MOLECULAR RELATEDNESS OF ENTERIC PATHOGENS ISOLATED FROM WATER SOURCES AND HIV/AIDS PATIENTS WITH DIARRHOEA IN RURAL COMMUNITIES IN THE LIMPOPO AND EASTERN CAPE PROVINCES

Report to the
Water Research Commission

by

CL Obi, J Ramalivhana, B Onabolu, MNB Momba, JO Igumbor, EL Janse van Rensburg, M Lukoto, E Green, S Ndou, PO Bessong and TB Mulaudzi

on behalf of

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EXECUTIVE SUMMARY

BACKGROUND TO THE STUDY AND PROBLEM STATEMENT

Water is an essential resource, and potable water supply remains a fundamental incentive for socio-economic development of communities. The significance of safe water supply is judged as essential and endorsed as one of the eight principal components of Primary Health Care. However, a marked inaccessibility to adequate supply of potable water still exists in most South African rural communities. These rural communities are poverty- stricken and, for their daily needs, rely mainly on river, stream, well and pond water sources, which are usually untreated. Consequently, residents are exposed to water-borne or diarrhoeal diseases and their complications. The HIV and AIDS epidemic further compounds this problem. In South Africa, the epidemic has almost reached crisis point. The statistics are staggering: 1 700 new cases of HIV infection each day, seroprevalence of about 30% among antenatal clinic attendees, 6-8 million people infected and a bourgeoning number of HIV/AIDS orphans who may drop out of school.

Water services are limited in communities with high prevalence of HIV/AIDS. Immune systems of HIV positive individuals are prone to a wider range of common illnesses and diseases than individuals whose immune systems are not compromised by HIV/AIDS. HIV-infected individuals therefore have greater requirements for potable water than un-infected individuals. The most affected are the poor, who represent the fastest growing sector of HIV/AIDS afflicted and are very likely to suffer from diarrhoeal diseases caused by poor microbial quality.

One of the hallmarks of HIV/AIDS is diarrhoea and about 90% of HIV/AIDS patients in Africa suffer from chronic diarrhoea. The substantial degree of morbidity and mortality due to diarrhoeal diseases in developing countries is thus compounded by the HIV/AIDS epidemic. Diarrhoeagenic enteropathogens include bacteria such as *Campylobacter, Salmonella, Shigella, Vibrio, Escherichia coli, Aeromonas* and *Plesiomonas.* These pathogens are water borne and their association with HIV/AIDS typifies a link which implicates water quality in some clinical manifestations of HIV/AIDS.

In addition, high infection burdens of diarrhoeagenic bacteria have been reported in developing countries, especially in communities with high HIV and AIDS prevalence, poor sanitation and inadequate water treatment. Poor microbial water quality and breast-feeding is a dilemma of mothers living with HIV/AIDS. HIV positive mothers without access to water of safe microbial quality are at a crossroad as to whether to pass on the infection to their babies or deny them breast milk. Unsafe water used in infant feed increases the risk of diarrhoeal diseases and deaths in infants. Safe water is also needed for HIV medications. Antiretrovirals (ARVs) or treatment for opportunistic infections in HIV/AIDS requires the use of safe water. Several links therefore exist between HIV/AIDS and water quality.

One of the effective ways of managing bacterial diarrhoea, particularly in immuno-compromised individuals is the use of antibiotics. However, antibiotic resistance and recurrent infections have been observed among enteric bacterial pathogens. Antibiotic susceptibility profiles of micro-organisms have been documented to vary from country to country, province to province, town to town and even from hospital to hospital in the same area as well as between public and private health care facilities in the same town

The Limpopo Province is predominantly rural and ranks as one of the poorest provinces in South Africa, with estimated HIV prevalence rates of 15.6 to 23.6% in different districts. In spite of the linkage between HIV/AIDS and water quality, no studies on the comparison of relatedness of enteric bacteria, isolated from household drinking water and from HIV/AIDS patients as well as their antimicrobial sensitivity profiles in rural communities in the Limpopo Province, could be found in the literature. There is also a dearth of knowledge by rural residents on the intricate link between water quality, diarrhoea and HIV/AIDS.

This study has unravelled the linkages, scope, frequencies and antibiograms of enteric bacteria isolated from HIV positive and -negative individuals with and without diarrhoea and their household drinking water.

Project Objectives

- To isolate and identify bacterial enteropathogens from stool samples of HIV positive individuals with and without diarrhoea and controls as well as from household drinking water of the respective study cohorts in rural communities in the Limpopo Province.
- To establish any epidemiologic linkage between bacterial enteropathogens from HIV/AIDS patients with diarrhoea and their household drinking water in rural communities in the Limpopo Province.
- To determine the antimicrobial susceptibility profiles of enteric isolates from water in comparison with diarrhoeagenic pathogens from HIV/AIDS patients in order to guide clinicians on the empiric treatment of diarrhoea cases requiring antibiotic therapy in HIV/AIDS patients.

Accomplishment of Objectives

The objectives mentioned above have all been realised. The scope and frequencies of bacterial enteric pathogens from HIV positive and HIV negative individuals with and without diarrhoea and their household drinking water in rural communities of Limpopo Province were determined. Bacterial enteropathogens isolated , in varying frequencies, from the different study groups, included *Salmonella, Shigella, Campylobater, Aeromonas, Escherichia coli*, and *Plesiomonas shigelloides*. The antimicrobial susceptibility profiles of enteric bacterial pathogens isolated from the different study cohorts and their household drinking water were also established.

The use of polymerase chain reaction was employed in establishing correlation of bacterial isolates from stool samples of HIV positive and negative individuals with and without diarrhoea and their respective household drinking water. Accomplishment in terms of capacity building included involvement and training of several postgraduate students.

MATERIALS AND METHODS

Areas for the collection of samples were stratified according to the six districts in Limpopo province. Three districts were selected for the study, based on familiarity with the area, HIV

prevalence and other parameters. The selected districts consisted of Vhembe, Waterberg and Capricorn.

Stool and water samples were collected from the study cohorts and transported for microbiological analysis to the base laboratory, Department of Microbiology, University of Venda.

The scope and frequencies, antibiograms and the correlation of the enteric bacterial pathogens isolated from clinical and environmental samples of the various study groups were ascertained over a period of five months.

The presence of enteric bacterial pathogens such as *Salmonella, Shigella, Campylobater, Aeromonas, Escherichia coli* and *Plesiomonas shigelloides* was ascertained using standard microbiological methods. Antibiogram profiles of isolates from water and HIV positive patients were determined using the Kirby-Bauer disc diffusion method. Screening for HIV sero-status was preformed using the OraQuick HIV-1 and HIV-2 (Ora Sure Technology, USA) test kit as described by the manufacturers and as previously reported. Correlation of enteric bacterial pathogens isolated from stools samples of HIV positive and HIV negative individuals with and without diarrhoea and their household drinking water were established, using the polymerase chain reaction.

SUMMARY OF MAJOR FINDINGS

Scope and Frequency of Enteric Bacterial Pathogen Isolated from HIV/AIDS Patients and their Household Drinking Water in Limpopo Province

Stool Samples

- Age and sex distributions of study participants showed that 126 (38%) and 204 (62%) of the HIV positive individuals were males and females respectively.
- HIV infection was predominantly in the age group 21-30 (31.5%) followed by the 11-20 and 31-40 years age groups.

- For the HIV negative individuals, 69 (43%) and 91 (57%) of the 160 HIV negative study group were males and females respectively.
- Higher frequencies of enteric bacterial pathogens isolated from HIV positive individuals with diarrhea when compared to those without diarrhea.
- The same types of enteric bacterial pathogens were also isolated from HIV negative individuals with and without diarrhoea but at lower frequencies.

Water Samples

- Profiles of enteric pathogens from household drinking water of HIV positive study cohorts 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 their respective study cohorts.
- 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.

Antibiotic Resistance Profiles and Relatedness of Enteric Bacterial Pathogens Isolated From HIV/AIDS Patients and their Household Drinking Water in Limpopo Province

- 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 and amoxicillin.
- Conversely almost all the organisms were sensitive to ciprofloxacin, gentamycin, meropenem and imipenem.
- Relative to the other organisms, more of the *E. coli isolated* showed multiple antibiotic resistance to penicillin, amoxicillin, ampicillin, erythromycin, tetracycline, doxycycline and cotrimoxazole.
- Over 30% of the *Campylobacter* isolates were resistant to erythromycin, ampicillin, tetracycline, cotrimoxazole, and ceftazidime whereas over 85% of them were susceptible to ciprofloxacin, ofloxacin, gentamycin, amikacin, meropenem, and nalidixic acid.

SUMMARY OF CONCLUSIONS REACHED

- The high rate of isolation of various enteric pathogens from HIV positive individuals with diarrhoea strongly incriminated them in the aetiology of diarrhoeal diseases in HIV/AIDS patients.
- Age and sex distributions of HIV seropositive individuals showed prevalence of HIV seropositivity in all age groups studies and with higher rates among females than males.
- The presence of enteric bacterial pathogens and the high rates of multiple antibiotic resistance of isolates from stool and water samples could be a potential public health threat.
- The study also revealed the usefulness of some antibiotics such as meropenem, imipenem, gentamicin, ciprofloxacin, piperacillin-tazobactam, amikacin and nalidixic acid in the empiric management of diarrhoeal cases.
- The demonstrated correlation of enteric bacterial pathogens from HIV positive individuals and their household drinking water unraveled the impact of water quality on HIV/AIDS and warrant some risk assessment studies. The most prevalent enteropathogens were *Salmonella*, *Campylobacte*, *E. coli and Aeromonas* species.

RECOMMENDATIONS FOR INTERVENTION STUDIES

- Studies on home-based care providers to educate them on sanitation, hygiene and water quality and how these impinge on the HIV/AIDS epidemic.
- Integration and effective coordination of activities of support groups, home-based care providers, NGO's, CBO's, people living with HIV/AIDS (PLWHA) and environmental health workers in order to foreground and address issues related to water quality and HIV/AIDS.
- Periodic monitoring of the scope, frequencies and antibiograms of enteric bacterial pathogens isolated from water sources and HIV/AIDS patients in order to unravel new trends and update known profiles.
- Interventions should also include strategies to ensure the sustainability of water quality in child-headed households, institutionalised orphanages and home-based care settings.

RECOMMENDATIONS FOR FUTURE RESEARCH

The following research agenda is recommended in order to strengthen the thrust of this project.

- Conduct extensive molecular epidemiological studies of bacterial enteric pathogens from stool samples of HIV/AIDS patients and water sources in order to determine the relatedness of bacterial stool pathogens from HIV/AIDS cases and water pathogens.
- Determine the array of factors that may impact on household water quality and diarrhoeal cases among HIV/AIDS households and controls.
- Determination of the virulence markers of enteric bacterial pathogens isolated from HIV/AIDS patients and water samples.

CAPACITY BUILDING

Capacity building activities targeted previously disadvantaged students. The specific activities included proposal development, scientific reporting, public health issues, culturing, isolation and identification of bacterial pathogens, HIV testing, polymerase chain reaction (PCR) method, compilation and interpretation of data, amongst others. Mr Jeffrey Ramalivhana obtained his MSc degree on the project.

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Published Paper(s)

Obi CL, Onabolu B, Momba NMB, Igumbor OJ, Van Rensburg EJ, Lukoto M, Green E and Mulaudzi TB (2006). The Interesting Cross-paths of HIV/AIDS and Water in Southern Africa with Special Reference to South Africa. *Water SA*, 32(3): 323 - 343

Papers Submitted for Publication

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Obi CL, Ramalivhana J, Onabolu B, Momba NMB, Lukoto M, Mulaudzi TB, Bessong PO, Green E, Jansen van Rensburg EL , Igumbor JO, and Ndou S. Scope and Frequency of Enteric Bacterial Pathogens Isolated from HIV/AIDS Patients and their Household Drinking Water in Limpopo Province. *Journal of Health Population and Nutrition*

Conference Presentation:

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Proposal for Archiving of Data

Data obtained will be kept at the Department of Microbiology, University of Venda, Thohoyandou, South Africa

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The Water Research Commission Reference Group Members responsible for this project:

Mrs APM Moolman	Water Research Commission (Chairperson)
Dr PO Bessong	University of Venda
Ms B Genthe	CSIR (Stellenbosch)
Dr NP Mjoli	Hlathi Development Services
Ms EJ Ncube	Rand Water
Prof SN Venter	University of Pretoria
Prof P Jagals	University of Johannesburg

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LIST OF ABBREVIATIONS

A	:	Amoxicillin
AIDS	:	Acquired Immunodeficiency Syndrome
AK	:	Amikacin
AP	:	Ampicilin
ARV	:	Anti-retroviral
C	:	Cholramphenicol
CAZ	:	Ceftazidem
CIP	:	Ciprofloxacin
CPM	:	Cefepime
CRO	:	Ceftriaxone
CTL	:	Cytotoxic T Lymphocyte
CTX	:	Cefotaxime
DOH	:	Department of Health
DXT	:	Doxycyclin
E	:	Erythromycin
EC	:	Eastern Cape
FOX	:	Cefoxitin
FS	:	Free State
GM	:	Gentamicin
GP	:	Gauteng
HDI	:	Human Development Index
HIV	:	Human Immunodeficiency Virus
HLA	:	Human Leukocyte Antigen
HSRC	:	Human Sciences Research Council
IMI	:	Imipenem
KN	:	Kanamycin
KZN	:	KwaZulu-Natal
LP	:	Limpopo
MEM	:	Meropenem

MP	:	Mpumalanga
NA	:	Nalidixic Acid
NC	:	Northern Cape
Ne	:	Neomycin
NOR	:	Norfloxacin
NW	:	North West
P	:	Penicillin G
PCR	:	Polymerase Chain Reaction
PMTCT	:	Prevention of Mother-To-Child Transmission of HIV
PTZ	:	Pipevacillin+TazoBactam
SADC	:	Southern African Development Community
Spp	:	Specie(s)
SWTPs	:	Small Water Treatment Plants
T	:	Tetracycline
TS	:	Cotrimoxazole
WC	:	Western Cape
WRC	:	Water Research Commission

CHAPTER 1
REVIEW OF HIV/AIDS AND WATER ISSUES

1.1 GENERAL INTRODUCTION

The linkage between HIV/AIDS and water may appear superficial but is indeed an in-depth association which reflects the ripple effects of the scourge of HIV and AIDS on society and the undisputed implications for the provision of water and water resource management. Water resource management and water supplies to communities operate within the context of a matured and generalized HIV and AIDS epidemic. Worldwide, HIV/AIDS and water borne disease account for a substantial degree of morbidity and mortality in different age groups. Incontrovertibly, water supply to communities in South Africa must take into account the HIV and AIDS epidemic in their design and operations. However the interaction between HIV/AIDS and water has received only a cursory attention in South Africa. In trying to unravel the intricate linkages between HIV/AIDS and water, this report first documents issues around HIV and AIDS, followed by matters of safe water supply. Thereafter, the various perspectives on the linkages and the importance of HIV and AIDS on the demand for water and sanitation services are presented.

1.2 REGIONAL AND COUNTRY HIV/AIDS PROFILE

Countries of sub-Saharan Africa are known to be the most severely affected by the HIV pandemic, by being host to over 24 million infections (UNAIDS, 2006). UNAIDS reports show that “Southern Africa is home to about 30% of people living with HIV/AIDS worldwide” even though it represents less than 2% of the worlds population (UNAIDS, 2003).

Overwhelming evidence indicate that the current trend in Southern Africa will have a marked impact on the future of infants, children, maternal mortality, life expectancy and economic growth in the area (World Bank, 2001). It has been reported that infection rates among adults in the Sothern African Development Community (SADC) ranged between 25% - 30% and this

has been attributed to the system of migrant labour, cross boarder transportation and trade (Lurie, 2000)

In South Africa, HIV infection prevalence rose from less than 1% among pregnant women attending antenatal clinics in 1990 to over 29% in 2004 (DOH, 2004). It was also estimated that the about 5.5 million South Africans were living with HIV at the end of 2004 (DOH, 2004). Diarrhoea, which is often water borne is one of the hallmarks of HIV/AIDS in developing countries. Consequently, provision of safe water is essential in HIV/AIDS care and management. For example, safe water is needed during HIV medication, and preparation of feeds.

1.3 WATER ISSUES

1.3.1 The Importance of Water and Sanitation

The significance of safe water supply to populations is endorsed as one of the eight principal components of Primary Health Care (WHO, 1978). UNDP (2005) noted that the global Millennium Development Goal of connecting all households to a reliable source of water that is reasonably protected from contamination will be an important step towards improving health and reducing the time spent collecting water. Access to safe water is a fundamental human right which the United Nations Committee on Economic, Social and Cultural Rights adopted on the 26th of November 2006. The South African Constitution states that every citizen has a right of adequate access to water that is not harmful to health (RSA, 1996). Access to improved water supply is not only a fundamental need and human right, it possesses considerable health and economic benefits and inadequate access to water supply and sanitation limits opportunities to escape poverty and exacerbates the problems of vulnerable groups, especially those affected by HIV/AIDS and other diseases (DWAF, 2003;WHO, 2004).

In South Africa, water infrastructures are well developed in urban areas and majority of the urban population utilise potable water. In rural communities, water infrastructures are either poorly developed or non existent and majority of the populace rely on water sources such as rivers and ponds for their drinking water (Obi et al., 2003 a, b). These water sources are

usually not treated, faecally contaminated and unsafe for human consumption (Obi et al., 2002; 2003 a, b; Momba et al., 2000; Muyima and Ngcakani 1998). Contaminated water sources are vehicles for the transmission of water-borne diseases such as cholera, shigellosis and Campylobacteriosis (Ashbolt 2004**).** Several bacterial enteropathogens namely *Campylobacter jejuni/coli, Salmonella, Shigella, Plesiomonas, Aeromonas, Vibrio cholerae* and *Escherichia coli* were also isolated from the river water sources (Obi et al., 2002). Isolation of water pathogens from water connotes a serious public health risk for consumers.

Approximately 3.1% of annual deaths (1.7 million) and 3.7% of the annual health burden (disability adjusted life years [DALYs]) world-wide (54.2 million) are attributable to unsafe water, sanitation and hygiene (WHO 2003).

Drinking water can also be contaminated by sewage (Ljungstrom and Castor, 1992). Sewage treatment plants are employed in the treatment of wastewater. Waste water is defined as water that carries wastes from homes, businesses and industries, for which disposal is more economical than use at the time and point of its occurrence. Wastewater components show different degrees of environmental nuisance and contamination hazard due to their chemical and microbiological characteristics (Bohdziewicz and Sroca, 2004). Outbreaks of cholera, Salmonellosis, Cryptosporidiosis and Giardiasis have been linked to the contamination of drinking water by sewage (Ljungstrom and Castor, 1992).

1.3.2 Water, Sanitation, Hygiene and Health

Poor water quality and lack of access to improved sanitation continue to pose a major threat to human health. Burden of disease analysis suggests that lack of access to safe water supply, sanitation and hygiene is the third most significant risk factor for poor health in developing countries with high mortality rates (WHO, 2002). Diarrhoea is one of the diseases associated with unsafe water supply, sanitation and hygiene and is a major cause of childhood morbidity in sub-Saharan Africa (Boadi and Kuitunen, 2005). According to the WHO/UNICEF Joint Monitoring program carried out in 2002, diarrhoeal diseases cause approximately 6,000 deaths mostly among children every day and between 1,085,000 and 2,187,000 deaths due to

diarrhoeal diseases can be attributed to the water sanitation and hygiene risk factor (UNICEF/WHO, 2002).

Water is related to disease in various ways. It serves as a route of transmission e.g. cholera; a breeding site of a stage of the lifecycle of the infective agent e.g. malaria; a harbor for the carrier of the infective agent e.g. schistosomiasis.

