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Prevalence of *Escherichia coli* O157:H7 among Diarrhoeic HIV/AIDS Patients in the Eastern Cape Province-South Africa

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Abstract: This study investigated the prevalence of *Escherichia coli* O157:H7 in the stool of confirmed and non-confirmed diarrhoeic HIV/AIDS patients. *Escherichia coli* O157:H7 was isolated by culture-based and immunomagnetic separation from three hundred and sixty stool swabs. Identification was by conventional IMViC, 20E API and molecular techniques. Confirmed and non-confirmed diarrhoeic HIV/AIDS patients had 56.5% (74/131) and 43.5% (57/131) respectively of *E. coli* O157:H7. Molecular results indicated that the prevalence of *E. coli* O157:H7 was 12.16% (9/74) and 8.77% (5/57) from stool swabs of confirmed and non-confirmed diarrhoeic HIV/AIDS patients. Antimicrobial resistance was higher for *E. coli* O157:H7 isolates from stools of confirmed HIV/AIDS than it was for non-confirmed HIV/AIDS patients. *Escherichia coli* O157:H7 might be a silent cause of diarrhoea in HIV/AIDS patients. It is recommended that HIV/AIDS patients with diarrhoea should be screened for *E. coli* O157:H7 and surveillance programmes for these bacteria should be established in both urban and rural areas of South Africa.

Key words: Prevalence, *Escherichia coli* O157:H7, diarrhoea and HIV/AIDS

INTRODUCTION

Diarrhoea is a universally occurring infectious disease found mostly in developing countries. Diarrhoeal diseases cause an estimated 5 483 deaths, mostly among children (CSE, 2006). A wide assortment of organisms cause diarrhoea and many of them have only been discovered in recent years. While *E. coli* is part of the normal faecal flora of humans and animals, some strains can cause life-threatening diarrhoea (Gray, 1995). Enterotoxigenic *E. coli*, mostly spread by means of contaminated food and water, remains a major public health problem of children and young infants and especially to the severely immuno-compromised (CSE, 2006). Of the well-known pathogenic enterotoxigenic *E. coli* is the serotype O157:H7, however, some non-O157:H7 have as well been reported to cause human disease outbreaks (Centers for Disease Control, 2004). Unlike non-O157:H7, which is mostly associated with cattle and other ruminants, *E. coli* O157:H7 is the only well known strain of *E. coli* associated with water and food-borne diarrhoea in humans (Renter *et al.*, 2003). Some *E. coli* O157:H7 are referred to as non-motile *E. coli* O157:H⁻, because they have lost their flagella characteristics due to deletion in the flagella regulatory gene $fliC_{H7}$ (Feng *et al.*, 1996). Diarrhoeal outbreaks, which are linked to *E. coli* O157:H7 infections; are characterized by blood in the stools (not in all the cases); cramping abdominal pain, fever, nausea and vomiting (Olorunshola *et al.*, 2000; Koyange *et al.*, 2004).

Approximately 93% of HIV/AIDS patients develop significant gastrointestinal complications at some point during the course of their illness (Gazzard, 1988). As HIV progresses and the patient become more immune-compromised, the occurrence of gastrointestinal symptoms increases (May *et al.*, 1993). In Sub-Saharan Africa, recent estimates suggest that 6 out of every 10 men, 5 out of every 10 women and 9 out of every 10

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children are positive for HIV/AIDS(Shisana *et al.*, 2005). South Africa is severely affected, being a home to 4.8 million people living with HIV/AIDS. This translates to an HIV prevalence of 10.8% among persons aged 2 years and older (Shisana *et al.*, 2005).

The Eastern Cape Province has one of the highest HIV growth rates increasing from 20.2% in 2000 to 29.5% in 2005 for antenatal clinic attendees (Shisana *et al.*, 2005). The predicted incidence of HIV infections in the Eastern Cape Province is at 1.3% and as at June 2006, 194 443 people had died of HIV/AIDS and another 64 095 people were already sick with the disease (Dorrington *et al.*, 2006). Diarrhoea is also another disease that threatens the lives of rural communities in the Eastern Cape (Bradshaw *et al.*, 2000). The situation is further exacerbated by the prevalence of extreme poverty in the rural areas. Awareness of the clinical and therapeutic aspects of watery diarrhoea suspected to have been caused by *E. coli* O157:H7 in HIV/AIDS patients is therefore vital in directing diagnostic evaluation of these patients and further research to improve human health. This strain of *E. coli* is a threat to human health, especially to immuno-compromised person such as HIV/AIDS patients, as the bacteria can be contracted from food and water consumed by these patients. Also because of a compromised immune system, such patients may develop diarrhoea attributed to *E. coli* O157:H7 than it would be the case for immuno-competent persons. This is the baseline on which this investigation of the prevalence of *E. coli* O157:H7 in confirmed and non-confirmed HIV/AIDS diarrhoeic patients was based.

