updated data for clinicians' in order to facilitate the use of appropriate and more effective treatment regimens. In order to curb the problem of resistance, indiscriminate use of antibiotics and over the counter sales of antibiotics should be avoided. In addition, antibiotics should mainly be given for cases of severe diarrhoeal episodes, such as bloody diarrhoea and cholera-like illness and be avoided for acute non-cholera watery diarrhoea. The search for alternative remedies concurrent with the quest for effective enteric vaccines should be paramount.

Although antibiograms may be used as epidemiological markers in terms of classifying organisms belonging to the same antibiogram clusters, the method is not as reliable as molecular methods. Several DNA molecular markers abound for use in epidemiology, for rapid differentiation of species and definition of strain relatedness or similarity from clinical and environmental samples (Babalola, 2003). Such molecular techniques involve DNA amplification fingerprinting (DAF), Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphisms (RFLPs), Amplified Fragment Length Polymorphisms (AFLPs) and Polymerase Chain Reaction (PCR) (Theron et al., 2001; Daly et al., 2000; Babalola, 2003; Vos et al., 1995).

In this preliminary study, PCR technique was employed to unravel some strain identification and relatedness of enteric bacterial pathogens isolated from stool samples of HIV positive and negative individuals and household drinking water of the same study cohorts. PCR has been described as a reliable and rapid method of detecting pathogens from clinical and environmental samples since it relies on the *in vitro* amplification of a DNA fragment and reveals a profound level of specificity (Rompre et al., 2002).

In the study involving the use of PCR technique, targeted species specific genes of *S. enteritidis*, *S. typhimurium, E. coli, Sh. dysenteriae* showed that the *Salmonella* and *E. coli* isolates from stool samples of HIV positive and HIV negative individuals with and without diarrhoea were also present in the household drinking water of the same study cohorts. This profile of results indicated that drinking water may have been the sources of the organisms in stool samples and lays credence to the possible linkage between enteric bacterial pathogens isolated from water and stool samples. Furthermore, by showing that those primers were able to amplify the genes in both clinical and environmental isolates, the link between the virulence was established. Although the linkage was demonstrated for enteropathogens from HIV positive and negative individuals with and without diarrhoea and the household drinking water of the study cohorts, water quality is a more critical factor for HIV positive cases. HIV positive individuals are immunocompromised and are thus more susceptible to even low grade pathogens than HIV negative individuals. The presence of enteropathogens and their multiple antibiotic resistance profiles are bound to have a more profound effect on the HIV /AIDS epidemic. For example, in South Africa, diarrhoea accounts for an annual estimated deaths of about 50,000, 3 million cases of illnesses and treatment cost of about R3.4 billion rands (Pegram et al., 1998; Mackintosh and Calvin, 2002). This will indeed complicate the gloomy picture of the HIV/AIDS epidermic, which has also caused great devastation in terms of morbidity and mortality.

In addition to diarrhoea, isolated bacterial enteropathogens are incriminated in other diseases. For example *E. coli* may cause neonatal meningitis, cystitis, pyelonephritis whereas *C. jejuni* is incriminated in the aetiology of Guillan Barre syndrome (GBS). GBS is an antoimmune disorder of the peripheral nervous system, which is characterised by acute flaccid paralysis (Coker et al., 2002). *C. jejuni* infection is the most frequently identified infection that preceeds GBS (Nachamkin et al., 1998). Such cases have also been reported in South Africa (Lastovica et al., 1997). *Aeromonas* species and *P. shigelloides* may cause septicaemia in immunocompromised patients, many of them with a fatal outcome .Other extra -intestinal infections include ocular infections, migratory polyarthritis, cholecystitis and cellulitis, a wound infection marked by acute inflammation of subcutaneous tissues with redness and induration (Podila and Sarima, 2002, Ulla-Britt et al., 2003). Myonecrosis and ecthyma, the two less commonly seen types of *Aeromonas* infections, are typically found in immunocompromised patients such as HIV/AIDS patients. Myonecrosis or bullous lessions is marked by liquefaction of the muscles with blackening of the tissue, which may be gangrenous with gas formation. These immunocompromised patients require aggressive antimicrobial therapy and debridement; those who fail to respond to these measures may require amputation (Janda, 1998).

The second type, ecthyma gangrenosum, is a cutaneous necrotic or gangrenous pustle, occuring secondary to sepsis and is usually fatal (Podila and Sarma, 2002). The untoward consequences of antibiotic resistance among these pathogens cannot be underestimated. The demonstrated correlation of enteric bacterial pathogens from HIV positive individuals and their household drinking water by polymerase chain reaction unravelled the impact of water quality on HIV/AIDS and warrants some risk assessment studies. Extensive studies on molecular epidemiology of isolates from water and stool samples by the use of restriction fragment length polymorphism or sequencing and generation of dendograms or phylogenetic analysis are warranted.

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