In recognition of the critical role water and sanitation plays in the quality of life of human populations, there is concerted effort, globally and locally to put in place various programs to eradicate the backlog of people without access to safe water and sanitation. The Millennium Development Goal's tenth target is the halving by 2015 of the proportion of people without sustainable access to safe drinking water and sanitation. The South African Government has also launched an accelerated water and sanitation delivery program with the aim of addressing the water and sanitation backlog by 2008 and 2010 respectively. In spite of the collaborative efforts to eradicate the backlogs of the people without access to water sanitation, a marked inaccessibility still exist in the country.

In view of limitations in resources and the vast number of people without adequate water and sanitation supply, there is a need in the sector to determine resource allocation and priorities for addressing the backlog. Moreover, HIV/AIDS is more prevalent in poor communities with limited water supply. There is agreement in the sector that the health and economic benefits of improved water and sanitation supply are compelling enough to support resource allocation towards eradicating the backlog of people without access to water and sanitation. This initiative is expected to stem the complication of water borne diseases in HIV/AIDS patients.

However, priorities need to be agreed upon and interventions have to be those which have the highest cost benefit. Some of the possible benefits include averted health costs, avoided lost days, and time from daily activities due to illness such as HIV/AIDS, time saving associated with having water and sanitation facilities closer to home.

1.3.3 Potential Health Impact of Improved Sanitation

There is agreement that improved sanitation has an impact on health and reduces diarrhoea morbidity by 32% (WHO, 2004). Sanitation facilities interrupt the transmission of faeco-oral disease at its most critical point. That is, the prevention of human fecal contamination of water. Studies suggest that sanitation is at least as effective in preventing disease as improved water supply. Hossain et al. (2003) in a study carried out between 1999 and 2000 to investigate the impact of sanitation and health education on intestinal parasites infection among school age children of Sherpur, Bangladesh found that intestinal parasite infection was significantly lower (P=0.05) among those who used a sanitary latrine and received health education. The association between stool disposal and diarrhoea has been investigated in a number of epidemiological studies. Indiscriminate defecation around living areas was found to be associated with an increased incidence of diarrhoea (Stanton and Clemens 1987; Han and Moe 1990). Baltazar and Solon (1989) found a 64% increase in pathogen positive diarrhoea in families where children's stools were inadequately disposed. Mertens et al. (1992) reported that unsafe stool disposal was associated with a 54% greater diarrhoea risk in Sri Lanka and deduced that if such practices were reduced from 91% to 50% of the population, then 12% of diarrhoeal episodes could be prevented. A further source of evidence for the importance of safe stool disposal is the literature on the impact of sanitation programs in developing countries. If the construction of latrines reduces diarrhoeal disease, then the effect is presumably due to the safe disposal of stools. Rahaman et al. (1985) found that post neonatal mortality rates were 68% percent lower in families with latrines than in those without. Daniels et al. (1990) suggested that the presence of a latrine may reduce diarrhoeal infection by a quarter, especially in households with good hygiene practices.

1.3.4 Integrated Water, Sanitation and Hygiene Provision

Water, sanitation and health are inseparably linked .The improvement of hygiene practices through key behaviors such as hand washing with soap, safer water handling and storage and safe disposal of children's faeces are effective means of reducing global burden of diarrhoeal diseases (Curtis et al., 2000;WHO 2004). In a study of the impact of hygiene on diarrhoea incidence in children, the prevalence of diarrhoea among children for whom unhygienic

behaviour was recorded was 2.2 times higher than in children in the hygienic group (Brooks et al., 2003; Strina, 2003).

In spite of the significant scope for improving hygiene practices at home to prevent infection and cross infection, personal and domestic hygiene practices cannot be improved without basic amenities such as water supply, waste water disposal and solid waste management. (Nath, 2003). An evaluation of the benefits of integrated water, sanitation and hygiene program (WASEP) showed that children not living in WASEP villages had a 33% higher adjusted odds ratio for having diarrhoea than children living in WASEP villages and interventions in hygiene , sanitation and water supply have been shown to control disease burden (Thompson et al., 2003).

1.3.5 Bacterial Pathogens in Water

The processes used for producing potable water are not intended to produce bacteria-free water; rather, they are concerned with removing microorganisms that are a potential health threat and making water aesthetically pleasing (Fricker, et al., 2003). The types of processes used to treat water appear to have relatively little effect on the types of bacteria that can pass through the treatment process, although disinfection with oxidizing disinfectants will tend to remove vegetative bacteria, whereas bacterial spores may well be unaffected. It is well understood that there are many factors that affect an organism's susceptibility to chlorine disinfection, and one of the major factors appears to be the degree of metabolic activity (Fricker, et al., 2003).

As our knowledge of clinical microbiology increases and epidemiological surveillance improves, the range of microorganisms that have been shown to cause waterborne diseases have grown. These include viruses such as rota viruses , norwalk viruses, calici-like viruses ; protozoans such as *Cryptosporidium*, *Entamoeba histolytica* and bacteria such as *Campylobacter , Salmonella , Shigella , Escherichia coli ,Vibrio cholerae* and *Aeromonas* .

Bacterial infections are usually treated with the use of antibiotics but these are now fraught with risks because of increasing resistance to antibiotics by bacterial pathogens (Lees, 2003).

Antibiotic resistance can be described as microbiological or clinical. Microbiological resistance exists when a bacterium possesses a resistance mechanism. Resistance mechanisms may include the production of enzymes that destroy active drug; changes in cell permeability; mutations in efflux mechanisms; alterations to structural target; bypass of metabolic pathway; or a combination of these. Clinical resistance can be explained as failure to achieve a concentration of antimicrobial that inhibits the growth of the organism in a particular tissue or fluid (Wickens and Wade, 2005).

In vitro antimicrobial activity is referred to in terms of minimum inhibitory concentration. Bacteria can be described as “sensitive”, “intermediately susceptible” or “resistant” depending on the level of drug required to inhibit growth of the organism (Lees, 2003). These phenotypes are commonly measured by the disc diffusion and E-test methods. In disc diffusion, discs containing know concentrations of antibiotic are placed on an agar plate that has been seeded with a pure culture of the bacterium under investigation. The plate is incubated overnight and examined for zones of inhibition around the antibiotic discs. Inhibited growth indicates susceptibility to the drug. The size of the zones is measured and reference tables are used to classify the organisms as sensitive, intermediately susceptible or resistant. Regarding the E-test, a strip containing an antibiotic at an increasing gradient is placed on an agar plate inoculated with bacteria, and this is incubated overnight. A zone of inhibition will appear as the organism grows around the strip. The minimum inhibitory concentration can be found where this zone intersects the graduated strip.

Bacterial resistance could be due to intrinsic factors or it can be acquired. Intrinsic resistance is the natural resistance bacterial possess to some antimicrobials (Sabath, 1972). For examples organisms may be naturally impermeable to some antibiotics due to their cell wall structure. A bacterium can acquire resistance to an antimicrobial to which it was previously sensitive. This can be due to chance mutation or the acquisition of resistance genes from other drug-resistant cells by conjugation, transformation or transduction processes.

Resistance profiles to antibiotics may different from region to region and also at different time intervals. 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. AIDS is a fatal disease characterized by numerous bacterial, viral and fungal infections, certain cancers, nutritional wasting and or deterioration of the central nervous system. Although many HIV-related infections only occur in the advanced stages of the disease, bacterial infections can occur at any time. Bacterial pneumonia and *Salmonella* water and food borne infections are particularly common problems for AIDS patients. When caused by penicillin-resistant bacteria, the mortality rate for AIDS patients is significantly higher compared to infections caused by bacteria that are fully or even partially sensitive to penicillin. HIV-infected patients are also at greater risk of developing serious water and food borne bacterial infections. For example, persistent infection with *Salmonella*, sometimes leading to septicaemia, is considered as one of the infections defining AIDS. Several other bacterial infections also plague HIV/AIDS patients. With antibiotic-resistant infections posing a particular threat, protecting the efficacy of these antibiotic drugs is critical in maintaining treatment options for these infections in persons living with HIV/AIDS.

Resistance patterns of enteric bacteria isolated from HIV/AIDS patients do not appear to be similar in different regions. In a case control study in Senegal, enteric bacterial such as *Salmonella* and *Shigella* 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% susceptibility in a hospital-based study (Awole, et al., 2002). Elsewhere, resistance to commonly used antibiotics such as ampicillin, tetracycline and erythromycin has been generally observed among enteric bacteria (Rahman, et al., 2001). Some of these enteric bacteria are discussed below:

1.3.5.1 *Aeromonas* spp.

The significance of these environmentally ubiquitous organisms in the etiology of human gastrointestinal diseases is documented (WHO 2002). While certain species, mainly *A. caviae*, *A. hydrophila* and *A. veronii* subspecies *sobria*, have been isolated from patients with diarrhoea (Ashiru et al., 1993; Obi et al., 2004), these organisms are also found frequently in

the faeces of subjects who are asymptomatic. There is no doubt that *Aeromonas* can frequently be detected in a variety of waters, and several investigators have described its detection in potable water (Havelaar et al. 1990) at levels of up to 1900 cfu/ml. However, even with modern tools for discrimination between bacterial isolates, it is difficult to determine if the types of organisms present in drinking-water are the same as those found in patients with diarrhoea. Havelaar and colleagues (Havelaar et al., 1992) used serotyping and cell wall fatty acid analysis to attempt to correlate the presence of *Aeromonas* in drinking-water and in patients with diarrhoea. The differences they encountered between the strains from the two sources led them to conclude that the strains isolated from water were essentially different from those isolated from human faeces. Similar studies (Hanninen 1994; Kirov et al., 1994) failed to confirm a link between the strains found in drinking-water and those isolated from human faeces.

Aeromonas is also frequently found in foods, and many studies have been carried out to determine its incidence. In all published studies, *Aeromonas* was found in a variety of foodstuffs with isolation rates of up to 84% and concentrations of up to 10^5/g, with ready-to-eat foods being frequently contaminated (Hanninen 1993). In human volunteer experiments, doses of 10^{10} failed to produce diarrhoea, and many subjects did not excrete the organism. However, the pathogenic traits of the inoculum were not clearly defined. However, Albert et al. (2000) examined the distribution of virulence genes such as the cytotoxic enterotoxin gene *act* (encoding Act) and the cytotonic enterotoxin genes *alt* (encoding Alt) and *ast* (encoding Ast) in aeromonad isolates from children with diarrhoea, healthy matched controls, and the environment. Their objective was to determine the association between these enterotoxin genes and to identify a new virulence property or properties that may, possibly in combination with other virulence traits, contribute to diarrhoea using polymerase chain reaction (PCR). The results showed that there were circulation of strains between humans and the environment.

1.3.5.2 *Salmonella*

An increase in the frequency and severity of non-typhoidal strains of *Salmonella* has been reported in patients with AIDS and may be an initial manifestation of AIDS (Sperber and

Schleupner, 1987). *Salmonella typhimurium* and *Salmonella enteritidis* were reported to be the most commonly isolated species from HIV/AIDS patients (Levine, et al., 1991).

In the developing world, HIV infection and AIDS are important risk factors for non-typhoidal salmonellosis and bacteraemia. Non-typhoidal Salmonellae are among the most common pathogens isolated from patients with diarrhoea. Musie (unpublished data) showed the similarity in banding patterns found in Salmonella species isolated from water sources and diarrhoeal patients using polymerase chain reaction.

1.3.5.3 *Campylobacter*

Campylobacter jejuni is the most common species isolated from HIV/AIDS infected people. The genus *Campylobacter* consist of several species including, *Campylobacter jejuni, C .coli, C. lari* and *C. upsaliensis* which are the most important species, with *C. jejuni* and *C. coli* accounting for the majority of human infections. Raw milk, untreated surface water, and poultry meat have been shown to be major sources of *C. jejuni* and *C. coli* infections (Corry and Atabay, 2001). A number of PCR protocols, targeting different genes within the genus *Campylobacter,* or specifically within the species *C. jejuni* and *C. coli*, have been developed and applied for detecting and identifying these organisms(Corry and Atabay, 2001).

1.3.5.4 *Vibrio*

Vibrio species are known to cause diarrhoea. However, the most important causes of diarrhoea in humans are *Vibrio cholerae* and *Vibrio parahaemolyticus*. Dependable discriminatory molecular methods have been developed for epidemiological examination of *V. parahaemolyticus*. Molecular typing of clinical and environmental isolates analysed by molecular methods showed that polymorphic patterns recognized were of the major types and were closely related to each other (Wong et al., 1999).

1.3.5.5 *Escherichia coli*

Obi et al., (2004) employed the use of polymerase chain reaction (PCR) to evaluate the genetic relatedness of isolates from patients with diarrhoea and from water sources. The results

indicated that *E. coli* genes found in patients' diarrhoeal stools were similar to those isolated from water sources. It was concluded that water could be the possible source of infection.

The PCR is a rapid and reliable tool for the molecular diagnosis of pathogens. It has been utilized to identify enteric pathogens in water and stool specimens (Obi, et al., 2004). Pulse field gel electrophoresis (PFGE) was used to genetically type isolates from stool samples of patients as well as isolates from environmental samples. The vast majority of the enteric pathogens such as *E. coli* O157:H7, *Vibrio*, *Salmonella and Aeromonas* isolates from stool samples of patients and water sources were of one related type, suggesting primary contamination of water (Hilborn, et al, 2000). This actually implicates water distribution systems as the main sources of exposure in outbreaks such as the typhoid fever outbreak in Delmas, Mpumalanga.

1.4 LINKAGES BETWEEN HIV/AIDS AND WATER, SANITATION AND HYGIENE

The HIV/AIDS epidemic has caused substantial morbidity and mortality in different age groups world wide. Water borne diseases, in turn, are responsible for a vast array of debilitation and death in different age groups world wide, particularly in rural communities.

At a superficial observation, issues around HIV/AIDS and water may appear not to be connected because HIV is transmitted sexually, whereas water is a renewable natural resource, a sine-qua-non for life. On a close analysis, the intricate web of relationship existing between water and HIV/AIDS cannot be underestimated. Indeed one of the easily discernable consequences of HIV/AIDS is the long term implications for effective water resource management and the provision of wholesome water supply to communities (Aston and Ramasar, 2002)

Consequently, an exploration of the linkages and perspectives between HIV and AIDS and water will enhance development of integrated approaches. Several aspects of the linkages between HIV/AIDS and water exist and are as follows:

1.4.1 The Health Aspects

The water supply sector is tasked with the responsibility of improving the heath of the populace by providing access to safe water. Water services are limited in communities with high prevalence of HIV/AIDS. These services, when adequate, may prevent fecal oral transmission of diarrhoea causing agents. People with compromised immune systems are prone to these diseases and without potable water supply may suffer from persistent diarrhoea. Immune systems of HIV positive individuals are prone to a wider range of common illnesses and diseases than individuals whose immune systems are not compromised by HIV/AIDS. HIV individuals therefore have greater requirement for potable water than un-infected individuals (Ashton, 2000). The most affected are the poor, who represent the fastest growing sector of HIV/AIDS afflicted and are very likely to suffer from diarrhoeal diseases, caused by unsafe water. While it is true that unsafe water is a public health risk, there is evidence that providing safe water has a positive impact on individuals living with HIV/AIDS. Improving water quality will also lead to a decline in childhood and adult deaths as well as diarrhoeal diseases in people living with HIV/AIDS (UNAIDS, 2003; UNICEF, 2003)

One of the hallmarks of HIV/AIDS is diarrhoea (Prasad, et all., 2000) and about 90% of HIV/AIDS patients in Africa suffer from chronic diarrhoea (Janoff, et al., 1998). The substantial degree of morbidity and mortality due to diarrhoea diseases in developing countries is thus compounded by the HIV/AIDS epidemic (Obi, et al., 2002). Diarrhoeagenic enteropathogens include bacteria such as *Campylobacter*, *Salmonella*, *Shigella*, *Vibrio*, *Escherichia coli*, *Aeromonas* and *Plesiomonas* (Obi, et al., 2003). These pathogens are water borne and their association with HIV/AIDS typifies a link, which implicates water quality in some clinical manifestations of HIV/AIDS. Campylobacter associated diarrhoea and bacteremia occur world wide in HIV/AIDS patients (Quin, 1997; Lastovica, et al., 2001). The incidence of clinical manifestations of campylobacteriosis is higher in HIV positive than in HIV negative patients, with substantial mortality and morbidity.

In addition, high infection burdens of diarrhoeagenic bacteria have been reported in developing countries especially in communities with high HIV and AIDS prevalence, poor sanitation and inadequate water treatment (Clark, 1999).

1.4.1.1 Infant Feeding

Water quality and breast-feeding is a dilemma of mothers living with HIV/AIDS. HIV positive mothers without access to safe water are at a crossroad as to whether to pass on the infection to their babies or deny them breast milk (UNICEF, 2004). Unsafe water used in infants feed increases the risk of diarrhoeal diseases and deaths in infants (Dunne, et al., 2001).

1.4.1.2 Anti-retroviral Treatment

Safe water is also needed during HIV medications. Antiretrovirals (ARVs) or treatment for opportunistic infections in HIV/AIDS requires the use of safe water. Several links therefore exist between HIV/AIDS and water quality: In fact providing safe water to people living with HIV/AIDS reduces AIDS related mortality (Lule, et al., 2004)

However it should be noted that the mere provision of safe water and sanitation may not guarantee improvement of health except if it is complimented with hygiene promotion and appropriate hygiene behaviors (IRC, 2004). Improved water handling and sanitation practices, personal, hygiene and safe water disposal and drainage are necessary adjuncts for the reduction of water borne diseases and these factors are more exerting for people living with HIV and AIDS and family members of HIV positive people. Hygiene education should therefore focus on care givers and volunteers involved in home based care but this is not as yet commonly practiced.

1.4.2 Access to Water and Latrines

In addition to providing safe water, supply points and latrines have to be close to points of use. This will reduce long distances that residents undertake to fetch water, particularly caregivers, weak or ill individuals and also reduces vulnerability to infection with HIV because it obviates the risk of girls and women being raped while fetching water.

Moreover, the design of water systems should be unique - pumping not too laborious for the immunocompromised (HIV and AIDS patients) children and the elderly who may be saddled with the task of fetching water. Consequently, improved access to water supply may result in labour saving benefits to HIV/AIDS affected households.

1.4.3 Burial Sites

The stigmatization of HIV and AIDS has made HIV infection to be shrouded in secrecy and this has precipitated 'the legacy of shame' attached to HIV infected and affected households. This has also made some family members of HIV and AIDS victims not to announce the death of relatives, with consequent burials in unofficial graveyards. Such graveyards may be sources of contamination of ground water supply. While this dictum is not suggestive of potential transmission of HIV to ground water, it may be associated with elevated nutrient levels and bacterial contamination from graves permeating ground water systems (Engelbrecht, 1998).

1.4.4 Poverty Alleviation Aspects

HIV and AIDS is an impediment to achieving global poverty reduction targets and development goals. Poverty enhances vulnerability to HIV infection and AIDS exacerbates poverty. Although HIV and AIDS affect the rich and the poor, the impact is higher among poor households, where female vulnerability is heightened because prostitution may be one of several coping strategies.

Poor access to basic amenities such as healthcare, water and sanitation which are also indices of poverty may increase vulnerability to HIV infection. In South Africa, the tension between the allocation of financial resources to combat HIV and AIDS and to solve low coverage of safe water supply and sanitation in rural poor communities must be cautiously mediated because water is of basic and strategic imperative for poor people and for HIV and AIDS infected people (IRC 2004). Adequate water supply has been reported to save labour and energy, reduce health expenditure, generate nutritional value and with other ripple effects on sustainable livelihood.

1.4.5 Gender Aspect

Gender imbalance is the critical hinge in the overlap between socio-cultural and economic impacts of HIV and AIDS. Oppressive customs and traditions may create reduced economic power for women and fan the spread of HIV. Women are affected disproportionately because of their socially defined roles such as caring for family members, partners, child bearing and rearing and other domestic responsibilities such as fetching water and transportation across

long distances. Water supply can therefore be of value to women in terms of meeting their practical needs (IRC, 2004). Safe water provision many also enhance sustainability of livelihoods

1.4.6 Community – Driven Development Aspect

IRC (2004) reported that in the water and sanitation sector, a relationship might exist between the level of community organization, empowerment and autonomy and the level of sustainability of water and sanitation interventions. Factors that are of importance in this connection are community cohesion, stable traditional leadership and respect for ethnic division. These factors also typify the ability of communities to cope with the impact of HIV and AIDS and to prevent new infection.