One of the effective ways of managing bacterial diarrhoea in immuno-compromised individuals is the use of antibiotics (Kelly *et al.*, 1996). Antibiotic susceptibility profiles of microorganisms have been documented to vary considerably (Sein *et al.*, 2005). There is no study known to our knowledge that has elucidated the presence of *E. coli* O157:H7 in HIV/AIDS patients, though recently Obi *et al.* (2007) isolated *E. coli* from stools of some HIV/AIDS patients with and without diarrhoea, *E. coli* O157:H7 was not investigated. The primary objective of this study was to investigate if *E. coli* O157:H7 is one of the bacteria involved in diarrhoea that habitually characterize HIV/AIDS patients. To guide clinicians in the Eastern Cape Province on the empirical treatment of diarrhoeal cases in HIV/AIDS patients, the anti-microbial susceptibility profiles of *E. coli* O157:H7 isolates obtained from the stools of these patients was also investigated.

MATERIALS AND METHODS

Sample size determination: A two-group continuity corrected χ^2-test (p = 0.050) one-sided significance and 95% power was used to estimate the sample size. A sample size of 186 in each group when β_1 is 0.060 for group 1 (confirmed HIV/AIDS patients) and β_2 is 0.160 for group 2 (non-confirmed HIV/AIDS patients) with odds ratio of 2.984; was found to be significant. This was estimated as a sample size that could bring out significance difference for the prevalence of *E. coli* O157:H7 in confirmed and non-confirmed HIV/AIDS patients (p = 0.05) (Fleiss *et al.*, 1980; Dixon and Massey, 1983). Hence equal sample sizes (180 for confirmed and 180 for non-confirmed diarrhoeic HIV/AIDS patients, which totals to 360) was chosen for the study (Fleiss *et al.*, 1980).

Study population and area: The study was conducted among confirmed HIV/AIDS and non-confirmed diarrhoeic HIV/AIDS patients visiting Frere Hospital for treatment of diarrhoea. Diarrhoea was diagnosed by the recruited nurse in the case of a patient experiencing 3 or more watery stools in 24 h. Frere Hospital is situated in the City of East London within the Amathole District in the Eastern Cape Province of South Africa. The Hospital basically carters for patients from the surrounding locations such as Mdantsane, King Williams Town, Berlin, Alice Butterworth, Umtata, City of East London, Sttutterheim, Gompo, Chulunna and other small villages. However, being a referral hospital, it also carters for special referral cases from other districts within Eastern Cape Province. The Amathole District has high levels of poverty and is one of the regions affected by the HIV/AIDS scourge, with about 8.6% of the population affected (Eastern Cape Department of Health, 2006).

Scientific ethics and informed consent: The University of Fort Hare's Govan Mbeki Research and Development Centre, the Provincial Department of Health (Bisho) and the Regional Eastern Cape Ethical Review Committee all approved the protocol that was used for the stool swab collection. Informed consent was obtained from patients or their guardians with the help of a nurse with Voluntary Counselling and Testing (VCT) skills and experience.

Stools swab collection procedure: Between March 2005 and August 2006, three hundred and sixty stool swabs were collected (180 from confirmed HIV/AIDS and another 180 from non-confirmed HIV/AIDS diarrhoeic patients). All the patients who consulted the hospital for diarrhoeal complaints and voluntarily consented by signing the informed consent form to give their stool specimen were recruited into the study. The confirmed HIV/AIDS patients had already been tested for HIV at the HIV/AIDS clinic of Frere Hospital and were known by the Hospital clinicians to be carriers of the HIV virus.

The stool swabs from non-confirmed HIV/AIDS diarrhoeic patients were used as controls. Sterile specimen bottles filled with 30 mL of sterile saline (Merck, SA) solution and sterile cotton swabs (Merck, SA) were taken to the HIV/AIDS and Outpatient clinics of Frere Hospital. The patients' biographical information such as gender, age, race, location and their diarrhoea and HIV/AIDS status was recorded. Anonymity of the patients was protected as much as possible.

The HIV/AIDS clinic provided stool swabs of confirmed HIV/AIDS patients with diarrhoea whereas the Outpatient clinic provided stool swabs of non-confirmed HIV/AIDS diarrhoeic patients. The swabbing of stools was done by the patients themselves. The patients were instructed by the nurse recruited to conduct sampling to touch the stools using the cottoned side of the swab. The patient then returned the cotton swab having the stool to the nurse. The stool swabs were then dipped into the sterile saline solutions in the specimen bottles, put into a cooler box filled with ice blocks and transported to the laboratory for the isolation of *E. coli* O157:H7. Specimens were duly processed within 1-4 h after their collection.

Culture-based isolation and identification of *E. coli* O157:H7 Enrichment and immunomagnetic separation recovery: One milliliter of each saline solution containing stool swabs was inoculated into 99 mL of modified *E. coli* (mEC) broth containing 20 µg mL^{-1} novobiocin (Merck, SA) (Cagney *et al.*, 2004). The suspensions were incubated in a shaking incubator (Gallenkamp, Loughborough, England) for 8 h at 37°C while rotating at 143×g. Twenty microlitres (20 µL) of the Dynabead (Dynal, Oslo) suspensions were incubated in a 1.5 mL Eppendorf tubes (Eppendorf, SA) with 1 mL aliquots of the pre-enriched samples at room temperature for 10 min with continuous mixing by vortex. This step was performed to allow the *E. coli* O157-specific antibodies coated onto the beads to bind to the target bacteria.

The bacterial-IMS bead complexes were separated using a magnetic particle concentrator, Dynal MPC-M (Dynal, Oslo) for 3 min (Dynal Product Brochure, 2006). After discarding the supernatants and washing the bead-particles using 0.02% (v/v) diluted (1:20) PBS-Tween 20 in distilled water (pH 7.2) (Merck, SA), the complete immunomagnetic separation and washing procedure were repeated twice (Dynal Product Brochure, 2006). The final bead-bacterial complexes were re-suspended in 1000 µL washing buffer (PBS-Tween 20) (Merck, SA). Fifty microlitres (50 µL) of the bacteria-IMS complex concentrate was transferred to *E. coli* O157 selective media cefixime (0.05 mg L^{-1}) and potassium tellurite

(2.5 mg L^{-1})-Sorbitol MacConkey (CT-SMAC) (Merck, SA) Agar and spread out into half of the plate using a sterile cotton swab. The swabbed portion using an inoculating loop was further streaked out onto the agar surface to achieve single isolated colonies (Dynal Product Brochure, 2006). The plates were then incubated at 37°C for 24 h. Sorbitol-non-fermenting colonies (up to 5 colourless colonies per plate per stool specimen) were randomly selected and further plated by streaking onto Eosin Methylene Blue (EMB) agar (Merck, SA) (Müller *et al.*, 2003; Cagney *et al.*, 2004). Seventy-four and 57 stools from confirmed and non-confirmed diarrhoeic HIV/AIDS patients, respectively, had presumptive *E. coli* O157 with greenish blue-black metallic sheen colour on EMB agar.

Identification of *E. coli* O157:H7: Presumptive *E. coli* O157 colonies were identified as described by Cagney *et al.* (2004). The Oxidase test was then conducted on colonies that were Gram negative before the conventional indole-methyl red-Voges-Proskauer-citrate (IMViC) test was performed (Feng, 1995; Heuvelink *et al.*, 1998; Radu *et al.*, 1998; Müller *et al.*, 2003). Out of 360 stool swabs (180 from confirmed HIV/AIDS and 180 from non-confirmed HIV/AIDS), 74 and 57 stool swabs were presumptively positive of *E. coli* O157.

One colony of presumptive *E. coli* O157, each representing the 74 and 57 stools from confirmed and non-confirmed diarrhoeic HIV/AIDS patients respectively, were subjected to IMViC test and further confirmed as *E. coli* with API 20E kits. The strips were then read and the final identification was secured using API LAB PLUS computer software (BioMérieux, Marcy-Etoile, France) (Momba *et al.*, 2006). Out of the 74 and 57 stools specimens, 25 and 29 presumptive *E. coli* O157 isolated from the stools of confirmed and non-confirmed diarrhoeic HIV/AIDS patients, respectively, demonstrated an identification profile for *E. coli* with API LAB PLUS computer software reader. These were further characterized using PCR.