In a nutshell, community management in the water sector equates to community HIV and AIDS competence. Community based organization may create social cohesiveness and a good platform for awareness and education campaigns, behavioral change communication and thus reduce vulnerability to HIV and water borne diseases

1.4.7 The Human Right Aspects

Stigmatization of HIV and AIDS has made the disease a human right issue because certain indices around HIV and AIDS containment, lack of accessibility to prevention methods, treatment, care and support are associated with human right violations. These indices include poverty, inequality, racialism and sexism. HIV and AIDS infected individuals are constrained to live a dignified and free life due to violations of their rights on the basis of their HIV status. International organizations such as UNAIDS, UNICEF, UNDP, UNESCO, WHO, ILO and the World Bank are entrenching human rights principles in HIV and AIDS prevention, treatment, care and support.

The water and sanitation sector also has a human right perspective. Access to safe water and sanitation is not just regarded as a mere basic need but also a human right. The water supply and sanitation collaborative council in its Vision 21 canvassed an encompassing approach regarding water, hygiene and sanitation as a human right juxtaposed in the context of human

development, poverty reduction, environmental sustainability and integrated management of water resources (IRC, 2004).

Furthermore, “the civil society Action Programme on Water” during the World Summit for Sustainable Development asserted that secure access to sufficient safe water and sanitation to meet basic human needs, including water for small scale production to support livelihoods is indeed a human right. The focus on provision of water as a human right is tangential to policy and programmatic strategies, particularly for countries with high prevalence of HIV and AIDS because it impinges on sustainable effort and increased financial outlay to realize total coverage

1.4.8 Productive Aspect of Water

It has been reported that food security is increased by access to water and this, in turn, enables people to stay healthy. Nutrition is also improved by making softer and easier to eat food by mixing with safe water. This is particularly good for people who have mouth ulcers or oral thrush as a result of HIV/AIDS and cannot eat solid foods .Water may also be useful for income generation such as in brewing, food production and tendering of livestock (Kamminga and Wegelin-Schuringa, 2003).

Consequent upon the examination of the various aspects of the links between HIV/AIDS and water, it would be pertinent to explore the impact of the disease on the water sector.

1.5 IMPACT OF HIV AND AIDS ON DEMAND FOR WATER AND SANITATION SERVICES

The demand for water and sanitation may be affected by the pandemic because of the skepticism around predictions of mortality due to HIV and AIDS and population growth rates may impact on the planning and implementation of water supply and sanitation systems (Ashton, 2000). Population growth rates and life expectancy are on the decline because HIV reduces fertility and more children die due to HIV and AIDS. In fact it was reported that the chances that boys of 15 years of age will die of AIDS in Kenya, Zambia, South Africa and Botswana were 50%, 60%, 70% and 90% respectively (UNAIDS, 2000). This gloomy

situation will certainly lead to a decrease for water supply and sanitation (Pallet 1997; Whiteside and Sunter 2000; Ashton, 2000). Indeed failure to place water demand estimates within the context of HIV and AIDS related mortality, would lead to an overestimation of water demands by between ten and thirty percent (Ashton and Ramasar, 2000) This scenario would bequeath unanticipated consequences for the water supply sector. It could also be argued that the demand for water may increase as a consequence of an urban-rural migration, because most of the infected individuals opt to return to rural home areas during the terminal stages of the disease (UNAIDS, 2002; White and Robinson, 2000). Migration of people to urban areas in search of employment also exists.

1.5.1 Faltering Payment of Water Services

HIV and AIDS is financially disempowering as a result of loss of income due to ill health, increased medical cost and general livelihood insecurity. This places expenditure on water in competition with medical bills, school fees and other survival instincts; with a consequent return of affected households to unprotected and unsafe water sources (Ashton and Ramasar, 2001). HIV and AIDS causes ecological collapse due to the fact that the middle generation are either severely ill or dead, leading to a shift of parenting to the very young and the elderly (Laurie, 2000; Karim, 2000). In such cases child headed households and elderly relatives become common. This creates enormous social problems because the very young and elderly are unable to engage in intensive productive ventures and thus rely on government support for their livelihoods (UNAIDS, 2000; Whiteside and Sunter, 2000). The consequences are unmet needs for basic services such as water supply and sanitation, electricity, housing and education. Child headed households may also be unaware of safe water handling procedures.

Household with economically active adults may experience a shift in expenditure on water and sanitation services to health care for sick family members. The ultimate consequence is that the state will be overburdened and water supply agencies would have to cope with the huge responsibilities of providing water supply and services.

1.5.2 Erosion of Social Capital and Waning Productivity

In this context, erosion of social capital embodies loss of knowledge and skills. Social capital is fuelled by people in the productive years (20 – 50 years) of age. Coincidentally, this is also the age group most affected by HIV and AIDS (UNAIDS, 2000; Williams et al., 2000). As a result of the anticipated reduction in the profile of skilled and semi-skilled workers due HIV and AIDS, the ripple effects will include the need to recruit replacements, high rates of employee turnover, heavy demand on available workforce, increased demand for training, decreased productivity, costly delays and unsafe work practices (Morris and Cheevers, 2000; Ashton, 2000; Williams, et al., 2000).

The aforementioned effects, while not exclusively confined to the water sector, would affect operators of water treatment works and sewage treatment works. The operators have been pin pointed because they play a crucial role in the management of water resources and provision of water supply for domestic and industrial use (Ashton and Ramsar, 2000). Increased turnover of water and sewage treatment operators, to whatever degree, will attract a corresponding increase in the recruitment and training of replacement operators (Ashton, 2000). Prevarication in training will affect the performance of treatment plants and the untoward decline in portable water quality and predisposition to water borne diseases (Ashton, 2000).

HIV and AIDS has also been reported to lead to a decline in productivity as a result of frequent absenteeism due to a progressive level of ill health and ultimate decline in worker productivity (UNAIDS, 2000; Crewe, 2000; World bank, 1999). Frequent attendance of funerals by employees causes depletion in annual working days and decline in productivity (Heywood, 1999). Additional burden to employers may include cost of treatment for opportunistic infections, funeral cost and payment of benefits.

1.6 MAINSTREAMING OF HIV/AIDS IN THE WATER SECTOR

Consequent upon the relationship between water and HIV/AIDS, the water sector should therefore strategise on policy and programmatic issues in terms of empowerment of local women, people living with HIV/AIDS and their care-givers and pro-poor financing mechanisms including focus on water for health and economic benefits. Others should include

development of workplace policies, adaptation and reorganisation of workload, development of strategies for reserve staff, adjustment of performance appraisal systems to manage impact on productivity and integration of HIV/AIDS into all training activities (Kamminga and Wegelin-Schuringa, 2003). Furthermore, how HIV/AIDS will affect water and sanitation programmes such as target groups, objectives, human and financial resources or vice-versa should be foregrounded.

1.7 RESPONSES TO HIV AND AIDS IN THE WATER SECTOR

There is a paucity of data to convincingly authenticate a concerted response to the multitude of implications of HIV and AIDS in the water resource management sector in Southern Africa (Ashton and Ramsar, 2002). Some government departments dealing with water related issues have called for national and regional appraisals of the implications of the pandemic for their activities whereas others have reiterated the need for additional training programmes in the wake of increased staff turn over, decreased worker productivity and associated HIV and AIDS related cost increases.

Educational campaigns to enhance public awareness of the pandemic and implications for individuals, communities and society and counseling services for infected and affected people have been foregrounded by national departments in most Southern African countries.

In South Africa, some activities of the state are associated with the linkages between HIV and AIDS and the water sector. These activities include efforts to provide safe water and sanitation services for poor rural and urban communities. Although these activities were initially undertaken to address historical antecedents that created lopsidedness in the distribution of basic amenities in the previously disadvantaged communities, they also reduce the risk of exposure of HIV and AIDS patients to poor water quality (Ashton and Ramsar 2002). The Water Research Commission (WRC) of South Africa has also initiated research and community programmes to address issues around HIV/AIDS and water (Personal communication).

Although responses to HIV and AIDS in South African Government Departments or Parastatals appear to be fragmented and somewhat devoid of the benefits of the advantages of integration, each departmental approach holds great promises (Ashton and Ramsar, 2002) and this further underpins the need for consolidation and integration efforts. However it should be noted that there is no single ideal response or universal blueprint, as a preferred or overriding choice to combat the scourge of the century.

Political commitment at the topmost level is imperative for stemming the impact of the pandemic. Commitment encourages disclosure, reduces stigma and enhances multi-sectoral approach in collaboration and partnership with civil society and private sector (Kamminga and Wegelin-Schuringa, 2003). The advantages of commitment at the highest level is exemplified by the reduction of HIV/AIDS prevalence in Uganda, Thailand and Senegal. Government's commitment may be complimented by involving people affected by HIV/AIDS in planning and execution of developmental issues.

Managers in the water resource sector should have reliable data or forecast for the demographic spread of water demand to ensure that poor communities, devoid of the ability to pay for water services are not neglected in terms of water supply.

In order to reduce the health impact of ineffective water treatment as a result of HIV and AIDS related mortality of water treatment operators, innovative water treatment processes that do not require regular or constant supervision or management activities should be explored, developed and eventually implemented (Ashton and Ramsar, 2002). Another responsibility of the water sector is to give impetus to awareness programmes aimed at unraveling the implications of untreated water and inadequate personal and environmental hygiene practices

Although a relationship exists between poor water quality, diarrhoea and HIV/AIDS, there is a dearth of knowledge or paucity of information on the intricate link between water quality, diarrhoea and HIV/AIDS in some communities in South Africa. This article attempts to narrow such gaps of knowledge of HIV/AIDS and water quality.

1.8 SCIENTIFIC METHODS OF ESTABLISHING LINKAGES BETWEEN ENTEROPATHOGENS ISOLATED FROM STOOL SAMPLES OF HIV/AIDS PATIENTS AND WATER

Identifying the source of bacterial pathogens is an important feature in the control of infections particularly in endemic and epidemic situations.

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 or determination of a source of contamination (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) (Babalola, 2003; Vos et al., 1995). Random amplification of polymorphic DNA (RAPD) has been used extensively to study genetic diversity and epidemiological relationships of *E. coli* and other food-borne pathogens. It offers an efficient and relatively inexpensive method to characterize large numbers of *E. coli* isolates.

PCR-restriction fragment length polymorphism (PCR-RFLP) analysis of the *fliC* gene, encoding the H antigen has also been used to characterize and to study genetic diversity of motile and non-motile *E. coil* strains (Aslam et al., 2003). DNA patterns obtained with RADP and PCR-RFLP are analyzed with appropriate software. Similarities between the DNA patterns of isolates based on band positions are determined by Dice similarity coefficient, and a dendogram can be constructed using arithmetic averages to reflect similarities in the matrix. Isolates are grouped into genetic subtypes which are considered genetically related based on DNA patterns. Similar methods have been applied for fingerprinting *Campylobacter*, *Aeromonas* and *Helicobacte*r (Linton et al., 1997; Marshall et al., 1999).

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).

1.9 IMPLICATIONS OF MICROBIAL WATER QUALITY AMONG HIV/AIDS PATIENTS

Indeed water services are limited in rural communities with a high prevalence of HIV/AIDS. Such rural communities are poverty-stricken and rely on contaminated water sources for their daily needs (WHO, 1993; Obi et al., 2002). Rural residents in South Africa are therefore exposed to water- borne diseases and their complications. These diseases including Campylobacteriosis, Salmonellosis, Shigellosis, Cholera, Cryptosporidiosis, Hepatitis and Giardiasis have debilitating effects and exacerbate the relentless saga of HIV/AIDS. Consequently, it is absolutely necessary to critically examine the inter-play of factors involving HIV/AIDS and water issues. HIV infected individuals have a greater need for potable water than un-infected individuals (Ashton 2000).

The impact reaches a plateau in the premises of the poor, who represent the growing sector of people living with HIV/AIDS and are prone to diarrhoeal diseases, due to the consumption of unsafe water. Unsafe water used in the preparation of infant feed by HIV seropositive mothers increases the risk of diarrhoeal diseases and deaths of infants (Dunne et al., 2001). Unsafe water is also used during anti-retroviral medications. Diarrhoea is reportedly one of the most common and crippling complications of HIV infection, occurring in about 50% of patients in North America and in up to 100% of patients residing in developing countries (Smith et al., 1992; Mayer and Wanke, 1994). Diarrhoea has been reported to affect up to 95% of persons with HIV/AIDS in developing countries causing malabsorption, profound weight loss (Slim disease) and increased mortality (Clerinx et al., 1995). Chronic diarrhoea with slim disease in an HIV positive person is considered by the World Health Organisation (WHO) as an AIDS defining criterion (Dallabeta and Mioti, 1992) and is pathognomic of AIDS in Africa (O'keefe and Wood; 1996). Diarrhoea is a compounding factor in HIV/AIDS because it results in significant morbidity and mortality, reduced quality of life and higher health care financial implications (Lubeck et al., 1993; Wilcox, 2000). Although HIV/AIDS patients are susceptible to the same spectrum of enteric pathogens, which may cause diarrhoea in immunocompetent

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, Lubeck et al.,1993). However, some microbial agents induce diarrhoea almost exclusively in HIV infected persons. These agents include *Mycobacterium avium* complex (MAC), Cytomegalovirus (Friedman; 1990, Baker et al., 1994). Microbial aetiologies of diarrhoea include viruses such as rotaviruses, norwalic viruses, calici - like viruses; parasites such as *Cryptosporidum*, *Giardia*, *Entamoeba histolitica*; fungi such as *Candida albicans* and bacteria, the focus of this study. Enteric bacterial pathogens are responsible for most diarrhoea episodes worldwide. Despite the linkage between HIV/AIDS, diarrhoea and water, no studies which has investigated the scope and frequency, antibiograms and relatedness of enteric bacterial pathogens isolated from HIV/AIDS patients with and without diarrhoea and their household drinking water in rural communities in the Limpopo Province could be found in Literature. This report marks the first attempt to provide such epidemiologic documentation and is also expected to guide clinicians in the management of bacterial diarrhoea in HIV/AIDS.

1.10 PROJECT OBJECTIVES

- To isolate and identify bacterial enteropathogens from stool samples of HIV positive individuals with and without diarrhoea and controls as well as from household drinking water of the respective study cohorts in rural communities in the Limpopo Province.
- To establish any epidemiologic linkage between bacterial enteropathogens from HIV/AIDS patients with diarrhoea and their household drinking water in rural communities in the Limpopo Province.
- To determine the antimicrobial susceptibility profiles of enteric isolates from water in comparison with diarrhoeagenic pathogens from HIV/AIDS patients in order to guide clinicians on the empiric treatment of diarrhoea cases requiring antibiotic therapy in HIV/AIDS patients.

In line with the above objectives, this report is presented in four parts, delineated into chapters as follows:

- Review of HIV/AIDS and water issues,

- Scope and frequency of enteric bacterial pathogens isolated from HIV/AIDS patients and their household drinking water in Limpopo province,
- Antibiotic resistance profiles and relatedness of enteric bacterial pathogens isolated from HIV/AIDS patients and their household drinking water in Limpopo province and
- Conclusions and recommendations.

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CHAPTER 2

SCOPE AND FREQUENCY OF ENTERIC BACTERIAL PATHOGENS ISOLATED FROM HIV/AIDS PATIENTS AND THEIR HOUSEHOLD DRINKING WATER IN LIMPOPO PROVINCE.

2.1 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 enteroaggregative *Escherichia coli, Shigella* species and *Salmonella enterica* respectively (Gassama et al., 2001). In Ethiopia the isolation rates from HIV/AIDS patients 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 species and 10% each for *Shigella, Salmonella* and *Escherichia* coli among HIV/AIDS patients had been reported (Obi and Bessong, 2002).

Although HIV/AIDS patients are susceptible to the same spectrum of enteric pathogens, which may cause diarrhoea in immunocompetent 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, Lubeck et al., 1993). Water borne bacterial pathogens are therefore strongly associated with HIV/AIDS. Water quality is therefore critical for HIV/AIDS patients. 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 part of the study was to determine the scope and frequency of enteric bacterial pathogens isolated from HIV/AIDS patients and their household drinking water in Limpopo Province.

Specific Objectives

Specific objectives of this section of the study were to:

1. determine the prevalence of enteric bacterial pathogens across HIV positive and negative individuals with and without diarrhoea and their household drinking water,
2. determine the sex and age distribution of HIV positive individuals with diarrhoea and
3. compare the proportions of enteric pathogens isolated from stool and water samples of HIV positive and negative individuals with and without diarrhoea.

2.2 MATERIALS AND METHODS

2.2.1 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 of 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/AIDS patients provided the platform to reach out to the cohorts.

2.2.2 Study Population

The present study was conducted prospectively between August 2005 to January 2006. Information on age, sex, 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 diarrhoic 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.

2.2.3 Ethical Approval

The Health, Safety and Ethics Committee of the University of Venda, Thohoyandou, 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.

2.2.4 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).

2.2.5 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 hours 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 4-6 hours of collection (Obi et al., 2002).

2.2.6 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.

2.2.7 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 hours. 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 hours. 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., 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.

2.2.8 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.

2.3 RESULTS

2.3.1 Demographic Characteristics of Study Cohorts:

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 sex distribution of the study participants are presented in Table 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 - 30 (31.5%) and in the 11 - 20 and 31 - 40 years age bracket (Table 1)

Table 2.1: Sex and Age Strata of HIV Positive and Negative Individuals with and without Diarrhoea

	SEX		**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 (31.0%)	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%)**

2.3.2 Prevalence of Enteric Pathogens across Study Cohorts

Figure 1 shows the prevalence rates of potential bacterial agents of diarrhoea 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 bacteria pathogens was also isolated from HIV negative individuals with and without diarrhoea but at lower frequencies (P = 0.0001).

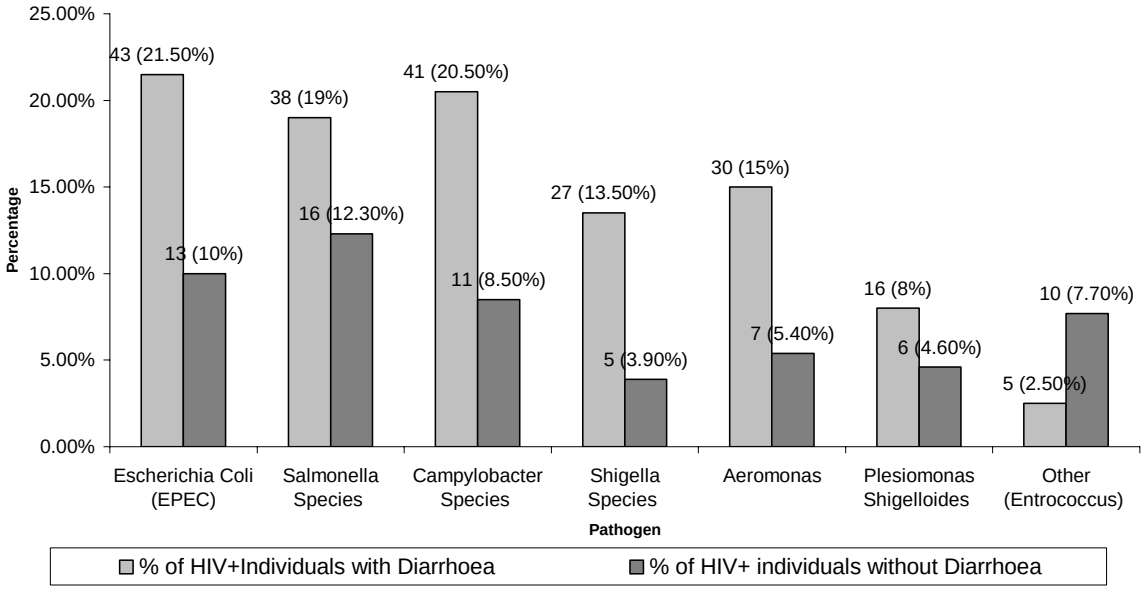

Figure 2.1: Isolation of Potential Bacterial Diarrhoea Agents from HIV Positive Individuals with and without Diarrhoea

Shigella spp (26.5%) and *Aeromonas* (20%) were more prevalent among HIV negative individuals (Figure 2). Profiles of enteric pathogen from household drinking water of HIV positive study cohorts 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 their respective study cohorts (Figure 3).