Molecular characterization of *E. coli* O157:H7 using Polymerase Chain Reaction (PCR)
Bacterial DNA extraction: DNA was extracted from colonies identified as *E. coli* and from a positive control strain (*E. coli* O157:H7, ATCC 43895) using direct lysis method according to Torres *et al.* (2003). A loop-full of overnight culture of *E. coli* colonies was suspended in 200 µL of sterile Milli-Q PCR grade water (Merck, SA) and the cells were lysed using a Dri-block DB.2A (Techne, Cape Town, SA) for 15 min at 100°C. The cell debris was

removed by centrifugation at 20000×g for 2 min using a MiniSpin microcentrifuge (Eppendorf, SA). The lysate supernatant was placed on ice for 5 min.

Amplification of $fliC_{H7}$, $rfbE_{O157}$ and eaeA genes: Oligonucleotide primers specific for the targeted $fliC_{H7}$, $rfbE_{O157}$ and eaeA genes used in the polymerase chain reaction (PCR) were similar to those used by Wang *et al.* (2002). The primers sequences that were used to identify the target genes were FliC-F 5'-TAC CAT CGC AAA AGC AAC TCC-3', FliC-R 5'-GTC GGC AAC GTT AGT GAT ACC-3' for $fliC_{H7}$, RfbE-F 5'-CTA CAG GTG AAG GTG GAA TGG-3', RfbE-R 5'-AATT CCT CTC TTT CCT CTG CGG-3' for $rfbE_{O157}$ and the primers for eaeA gene were EAE-R 5'-ATG CTT AGT GCT GGT TTA GG-3', EAE-R 5'-GCC TTC ATC ATT TCG CTT TC-3'. The expected amplification sizes for $fliC_{H7}$, $rfbE_{O157}$ and eaeA genes were 247, 328 and 248 base pairs, respectively.

The PCR assays for $fliC_{H7}$, $rfbE_{O157}$ and eaeA (A/E) genes were carried out in a 50 µL reaction volume. The reaction mixture contained 10× SuperTherm GOLD Buffer, 1.5 mM $MgCl_2$, each of the four deoxynucleoside triphosphates (dNTPs) (Southern Cross Biotechnology, Cape Town, SA) at a concentration of 0.25 mM, 100 pmol each of $fliC_{H7}$, $rfbE_{O157}$ and eaeA specific primers, 5U of *Taq* DNA polymerase (Southern Cross Biotechnology, Cape Town, SA). The reaction was carried out in the Eppendorf model AG 22331 Thermocycler (Merck, SA). PCR conditions for $fliC_{H7}$, $rfbE_{O157}$ and eaeA (A/E) genes were similar to those of Wang *et al.* (2002). Sterile Milli-Q PCR grade water (Merck, SA) and reference *E. coli* O157: H7, ATCC 43895 strains were included in each PCR assay as negative and positive controls, respectively.

DNA electrophoresis: The amplicons (10 µL aliquots) were resolved on a 2% (w/v) agarose gel (Merck, SA) in 1 U TAE buffer(40 mM Tris-HCl, 20 mM Na-acetate, 1 mM EDTA, pH 8.5) and stained with 0.5 µg mL^{-1} Ethidium Bromide (EtBr) (Merck, SA) (Gunasekera *et al.*, 2000; Wang *et al.*, 2002; Cagney *et al.*, 2004). The amplified products were visualized and photographed under the BioDoc-It System (UVP Upland, CA 91786, USA). A 100 bp DNA ladder (Promega, USA) was included on each gel as a molecular size standard. The electrophoresis was carried out at 76 V for 1 h.