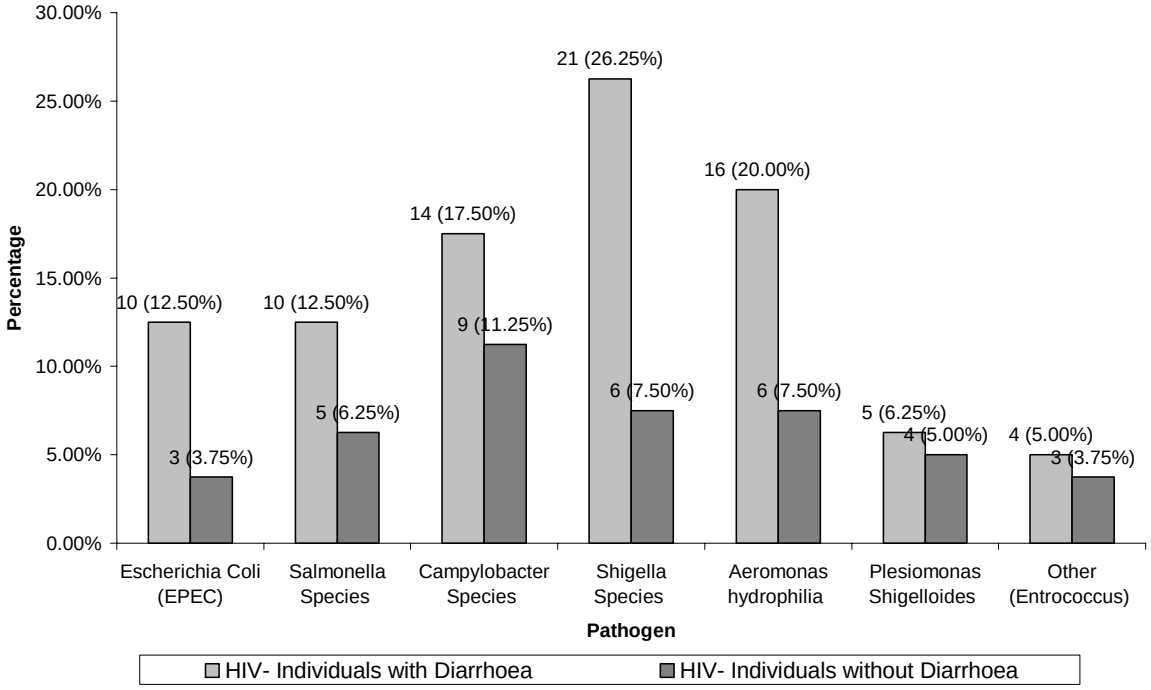

Figure 2.2: Isolation of Potential Bacterial Diarrhoea Agents from HIV Negative Individuals with and without Diarrhoea

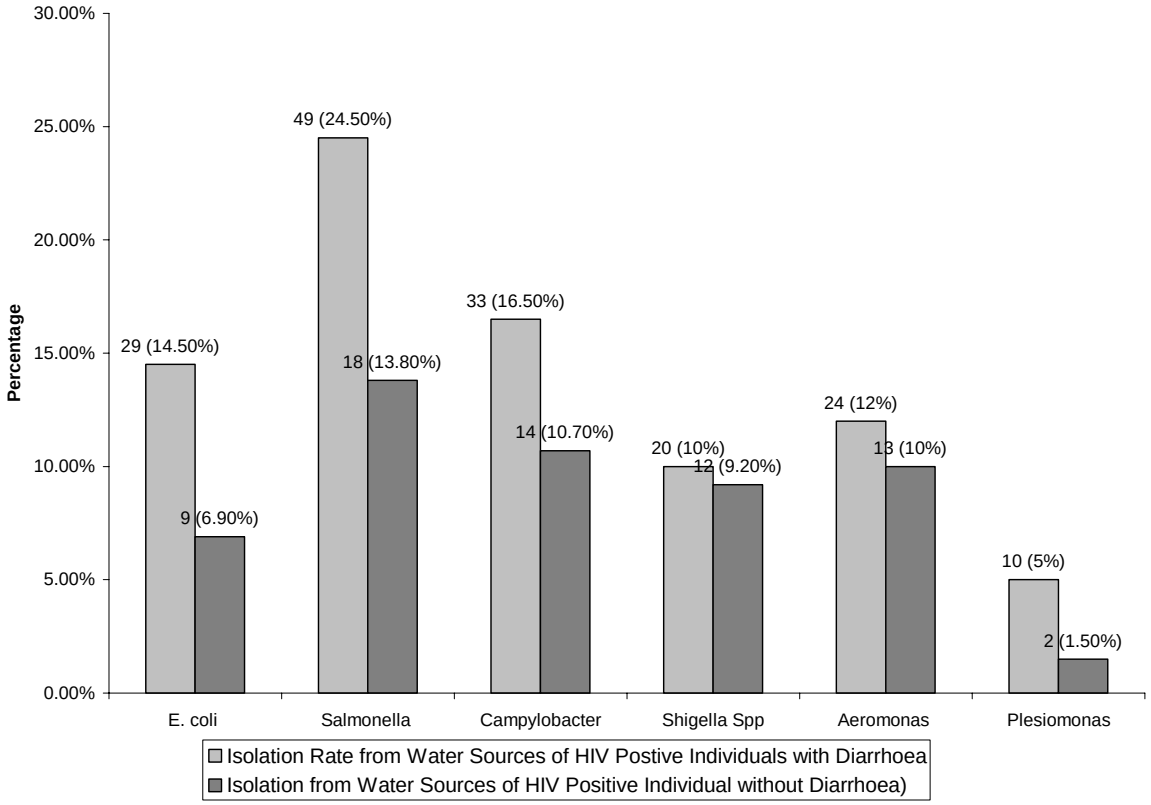

Figure 2.3: Profiles of Enteric Bacterial Pathogens Isolated from Household Drinking Water of HIV Positive Study Cohorts With and Without Diarrhoea

Data on enteric bacterial pathogens from HIV positive and negative individuals with diarrhoea accordingly to sex showed a significant association with diarrhoea for all pathogens except *Yersinia* and *Vibrio* species. Age distribution showed that enteropathogens were isolated from virtually all age groups (Table 2.2). For the control group (without diarrhoea) isolation according to sex and age groups were similar but to lesser frequencies (Table 2.3).

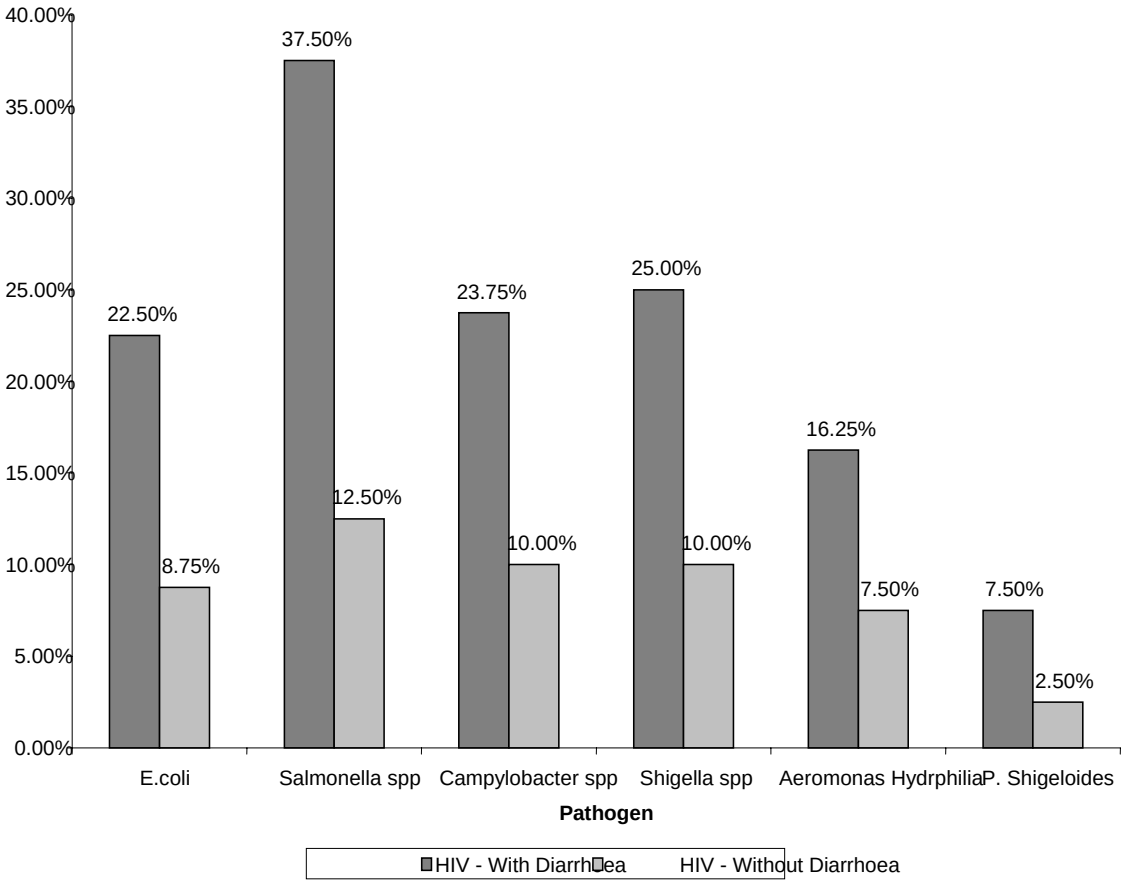

Figure 2.4: Profiles of Enteric Bacterial Pathogens Isolated from Household Drinking Water of HIV Negative Study Cohorts With and Without Diarrhoea

Table 2.2: Sex and Age Distribution of Enteric Bacterial Pathogens Isolated from HIV Positive and HIV Negative Individuals with Diarrhoea.

PATHOGENS BACTERIAL PATHOGENS	SEX		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%)

Table 2.3: Sex and Age Distribution of Enteric Bacterial Pathogens Isolated from HIV Positive and HIV Negative Individuals without Diarrhoea.

PATHOGENS BACTERIAL PATHOGENS	SEX		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%)

2.3.3 Comparison of Proportions of Enteric Pathogens Isolated from Stool and Water Samples of Study Cohorts:

Figures 2.5 and 2.6 below, shows 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).

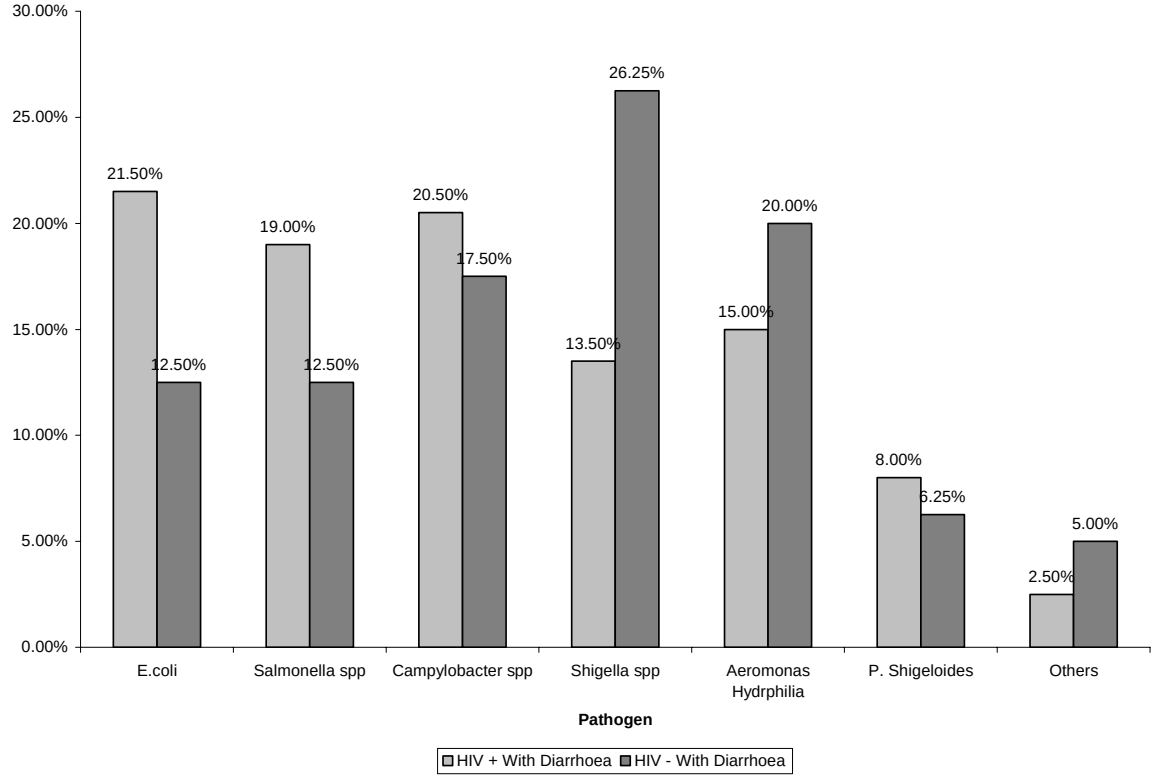

Figure 2.5: Profiles of Enteric Bacterial Pathogens Isolated from HIV Positive and Negative Individuals with Diarrhoea in Percentage

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 *Campylobacter* (25.0%). *Salmonella* (24.50%) was similarly 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.

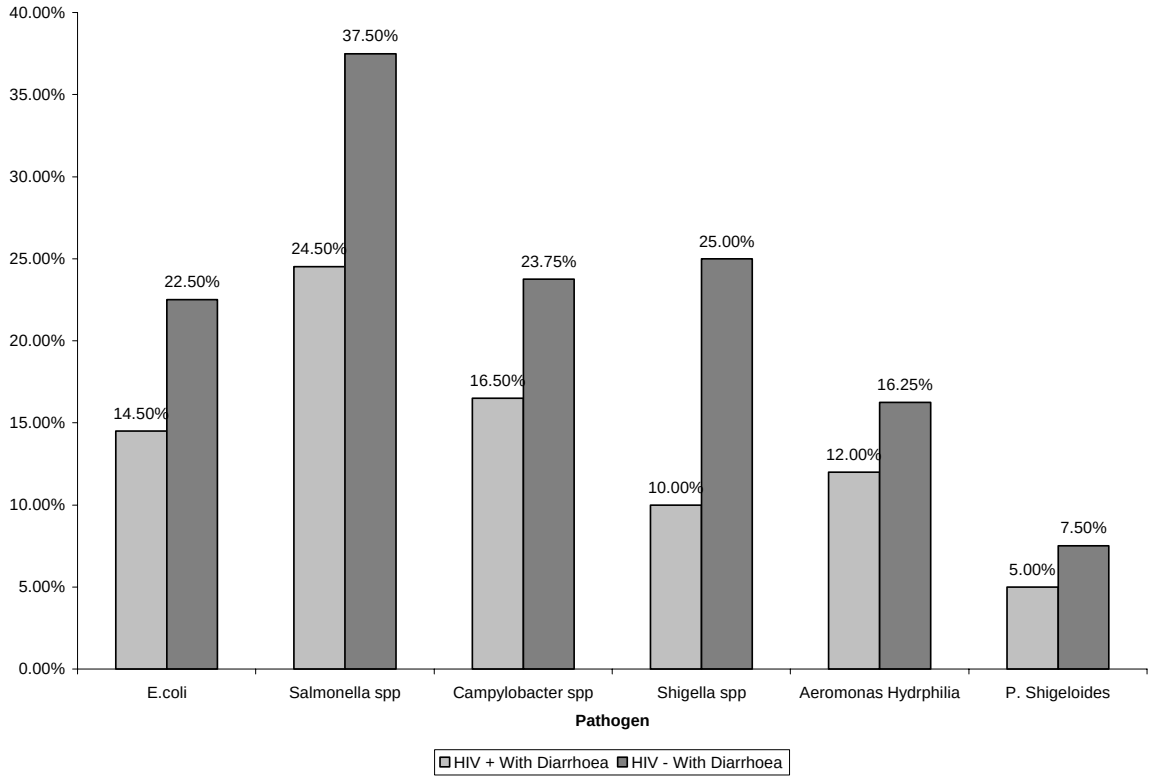

Figure 2.6: Profiles of Enteric Bacterial Pathogens Isolated from Water Samples of HIV Positive and Negative Individuals with Diarrhoea in Percentage

Further to the above figures, the chi-square analyses below tries 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 2.4 to 2.7). In both comparisons (1) HIV negative

without diarrhoea versus HIV positive individuals without diarrhoea and (2) HIV negative with diarrhoea versus 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 2.4). In this instant, *E. coli* was significantly more common among HIV positive individuals without diarrhoea.

Shigella and *Aeromonas* (Tables 2.8 and 2.9) 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 versus HIV positive individuals with diarrhoea (p = .011) (Table 2.9). In this comparison, *Shigella* species were significantly more prevent among HIV negative individuals with diarrhoea.

For the water samples, HIV negative individuals had higher percentages of all six 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 2.4 to 2.9). In these comparisons ((1) HIV negative without diarrhoea versus HIV positive individuals without diarrhoea and (2) HIV negative with diarrhoea versus HIV positive individuals with diarrhoea), HIV negative individuals with diarrhoea had significantly higher proportions of *Salmonella* (p = .029) (Table 2.5), *Shigella* (p = .001) (Table 2.8) and *Aeromonas* (p = .014) (Table 2.9) 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 2.10)

Table 2.4: **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 2.5: 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)

Table 2.6: 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 2.7: 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 2.8: 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 2.9: **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)

2.10: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

2.4 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 communities 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.

Sex 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 feminization of the epidemic (DOH, 2004). Feminization has been attributed to unequal power relations between men and women, 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 21-30 years age group (31.5%), followed by the age groups 11-20 (19.4%) and 31-40 (19.1%). The pooled affected age group (11-40 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 Carcamo 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 serostatus of patients. In immuno-suppressed HIV positive individuals with diarrhoea, the leading bacterial etiologies were Enteropathogenic *E.coli* (21.5%), *Campylobacter jejuni coli* (20.5%), *Salmonella* species (19%) and A*eromonas* species (15%). In immunocompetent individuals, *Campylobacter* (17.5%) and *Shigella* species (26%) were the main pathogens although Gassama et al. ,(2001) reported *Shigella* and *Salmonella* for immunocompetent individuals; E.coli, *Shigella* and S*almonella* for immunocompromised 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., 2002). 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., 1989).

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 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. Isolation of mixed pathogens from diarrhoic 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 pseudomembranous colitis (Kim et al., 1989; 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 were noted to be poor, toilet facilities were buzzing with flies and hand washing after using toilet facilities were not common. In some cases, faeces were not properly disposed as they were thrown into gutters or into surrounding veld. Drainage systems were poor in 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 counselors 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, *Vibrio cholerae* from diarrhoic 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.

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CHAPTER 3

ANTIBIOTIC RESISTANCE PROFILES AND RELATEDNESS OF ENTERIC BACTERIAL PATHOGENS ISOLATED FROM HIV/AIDS PATIENTS AND THEIR HOUSEHOLD DRINKING WATER IN LIMPOPO PROVINCE.

3.1 INTRODUCTION

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 immuno-competence.

Management of diarrhoea due to bacteria require the use of antibiotics which shortens the duration of diarrhoea, 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.

Antibiotic resistance among enteric bacteria pathogens complicates the heavy diarrhoea 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; Okeke et al., 2002) have been noted. In a case control study in Senegal, enteric bacteria such as *Salmonella* and *Shigella* 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% susceptibility 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 household drinking water samples of the study cohorts were determined using the polymerase chain reaction method.

The aim of this part of the study was to determine the antibiotic susceptibility profiles and linkage of enteric bacterial pathogens isolated from HIV/AIDS patients and their household drinking water in order to provide an updated reference epidemiological data.

Specific Objectives

The specific objectives of this section of the study were to:

1. determine the antibiograms of enteric bacterial isolates from HIV/AIDS patients with diarrhoea and their household drinking water.
2. determine the relatedness of enteric bacterial pathogens isolated from HIV/AIDS patients and their household drinking water by mens of the polymerase chain reaction.

3.2 MATERIALS AND METHODS

3.2.1 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/AIDS patients provided the platform to reach out to the cohorts.

3.2.2 Study Population

The present study was conducted prospectively between 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 diarrhoic 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.

3.2.3 Ethical Approval

The Health, Safety and Ethics Committee of the University of Venda, Thohoyandou, 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.

3.2.4 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).

3.2.5 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 hours 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 4-6hours of collection (Obi et al., 2002).

3.2.6 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.

3.2.7 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 hours. 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^{O}C for 72 hours. 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., 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.

3.2.8 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.

3.2.9 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 6h. 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 inoculum. The inoculum was 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 24h. 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, 30µg), Tetracycline (T, 30µg), Fusidic acid (FC, 30µg), Cloxacillin (CL, 30µg), Rifampicin (RF, 30µg), Meropenem (MEM, 10µg), Imepenem (IMI, 10µg), Chloramphenicol (C, 10µg), Amoxacillin/clavulanic acid (AMC, 30µg), Nitrofurantoin (NI, 200µg), Gentamycin (GN, 10µg), Amikacin (AK10µg), Ampicillin (AMP, 10µg), Cefoxitin (FOX, 30µg), Nalidixic acid (NA, 30µg), Piperacillin/Tazobactam (PTZ, 110µg), Doxycycline (DOX, 30µg), Novobiocin (NO, 30µg), Cotrimoxazole (TS, 25µg), Cefotaxine (CTX, 30µg), Cefepime (CPM, 30µg), Cefotrizone (CRO, 30µg)

Reference Strains: The following standard strains were included as controls; Esherichia coli ATCC 35218 beta-lactamase producer and Klebsiella pneumoniae ATCC 700603 Extended spectrum beta-lactamase (ESBL) producer. They were obtained from the Microbiology Department of the National Health Laboratory Services (NHLS), Johannesburg, South Africa. *Escherichia coli* ATCC strain 25922, *Salmonella typhimurium* ATCC strain 14028, *Salmonella enteritidis* ATCC strain 13076, *Shigella dysenteriae* ATCC strain 29027 were obtained from the American Type Culture Collection, Rockville, Md.

3.2.10 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 × *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 *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* (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 *Shigella 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.

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 (base pairs)	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

3.3 RESULTS

Results of antibiotic resistance profiles of enteric pathogens isolated from stool samples of HIV positive individuals with diarrhoea and their household drinking water are presented in figures 3.1 to 3.6. 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, doxycycline 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).

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.

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 3.3).

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) (Figure 3.4)

In addition to penicillin, amoxicillin, ampicillin and erythromycin, *Aeromonas* spp also showed marked resistance to chloramphenicol (Figure 3.5). *Aeromonas* spp from all the study cohorts were susceptible to ciprofloxacin, gentamycin, amikacin, meropenem, imipenem, nalidixic acid and pipevacillin-tazo bactam (CIP^S, GM^S, AK^S, MEM^S, IMI^S, NA^S and PTZ^S).

P. shigelloides isolates were markedly resistant to neomycin and chloramphenicol (figure 3.6). Across the various study cohorts, *P. shigelloides* 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 $TS^{S\ 50\%}$)

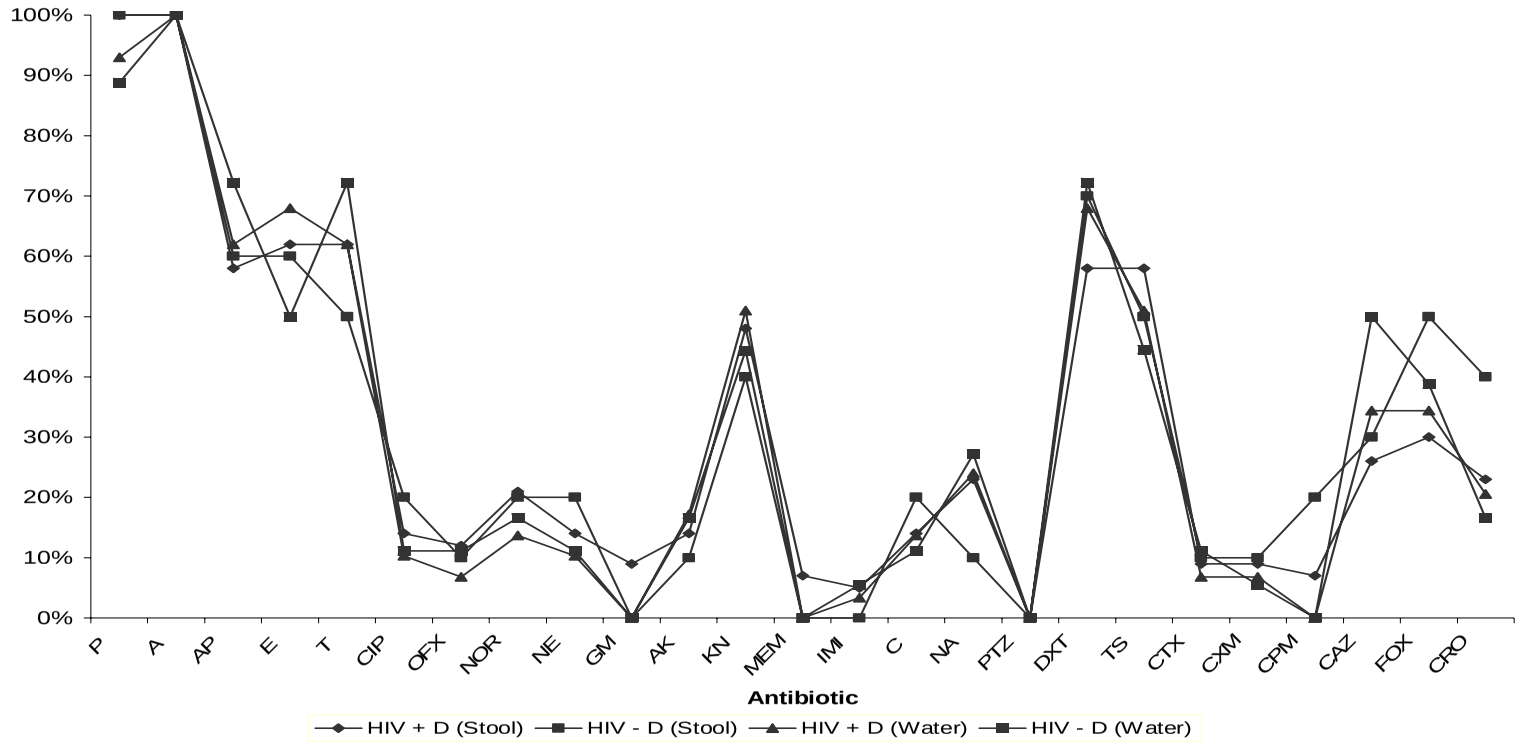


Figure 3.1: Antibiotic Resistance Profile of Escherichia coli Isolated from Stool and Household Drinking Water Samples HIV Positive and Negative Individuals with Diarrhoea

Penicillin (PG), Ciprofloxcain (CIP), Vancomycin (VA), Erythromycin (E), Tetracycline (T), Fusidic acid (FC), Cloxacillin (CL), Rifampicin (RF), Meropenem (MEM), Imepenem (IMI), Chloramphenicol (C), Amoxacillin acid (AMC), Nitrofurantoin (NI), Gentamycin (GN), Amikacin (AK), Ampicillin (AMP), Cefoxitin (FOX), Nalidixic acid (NA), Piperacillin/Tazobactam (PTZ), Doxycycline (DOX), Novobiocin (NO), Cotrimoxazole (TS), Cefotaxine (CTX), Cefepime (CPM), Cefotrizone (CRO)

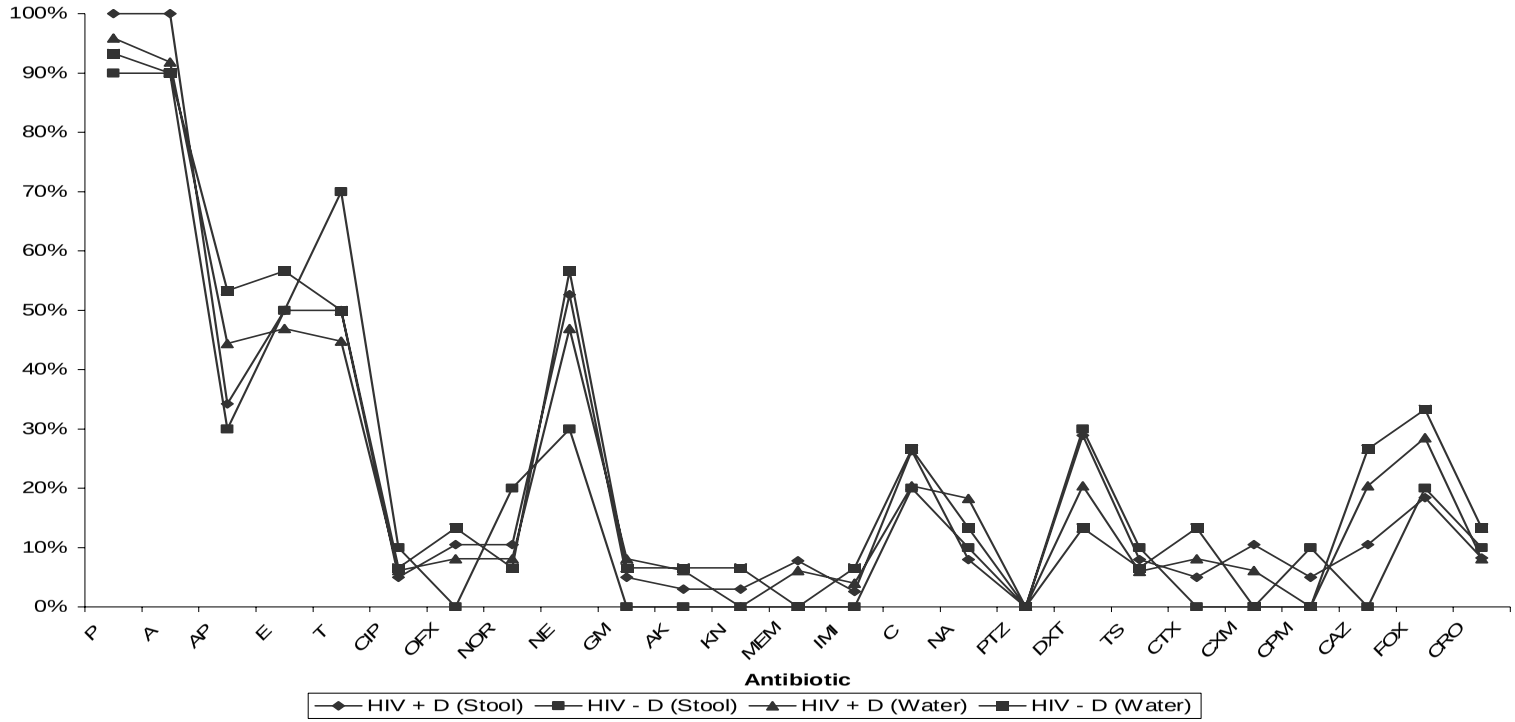


Figure 3.2: Antibiotic Resistance Profile of Salmonella Species Isolated from Stool and Household Drinking Water Samples HIV Positive and Negative Individuals with Diarrhoea

Penicillin (PG), Ciprofloxcain (CIP), Vancomycin (VA), Erythromycin (E), Tetracycline (T), Fusidic acid (FC), Cloxacillin (CL), Rifampicin (RF), Meropenem (MEM), Imepenem (IMI), Chloramphenicol (C), Amoxacillin acid (AMC), Nitrofurantoin (NI), Gentamycin (GN), Amikacin (AK), Ampicillin (AMP), Cefoxitin (FOX), Nalidixic acid (NA), Piperacillin/Tazobactam (PTZ), Doxycycline (DOX), Novobiocin (NO), Cotrimoxazole (TS), Cefotaxine (CTX), Cefepime (CPM), Cefotrizone (CRO)

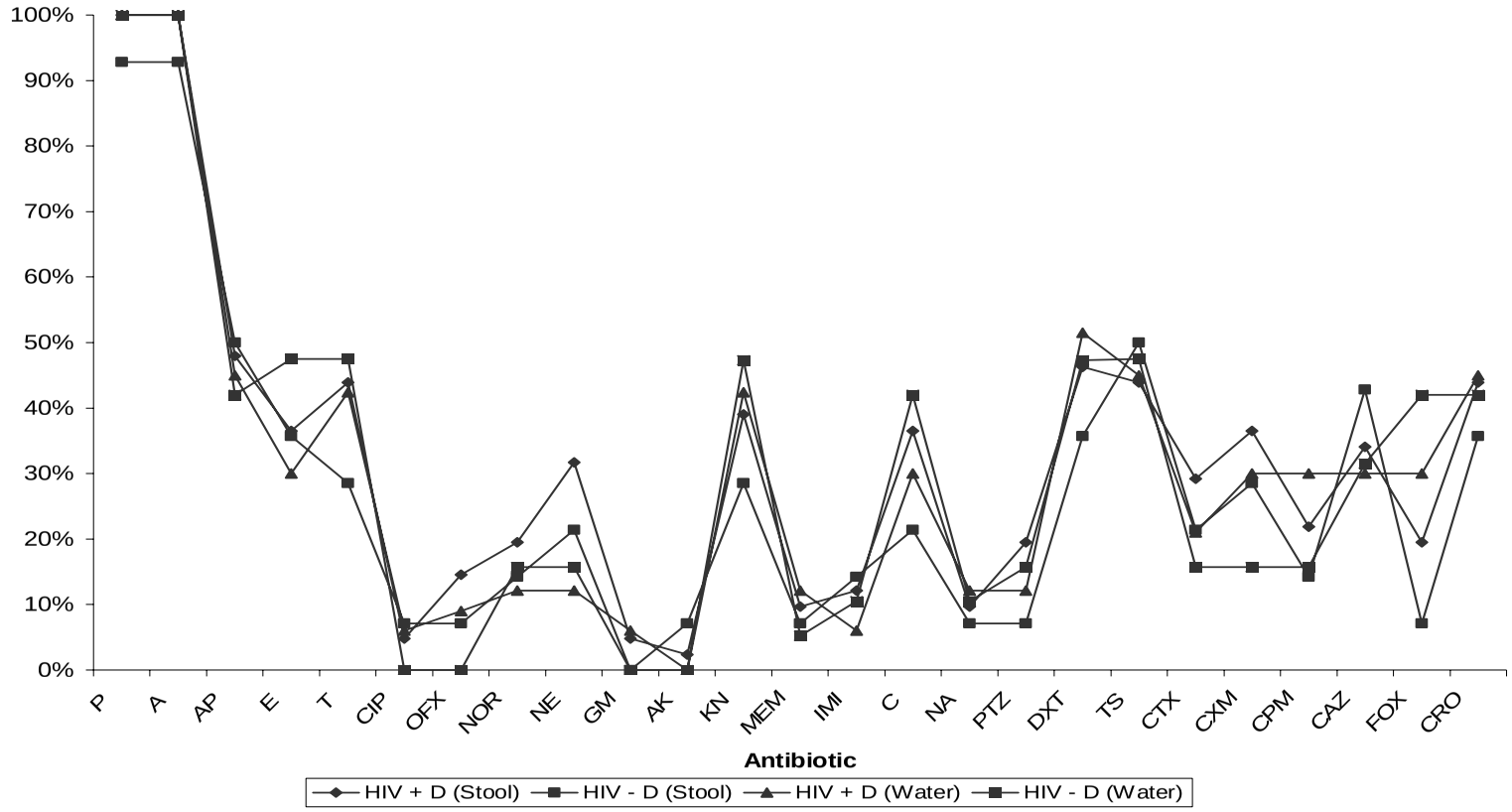


Figure 3.3: Antibiotic Resistance Profile of Campylobacter Species Isolated from Stool and Household Drinking Water Samples HIV Positive and Negative Individuals with Diarrhoea

Penicillin (PG), Ciprofloxcain (CIP), Vancomycin (VA), Erythromycin (E), Tetracycline (T), Fusidic acid (FC), Cloxacillin (CL), Rifampicin (RF), Meropenem (MEM), Imepenem (IMI), Chloramphenicol (C), Amoxacillin acid (AMC), Nitrofurantoin (NI), Gentamycin (GN), Amikacin (AK), Ampicillin (AMP), Cefoxitin (FOX), Nalidixic acid (NA), Piperacillin/Tazobactam (PTZ), Doxycycline (DOX), Novobiocin (NO), Cotrimoxazole (TS), Cefotaxine (CTX), Cefepime (CPM), Cefotrizone (CRO)

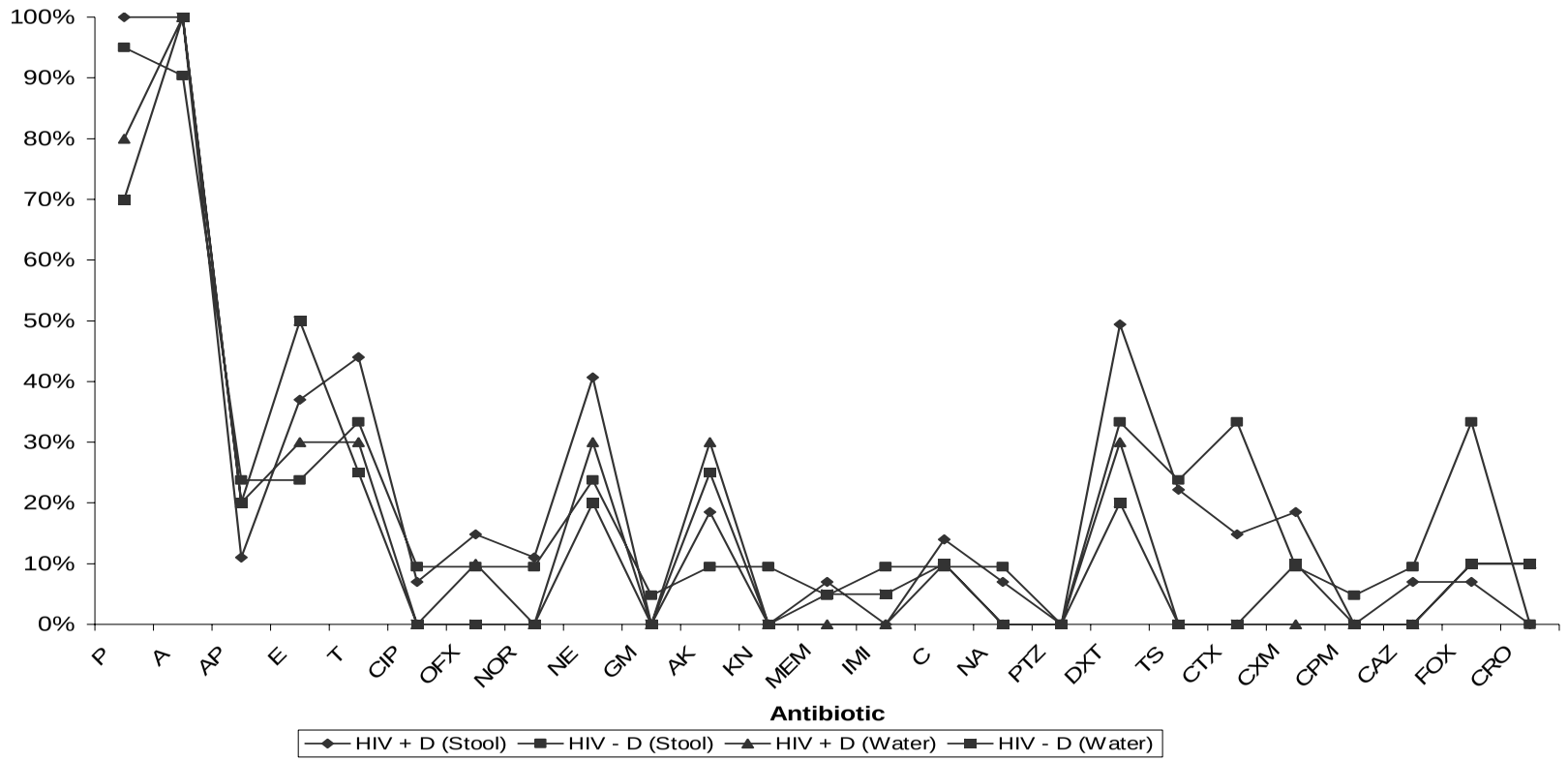


Figure 3.4: Antibiotic Resistance Profile of Shigella Species Isolated from Stool and Household Drinking Water Samples HIV Positive and Negative Individuals with Diarrhoea