Anti-microbial susceptibility test: The Antimicrobial susceptibility test was determined using Bauer and Kirby disk diffusion technique on Mueller-Hinton Agar (Merck, SA) (Jorgensen *et al.*, 1999). Antibiotics that were used for the antimicrobial susceptibility test are listed in Table 1. For the inocula development the bacterial isolates

Table 1: Antibiotic susceptibility distribution of *E. coli* O157:H7 isolated from stools of confirmed HIV/AIDS and non-confirmed HIV/AIDS patients

Antibiotic agent	Antibiotic disc content (µg)	Susceptibility distribution (%)		
		R	I	S
Gentamycin	10	100(100)	0(0)	0(0)
Ampicillin	10	33.33(40)	3.70(40)	62.97(20)
Chloramphenicol	30	0(0)	88.88(80)	11.12(20)
Nalidixic acid	30	0(0)	77.80(80)	22.22(20)
Amikacin	30	0(0)	0(0)	100(100)
Ceftriaxone	30	0(0)	77.80(20)	22.22(80)
Erythromycin	15	100(100)	0(0)	0(0)
Tetracycline	30	100(40)	0(60)	0(0)

[a]R = Resistant, I = Intermediate, S = Susceptible. [b]The numbers in parenthesis are susceptibilities of *E. coli* O157:H7 isolated from stools of non-confirmed HIV/AIDS patients with diarrhoea whereas unparenthesised numbers are susceptibilities of *E. coli* O157:H7 isolated from stools of confirmed HIV/AIDS patients with diarrhoea. [c]The susceptibility interpretation was based on the breakpoints of zone diameters of individual antibiotic (NCCLS, 1999)

were grown overnight on nutrient broth at 37°C for reactivation. The cells were harvested by centrifugation at 2200 xg using a Beckman model TJ-6 centrifuge (Great Britain) and washed twice with 0.85% (w/v) saline water (NaCl) (Merck, SA). The bacterial cells were re-suspended in sterile saline solution to a final density of 10^7 cfu mL^{-1}. The cell suspensions were then swabbed onto a freshly prepared Mueller-Hinton Agar (MHA) (Merck, SA) in order to achieve a lawn of growth on incubation.

The antibiotic discs were placed on the surface of the inoculated Mueller-Hinton Agar (MHA) (Merck, SA) using a disc dispenser and the plates were incubated for 18 h at 37°C (Jorgensen *et al.*, 1999). Zones of growth inhibition were measured and results interpreted according to the guidelines of National Committee for Clinical Laboratory Standards for antimicrobial susceptibility testing (National Committee for Clinical Laboratory Standards, 1999). *Escherichia coli* ATCC 43895 was included as a positive control.

Statistical analysis: The statistical analysis was done using the Statistical Analysis System (SAS) (SAS Institute, Cary, USA) program. The Chi-square test was run at a statistical significance level of (p = 0.05).

RESULTS AND DISCUSSION

HIV/AIDS patients have a higher likelihood of developing diarrhoea than people with competent immune systems (Mitchell *et al.*, 1998; Hayes *et al.*, 2003). The results obtained by the culture-based method using IMS and selective media coupled with IMViC test indicated that 131 isolates of *E. coli* O157 were detected out of 360 stool swabs analyzed. About 56.5% (74/131) were from stool swabs of confirmed HIV/AIDS patients and

43.5% (57/131) were from non-confirmed HIV/AIDS patients. Confirmation of the identity of the isolates using API 20E showed that only 33.78% (25/74) of confirmed diarrhoeic HIV/AIDS patients and 50.88% (29/57) of the non-confirmed HIV/AIDS patients were *E. coli* O157:H7 positive. This was indeed contrary to the perception that confirmed HIV/AIDS patients would harbour more *E. coli* O157 than non-confirmed HIV/AIDS patients.

The prevalence of presumptive *E. coli* O157 in patients by gender was of profound interest. The level of presumptive *E. coli* O157 was found to be higher in females than in males in both confirmed and non-confirmed HIV/AIDS patients. Out of the 74 confirmed HIV/AIDS patients who were presumptively positive of *E. coli* O157, 73.3% (54/74) were females whereas 26.7% (20/74) were males. Moreover, out of the 57 non-confirmed HIV/AIDS patients who were presumptively positive of *E. coli* O157, 84.2% (48/57) were females whereas 15.8% (9/57) were males. The higher prevalence of *E. coli* O157 in females than in males has been observed in most epidemiological studies. A similar trend was reported in Cameroon in 1998 when an epidemic of diarrhoea due to *E. coli* O157 was investigated (Cunin *et al.*, 1999).