Penicillin (PG), Ciprofloxcain (CIP), Vancomycin (VA), Erythromycin (E), Tetracycline (T), Fusidic acid (FC), Cloxacillin (CL), Rifampicin (RF), Meropenem (MEM), Imepenem (IMI), Chloramphenicol (C), Amoxacillin acid (AMC), Nitrofurantoin (NI), Gentamycin (GN), Amikacin (AK), Ampicillin (AMP), Cefoxitin (FOX), Nalidixic acid (NA), Piperacillin/Tazobactam (PTZ), Doxycycline (DOX), Novobiocin (NO), Cotrimoxazole (TS), Cefotaxine (CTX), Cefepime (CPM), Cefotrizone (CRO)

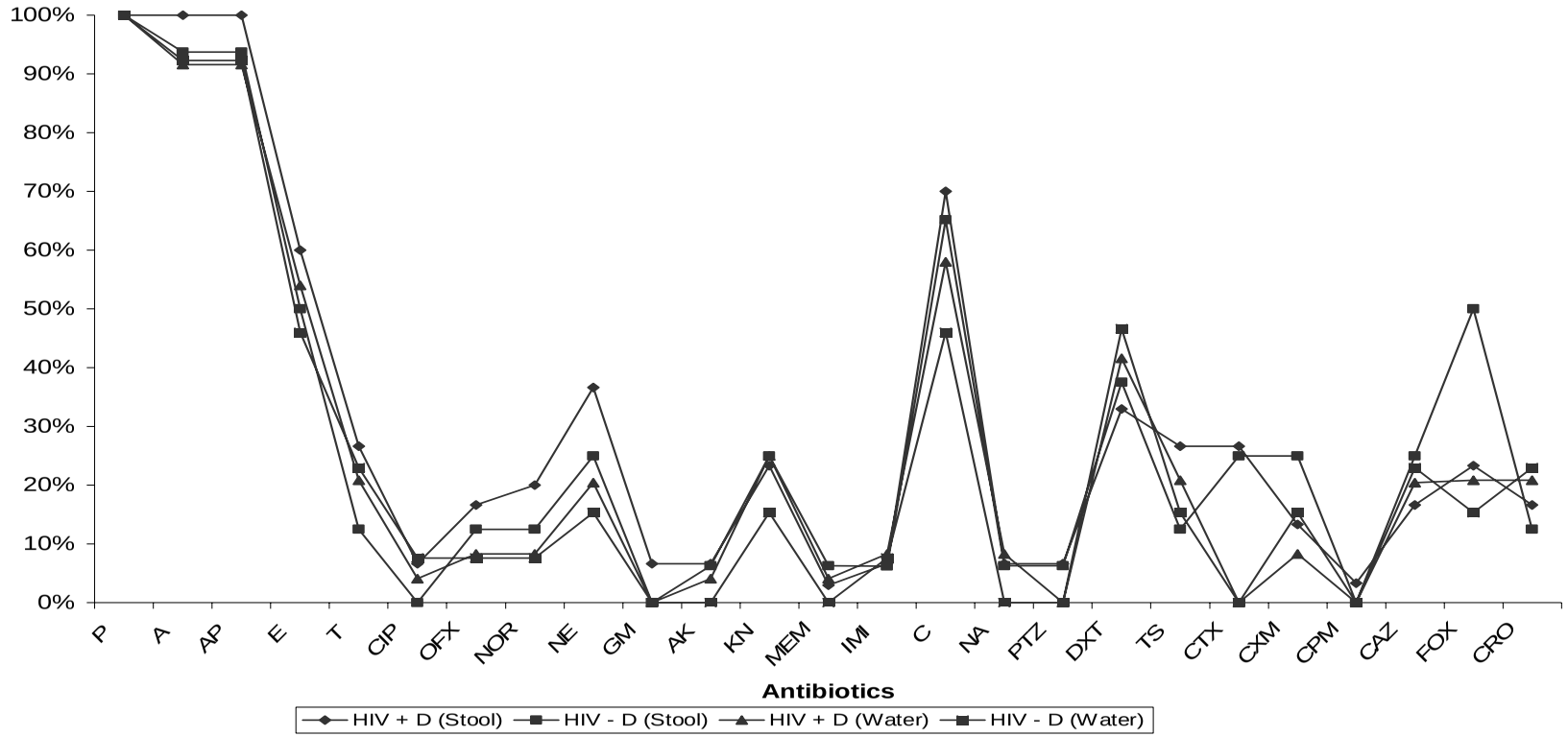


Figure 3.5: Antibiotic Resistance Profile of Aeromonas Species Isolated from Stool and Household Drinking Water Samples HIV Positive and Negative Individuals with Diarrhoea

Penicillin (PG), Ciprofloxcain (CIP), Vancomycin (VA), Erythromycin (E), Tetracycline (T), Fusidic acid (FC), Cloxacillin (CL), Rifampicin (RF), Meropenem (MEM), Imepenem (IMI), Chloramphenicol (C), Amoxacillin acid (AMC), Nitrofurantoin (NI), Gentamycin (GN), Amikacin (AK), Ampicillin (AMP), Cefoxitin (FOX), Nalidixic acid (NA), Piperacillin/Tazobactam (PTZ), Doxycycline (DOX), Novobiocin (NO), Cotrimoxazole (TS), Cefotaxine (CTX), Cefepime (CPM), Cefotrizone (CRO)

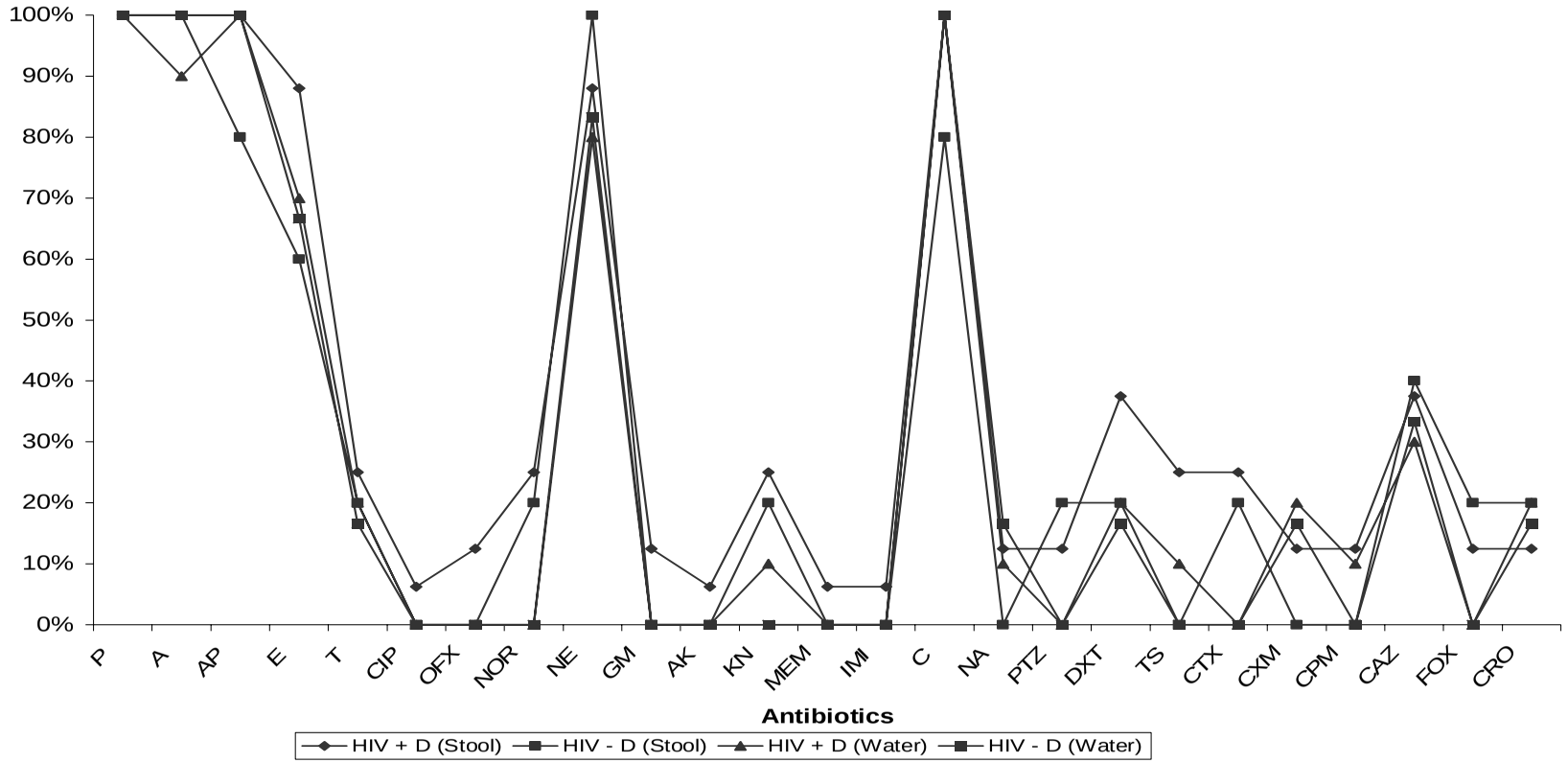
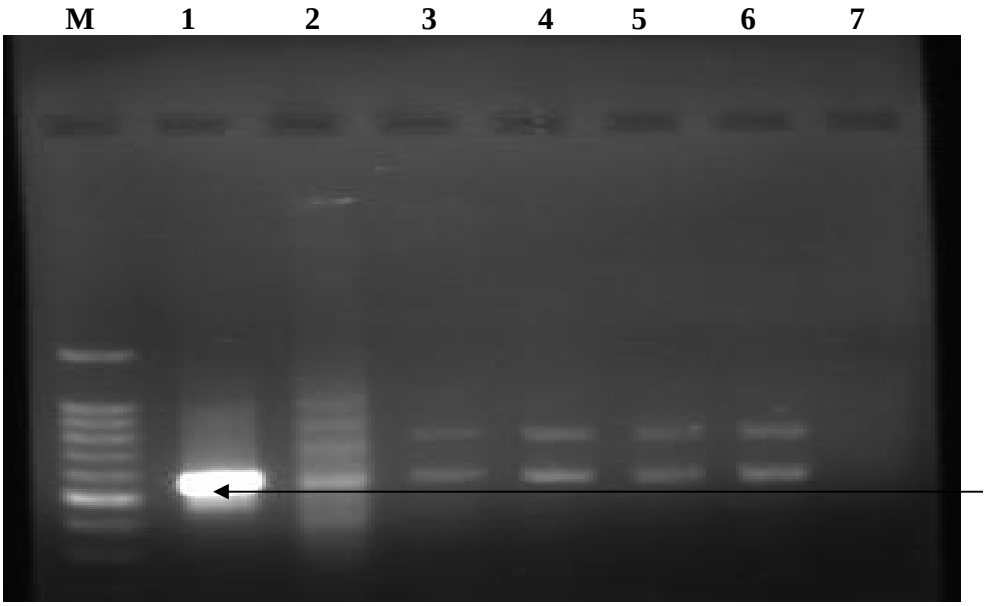


Figure 3.6: Antibiotic Resistance Profile of Plesiomonas Species Isolated from Stool and Household Drinking Water Samples HIV Positive and Negative Individuals with Diarrhoea

Penicillin (PG), Ciprofloxcain (CIP), Vancomycin (VA), Erythromycin (E), Tetracycline (T), Fusidic acid (FC), Cloxacillin (CL), Rifampicin (RF), Meropenem (MEM), Imepenem (IMI), Chloramphenicol (C), Amoxacillin acid (AMC), Nitrofurantoin (NI), Gentamycin (GN), Amikacin (AK), Ampicillin (AMP), Cefoxitin (FOX), Nalidixic acid (NA), Piperacillin/Tazobactam (PTZ), Doxycycline (DOX), Novobiocin (NO), Cotrimoxazole (TS), Cefotaxine (CTX), Cefepime (CPM), Cefotrizone (CRO)

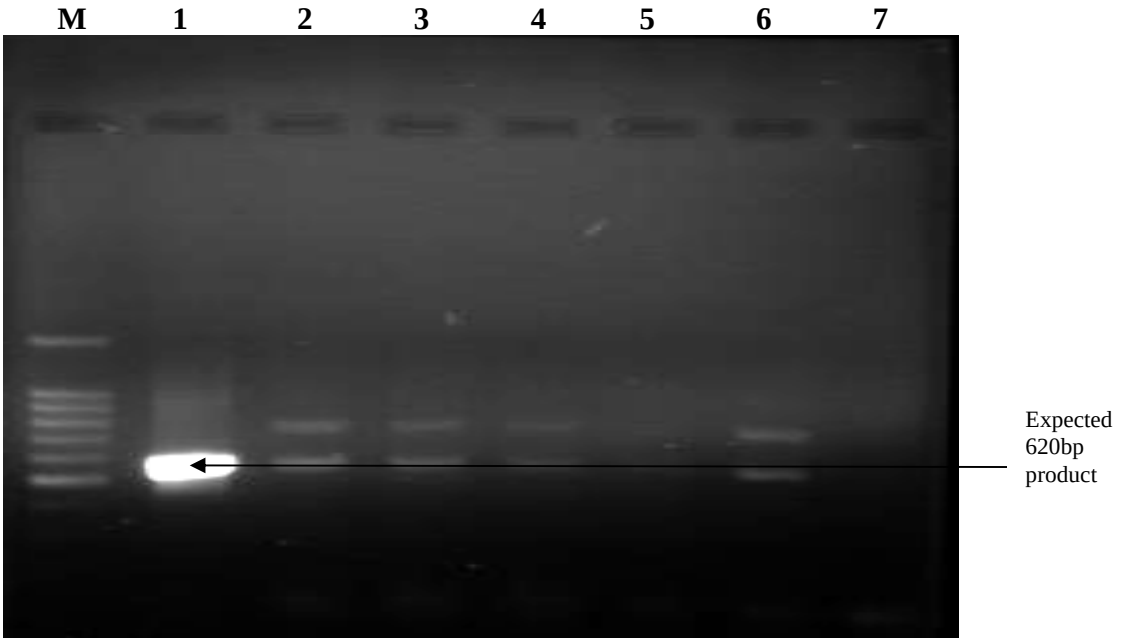
Representative gel electrophoretic profiles of amplified products of the target genes are presented in Figures 3.7 to 3.21. Using primers for the target genes, as indicated for *Salmonella, Shigella dysentriae and E.coli*, electrophoretic profiles of stool and water samples for the various study cohorts were similar.

PCR Results



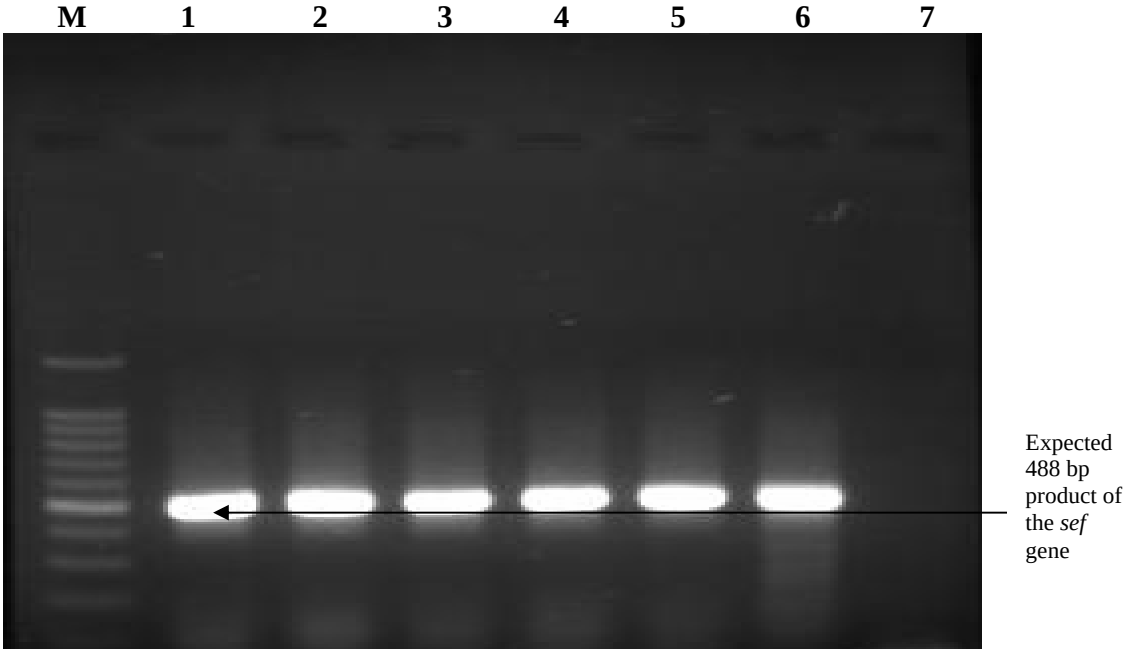
Lane M: Marker, Lane 1: *S. typhimurium* (Positive control), Lane 2: S1, Lane 3: S2, Lane 4: S3, Lane 5: S4, lane 6: S5, Lane 7: Negative control.

Figure 3.7: Sample - S1 - S5 HIV + H_2O S1 – S5 HIV+ H_2O (Tested for *Salmonella typhimurium*)



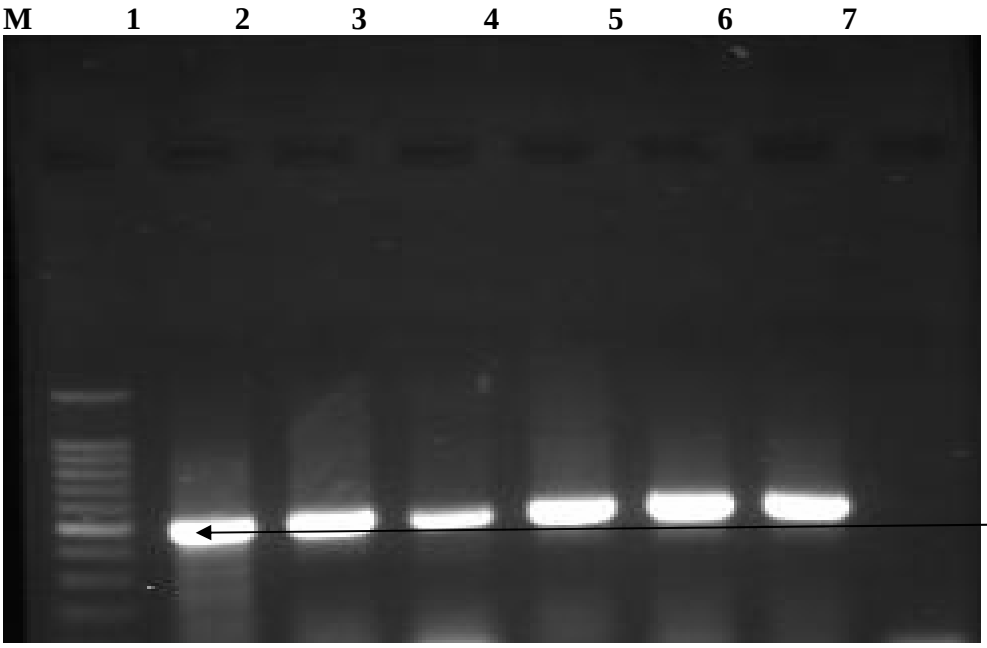
Lane M: Marker, Lane 1: *S. typhimurium* (Positive control), Lane 2: S1, Lane 3: S2, Lane 4: S3, Lane 5: S4, lane 6: S5, Lane 7: Negative control.

Figure 3.8: Sample S1 – S5 HIV– H_2O (Tested for *Salmonella typhimurium*)



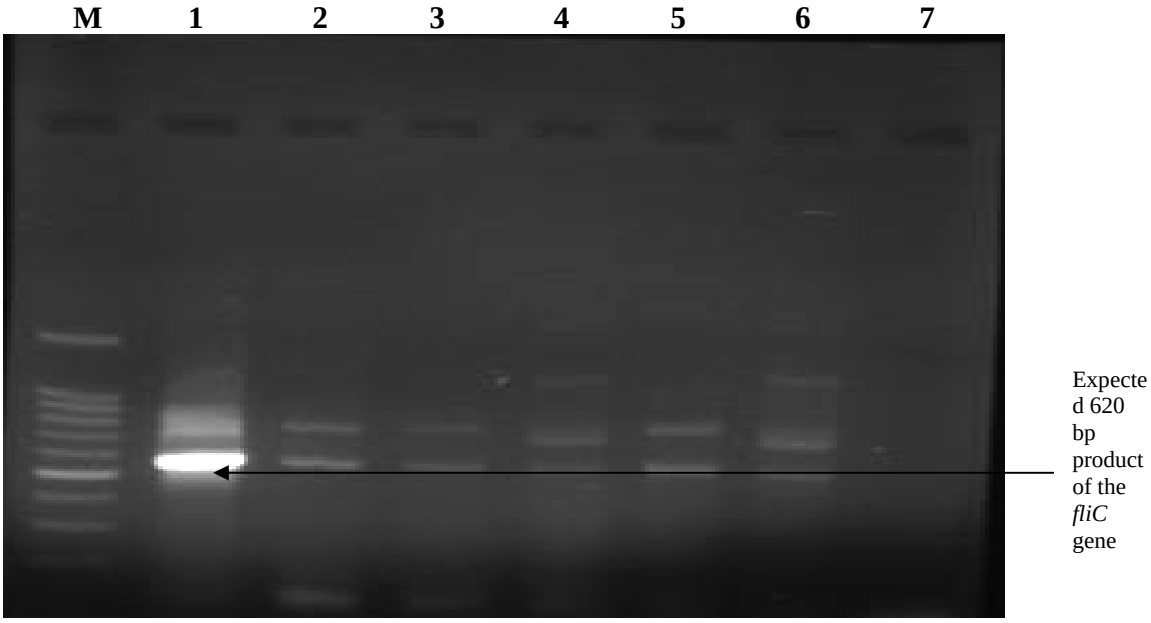
Lane M: Marker, Lane 1: *S. enteritidis* (Positive control), Lane 2: S1, Lane 3: S2, Lane 4: S3, Lane 5: S4, lane 6: S5, Lane 7: Negative control.

Figure 3.9: Sample - S1 – S5 HIV+ H_2O (Tested for *Salmonella enteritidis)*



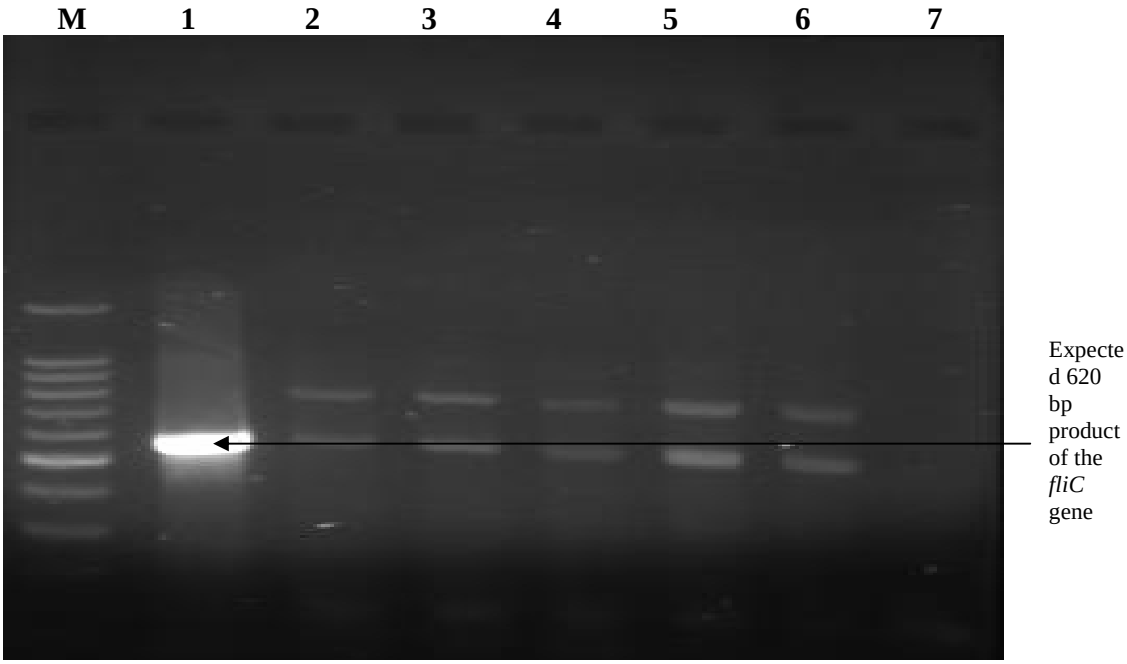
Lane M: Marker, Lane 1: *S. enteritidis* (Positive control), Lane 2: S1, Lane 3: S2, Lane 4: S3, Lane 5: S4, lane 6: S5, Lane 7: Negative control.

Figure 3.10: Sample S1 – S5 HIV- H_2O (Tested for *Salmonella enteritidis)*



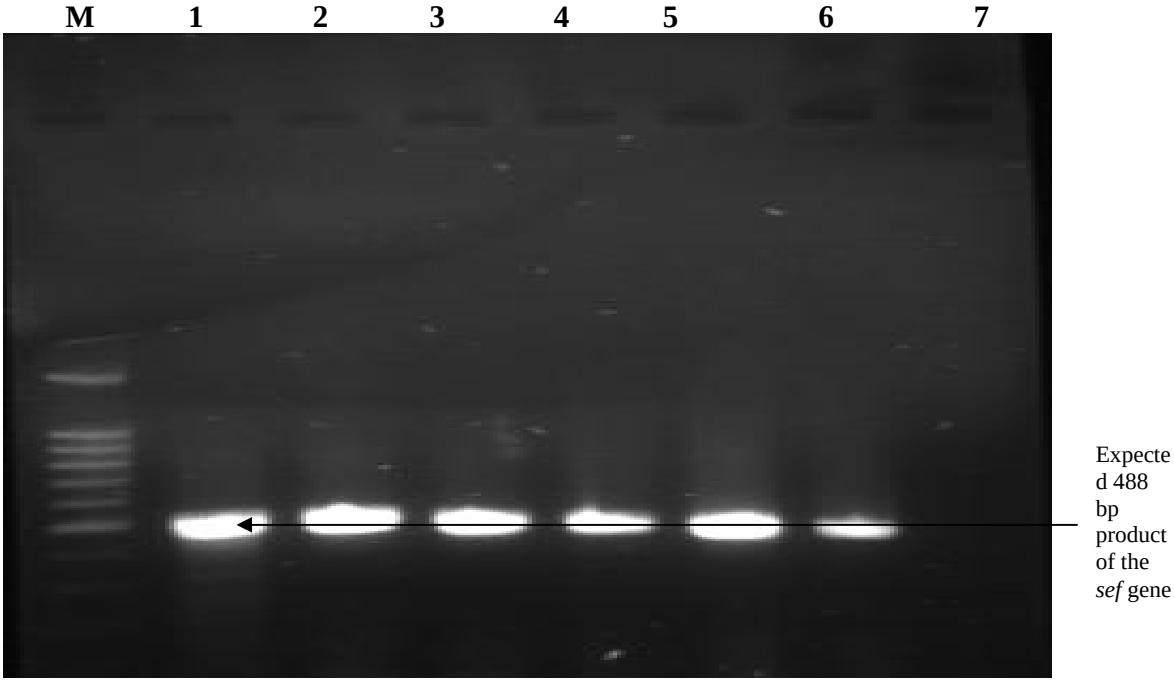
Lane M: Marker, Lane 1: *S. typhimurium* (Positive control), Lane 2: S1, Lane 3: S2, Lane 4: S3, Lane 5: S4, lane 6: S5, Lane 7: Negative control.

Figure 3.11: Sample S1 – S5 HIV+ stool (Tested for *Salmonella typhimurium)*



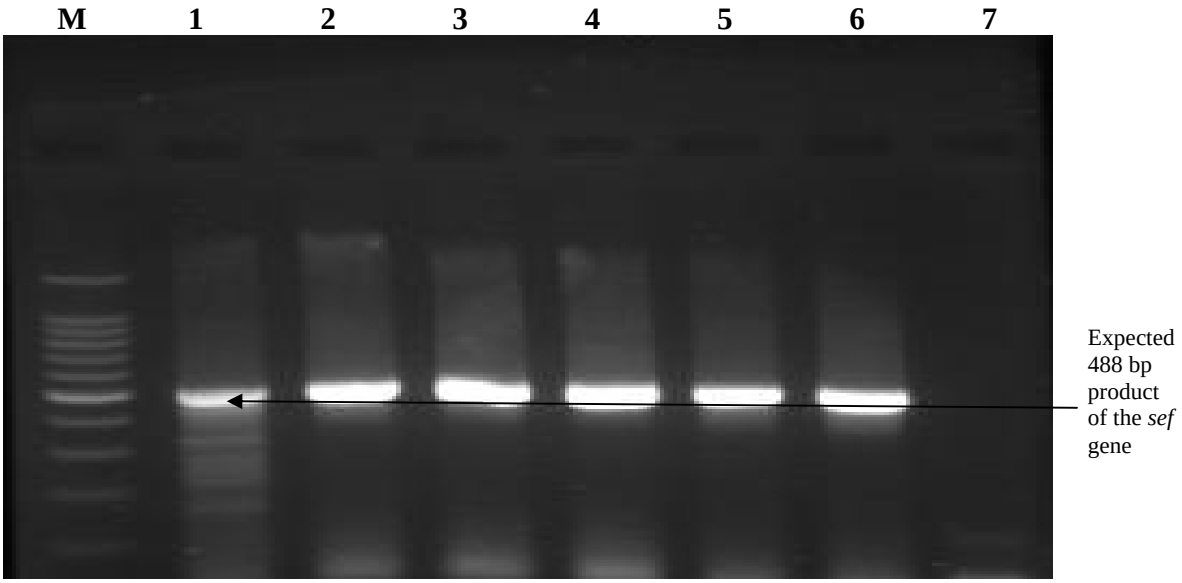
Lane M: Marker, Lane 1: *S. typhimurium* (Positive control), Lane 2: S1, Lane 3: S2, Lane 4: S3, Lane 5: S4, lane 6: S5, Lane 7: Negative control.

Figure 3.12: Sample S1 – S5 HIV- stool (Tested for *Salmonella typhimurium)*



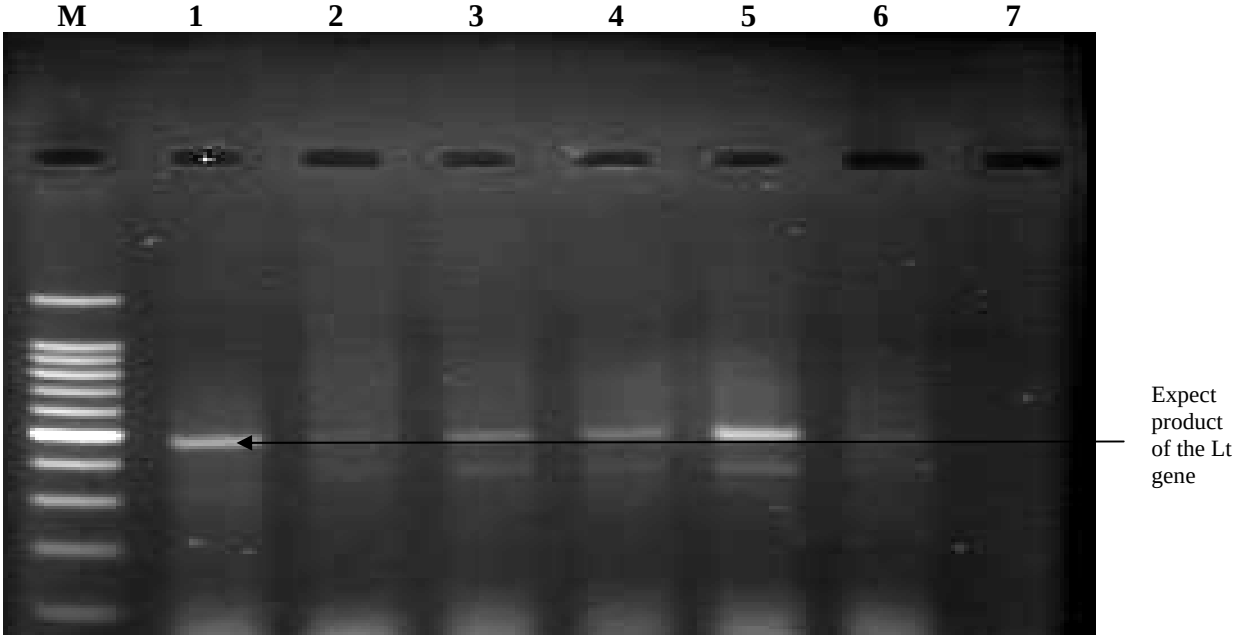
Lane M: Marker, Lane 1: *S. enteritidis* (Positive control), Lane 2: S1, Lane 3: S2, Lane 4: S3, Lane 5: S4, lane 6: S5, Lane 7: Negative control.

Figure 3.13: Sample- S1 – S5 HIV+ stool (Tested for *Salmonella enteritidis)*



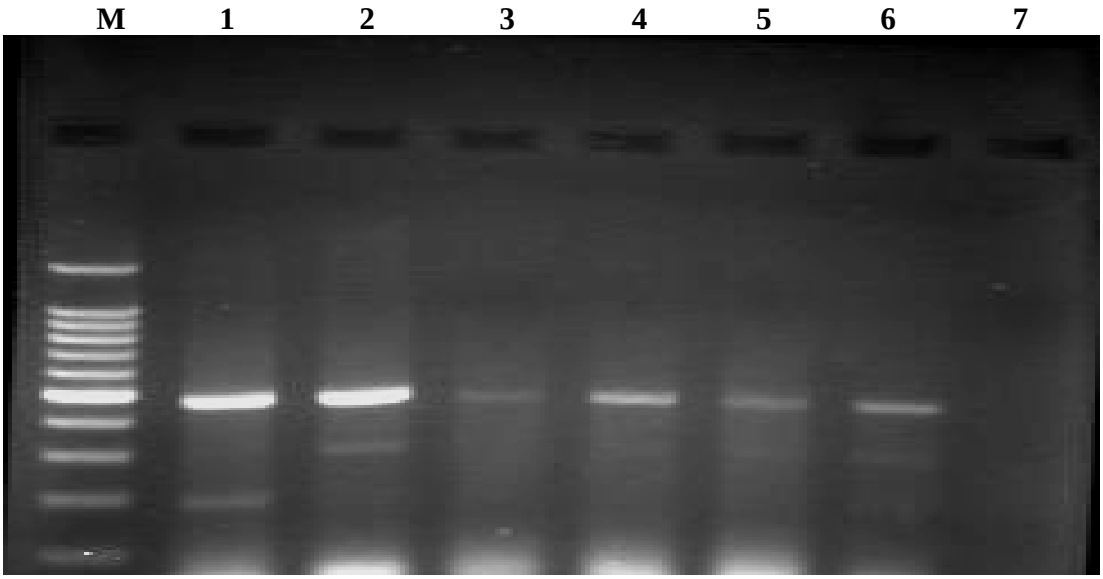
Lane M: Marker, Lane 1: *S. enteritidis* (Positive control), Lane 2: S1, Lane 3: S2, Lane 4: S3, Lane 5: S4, lane 6: S5, Lane 7: Negative control.

Figure 3.14: Sample S1 – S5 HIV- stool (Tested for *Salmonella enteritidis)*



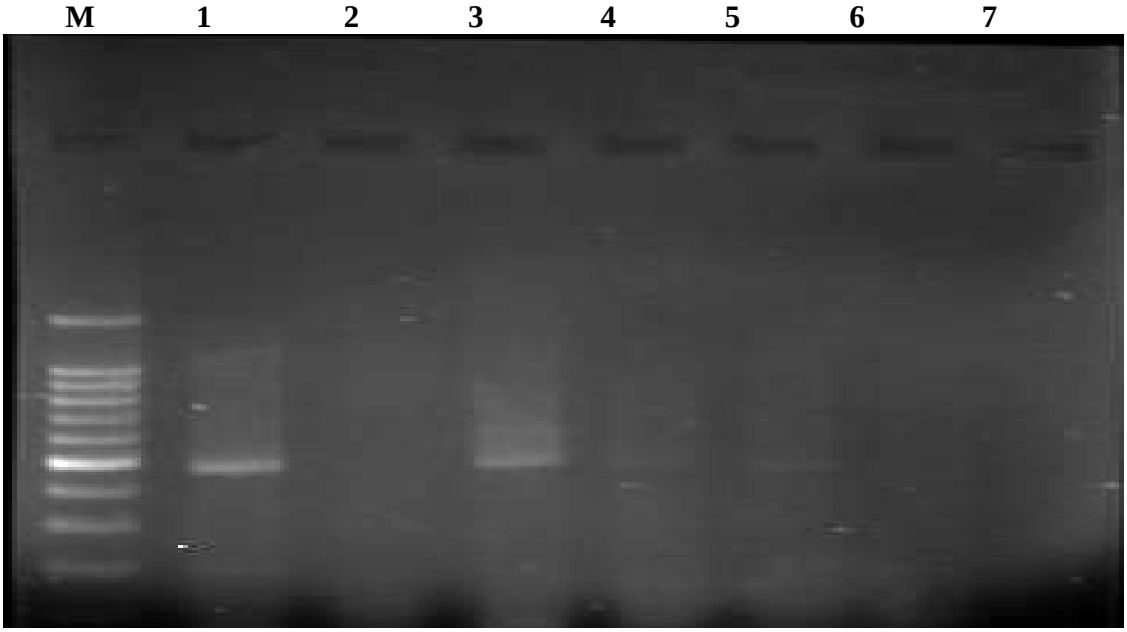
Lane M: Marker, Lane 1: *Esc20* (Positive control), Lane 2: 1, Lane 3: 2, Lane 4: 3, Lane 5: 4, lane 6: 5, Lane 7: Negative control.

Figure 3.15: Sample 1 – 5 HIV+ H_2O (Tested for *Escherichia coli* strain 20)



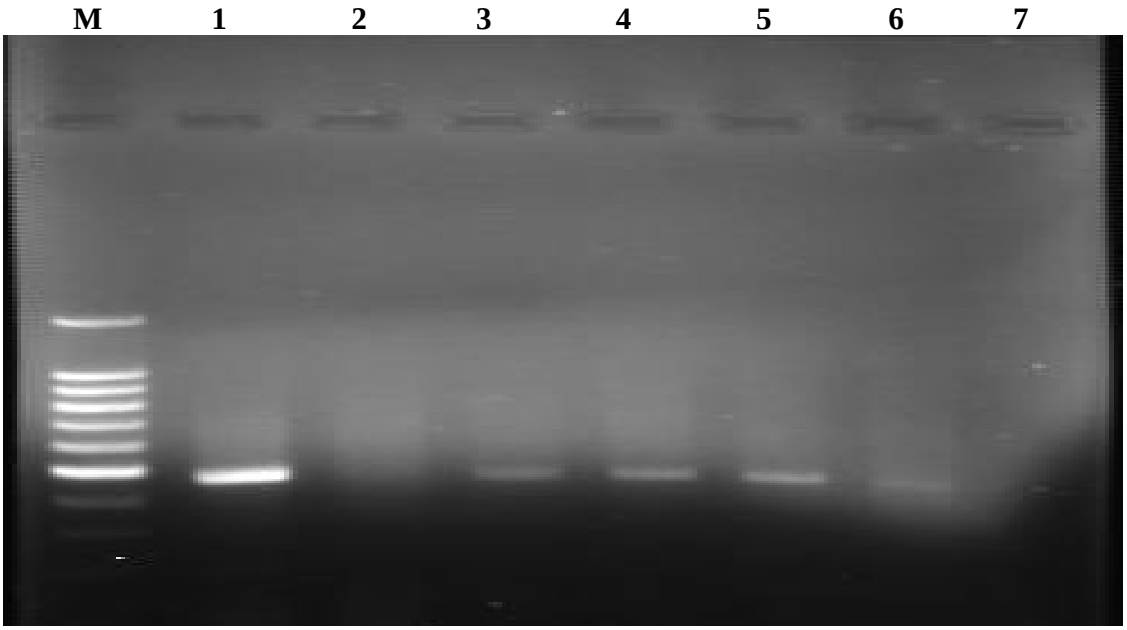
Lane M: Marker, Lane 1: *Esc20* (Positive control), Lane 2: 1, Lane 3: 2, Lane 4: 3, Lane 5: 4, lane 6: 5, Lane 7: Negative control.

Figure 3.16.: Sample 1 – 5 HIV- H_2O (Tested for *Escherichia coli* strain 20)



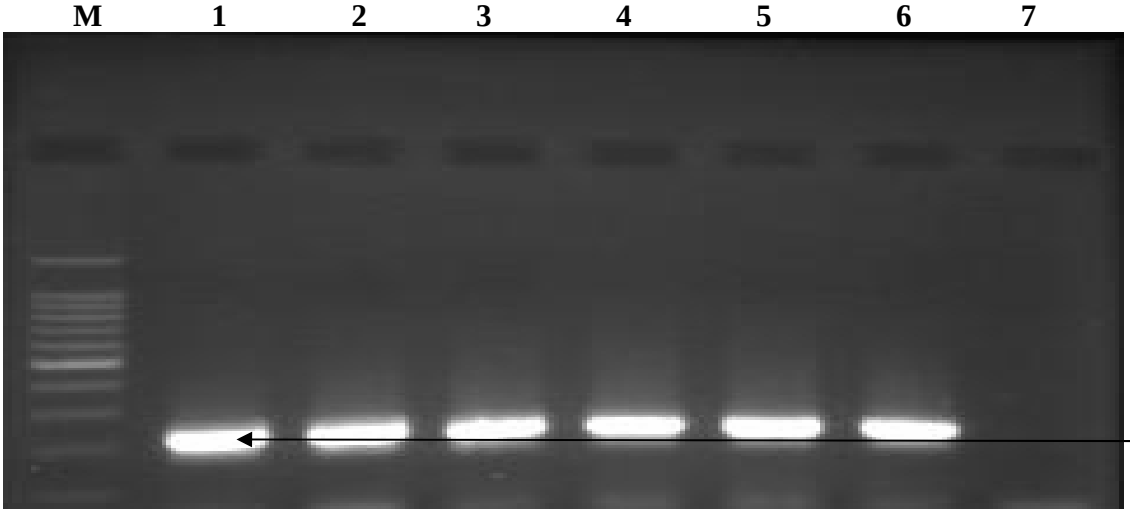
Lane M: Marker, Lane 1: *Esc20* (Positive control), Lane 2: 1, Lane 3: 2, Lane 4: 3, Lane 5: 4, lane 6: 5, Lane 7: Negative control.

Figure 3.17: Sample 1 – 5 HIV+ Stool (Tested for *Escherichia coli* strain 20)



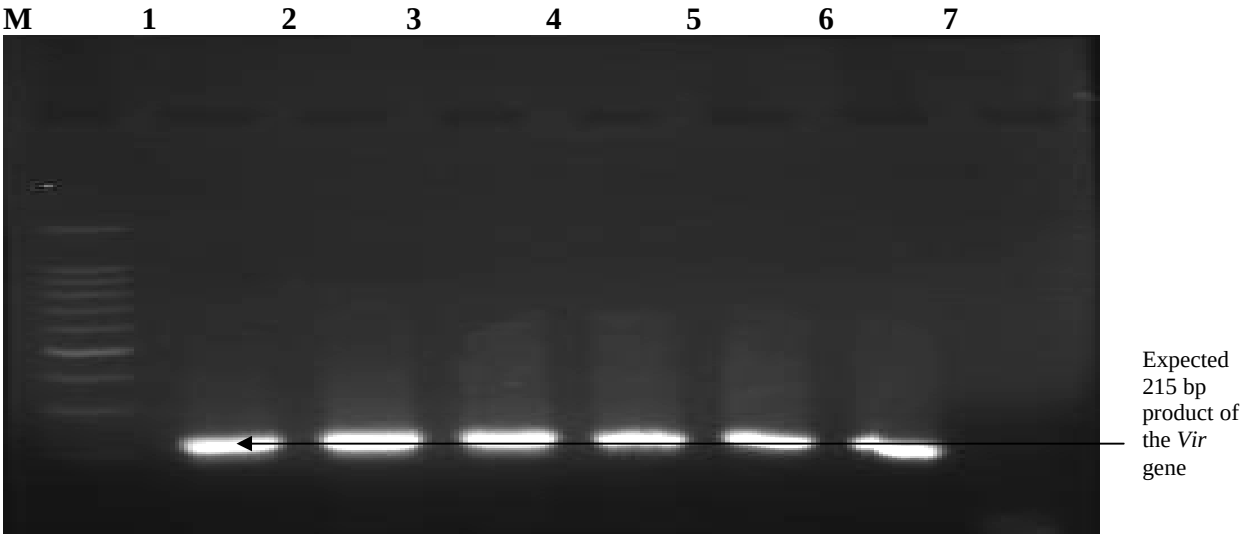
Lane M: Marker, Lane 1: *Esc20* (Positive control), Lane 2: 1, Lane 3: 2, Lane 4: 3, Lane 5: 4, lane 6: 5, Lane 7: Negative control.

Figure 3.18: Sample 1 – 5 HIV- Stool (Tested for *Escherichia coli* strain 20)



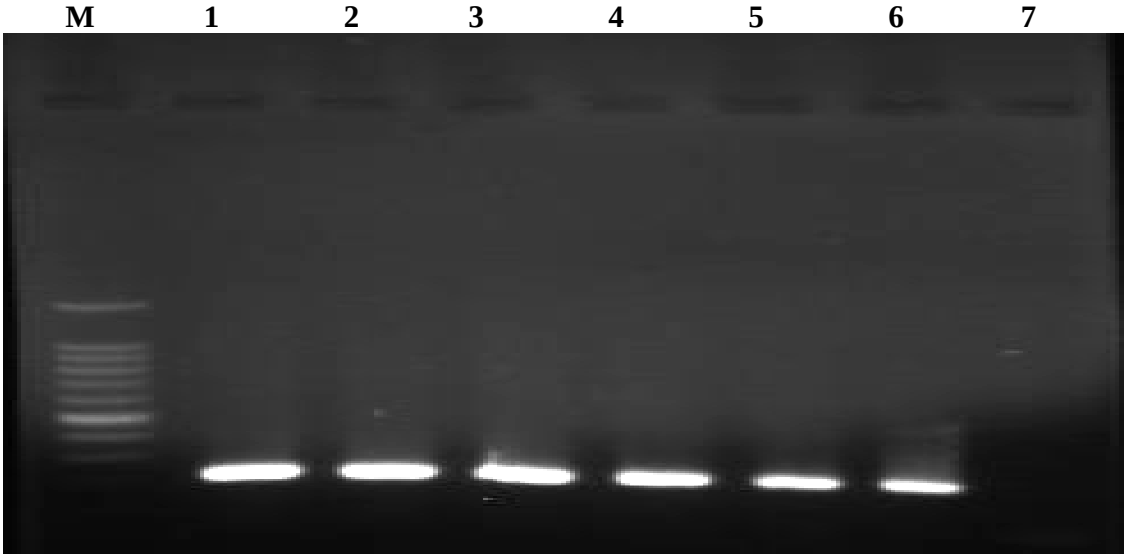
Lane M: Marker, Lane 1: *Shigella dysenteriae* (Positive control), Lane 2: SD1, Lane 3: SD2, Lane 4: SD3, Lane 5: SD4, lane 6: SD5, Lane 7: Negative control.

Figure 3.19: Sample - SD1 – SD5 HIV+ H_2O



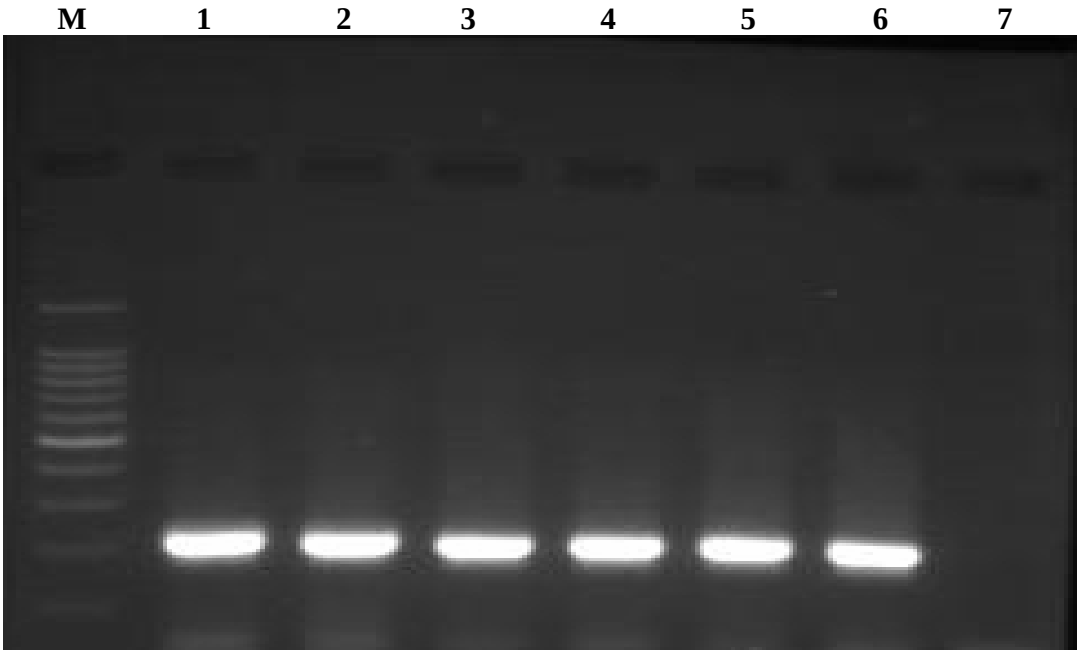
Lane M: Marker, Lane 1: *Shigella dysenteriae* (Positive control), Lane 2: SD1, Lane 3: SD2, Lane 4: SD3, Lane 5: SD4, lane 6: SD5, Lane 7: Negative control.

Figure 3.20: Sample SD1 – SD5 HIV- H_2O



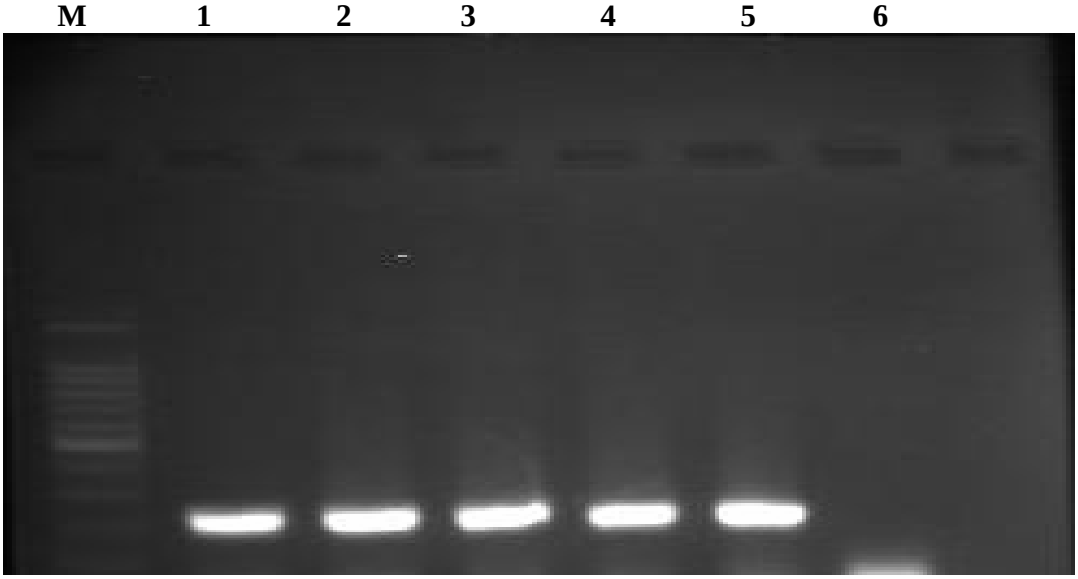
Lane M: Marker, Lane 1: *Shigella dysenteriae* (Positive control), Lane 2: SD1, Lane 3: SD2, Lane 4: SD3, Lane 5: SD4, lane 6: SD5, Lane 7: Negative control.

Figure 3.21: 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.

Figure 3.22: Sample SD1 – SD5 HIV+ Stool



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

Figure 3.23: Sample SD1 – SD5 HIV+ H_2O

3.4 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 Province, South Africa. For example, virtually all the enteric bacterial pathogens isolated from HIV positive cases (70 -100%) showed multiple resistance 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/AIDS. 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).

The multiple antibiotic resistance 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 resistance 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 ripple effects of these developments connotes that for certain strains, there are few or no effective antibiotics.

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 *Escherichia coli* showed multiple resistance including resistance to drugs of choice, such as the fluoroquinolones. The resistance to fluoroquinolones in E. coli is mainly associated with mutation in the chromosomal 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 chloramphenicol 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 reportedly associated with decreased permeability of the cell envelop in enteric bacteria, including plasmid mediated mechanisms (Isenbarger, et al., 2000). 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.

Among Campylobacters, multiple antibiotic resistance was 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.

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 epidemiologic 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 empiric 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 entero-pathogens typifies the growing concern among health care workers on the continued effectiveness of antibiotics in the empiric 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 foregrounded.

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 *Salmonella enteritidis, Salmonella typhimurium, E. coli, Shigella 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 3.4 billion Rands (Pegram et al., 1998; Mackintosh and Calvin, 2002). This will indeed complicate the gloomy picture of the HIV/AIDS epidemic, 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 *Campylobacter jejuni* is incriminated in the etiology of Guillan Barre syndrome (GBS). GBS is an antoimmune disorder of the peripheral nervous system, which is characterised by acute flaccid paralysis (Coller et al., 2002). *C.jejuni* infection is the most frequently identified infection that precedes GBS (Nachamkin et al., 1998). Such cases have also been reported in South Africa (Lastovica et al., 1997). *Aeromonas* species and *Plesiomonas 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 lesions 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, occurring 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 unraveled 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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CHAPTER 4
CONCLUSIONS AND RECOMMENDATIONS

4.1 CONCLUSIONS

The following conclusions and recommendations are derivable from the study:

4.1.1 Scope and Frequency of Enteric Bacterial Pathogens Isolated from HIV/AIDS Patients and their Household Drinking Water in Limpopo Province

- Sex and Age distributions showed that 126 (38%) and 204 (62%) of the HIV positive individuals were males and females whereas HIV infection was predominantly in the age group 21-30. Enteropathogens were isolated from virtually all age groups.

- *Escherichia coli*, *Salmonella* species, *Campylobacter* spp., *Shigella* spp. and *Aeromonas* spp. were strongly associated with diarrhoea among HIV positive individuals.

- Profiles of enteric bacterial pathogens isolated from HIV positive individuals with and without diarrhoea were similar to those isolated from HIV negative individuals but at lower frequencies.

- Higher percentages of enteric pathogens were isolated from household drinking water of HIV negative individuals with diarrhoea as compared with those isolated from household drinking water of HIV positive individuals, though this was not of statistical significance.

- *Plesiomonas shigelloides* strains were the least isolated organisms in household drinking water of HIV positive and HIV negative individuals with and without diarrhoea.

- 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. shigelliodes* (1.3) and *Campylobater* (1.2). Thereore HIV positive individuals with diarrhoea had a higher likelihood of having diarrhoea due to *E. coli.*

4.1.2 Antiobiotic Resistance Profiles and Relatedness of Enteric Bacterial Pathogens

- Antibiotic resistance profiles showed that over 70% of all enteric bacterial pathogens isolated from HIV positive and negative individuals with and without diarrhoea and their household drinking water showed multiple antibiotic resistance to ampicillin, penicillin and amoxacillin.

- Antibiograms also showed that the majority of the enteric bacterial isolates from the various study groups were susceptible to ciprofloxacin, meropenem, imipenem, gentamicin, cefepime, nalidixic acid and piperacillin/tazobactam.

- The demonstration of the presence of enteric bacterial pathogens such as *Salmonella* species, *Escherichia coli and Shigella*-species in stool and water samples of the various study cohorts unraveled a linkage between HIV/AIDS and water. Therefore microbial water quality may be an important factor in the management of HIV/AIDS.

4.2 Recommendations

- Studies on home-based care providers to educate them on sanitation, hygiene and water quality and how these impinge on the HIV/AIDS epidemic should be conducted by relevant researchers and monitored by the relevant government department.

- Integration and effective coordination of activities of support groups, home-based care providers, NGO's, CBO's, people living with HIV/AIDS (PLWHA) and environmental health workers in order to foreground and address issues related to water quality and HIV/AIDS should be initiated by the WRC in conjunction with the National Department of Health.

- Periodic monitoring of the scope, frequencies and antibiograms of enteric bacterial pathogens isolated from water sources and HIV/AIDS patients in order to unravel new trends and update known profiles should be researched in conjunction with the designated government department or the National Research Foundation.

- Interventions should also include strategies to ensure the sustainability of water quality in child-headed households, institutionalised orphanages and home-based care settings.

- Extensive molecular epidemiological studies of bacterial enteric pathogens from stool samples of HIV/AIDS patients and water sources in order to determine the relatedness of bacterial stool pathogens from HIV/AIDS cases and water pathogens are necessary for clinical-epidemiological purposes.

- Determine the array of factors that may impact on household water quality and diarrhoeal cases among HIV/AIDS households and controls

- A study on the WRC strategies, challenges and sustainability of programmes designed to mitigate the impact of HIV/AIDS.

- Determination of the virulence markers of enteric bacterial pathogens isolated from HIV/AIDS patients and water samples.

Technology transfer and knowledge dissemination may be achieved through feedback to communities, viz organisation of seminars, focus group discussions and development of leaflets or by any other audio or visual means.