The non-confirmed diarrhoeic HIV/AIDS female patients had higher *E. coli* O157 prevalence (84.2%) than confirmed diarrhoeic HIV/AIDS females (73.3%). This observation was contrary to the expectation, based on the assumption that confirmed diarrhoeic HIV/AIDS patients have a compromised immune system and so are more vulnerable to *E. coli* O157 infections than non-confirmed diarrhoeic HIV/AIDS females. This discrepancy might be because non-confirmed HIV/AIDS patients were not tested for HIV/AIDS and so their HIV status was not known. Confirmed diarrhoeic HIV/AIDS males had a higher prevalence of *E. coli* O157 than non-confirmed diarrhoeic HIV/AIDS male patients did. This suggests that these confirmed diarrhoeic HIV/AIDS males might be more susceptible to *E. coli* O157 infections than non-confirmed diarrhoeic HIV/AIDS males.

The age distribution among *E. coli* O157 positive confirmed and non-confirmed HIV/AIDS patients indicated that confirmed and non-confirmed HIV/AIDS patients aged from 21 to 30 and 41 to 50, respectively had a higher prevalence of presumptive *E. coli* O157. There was a marked decrease in the prevalence of *E. coli* O157 in confirmed HIV/AIDS patients aged 21 to 30 from 41.9% down to 4.1% for those patients >50 years old. Contrarily for non-confirmed HIV/AIDS patients, there was an increase in the prevalence from 5.3% for those aged 21 to 30 up to 33.3% for those aged 41 to 50 (Fig. 1). This trend was also observed by Cunin *et al.* (1999). The prevalence of presumptive *E. coli* O157 by race was found
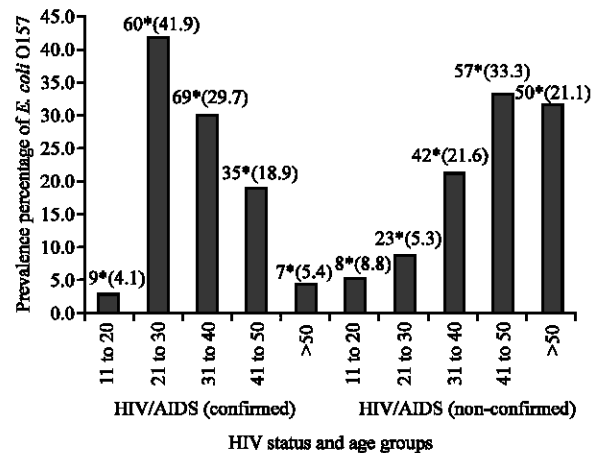

Fig. 1: Prevalence of *E. coli* O157 in confirmed and non-confirmed diarrhoeic HIV/AIDS patients by age. (*) sample size per age group
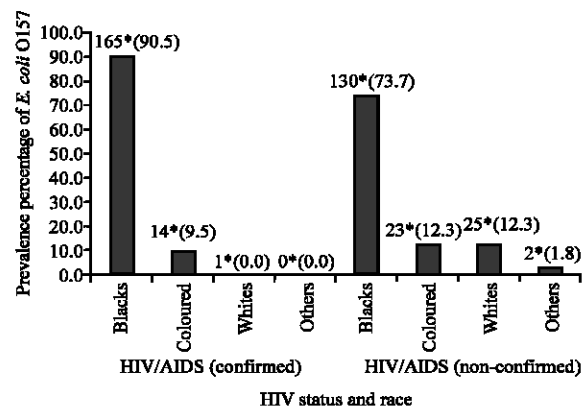

Fig. 2: Percentage prevalence of *E. coli* O157 in confirmed and non-confirmed diarrhoeic HIV/AIDS patients by race. (*) sample size per race

to be higher for the black race than the other races. This was at 90.5% for the confirmed HIV/AIDS patients and 73.7% for the non-confirmed HIV/AIDS patients of the black race. The prevalence of *E. coli* O157 in patients of the Colored race was 9.5% for confirmed diarrhoeic HIV/AIDS patients and 12.3% for the non-confirmed diarrhoeic HIV/AIDS patients of white race. No *Escherichia coli* O157 were noticed in stool from confirmed HIV/AIDS diarrhoeic patients of the White race. However, 12.3% of non-confirmed diarrhoeic HIV/AIDS patients of white race had *E. coli* O157 (Fig. 2). A clear picture has been highlighted with these results. The picture reveals an increase in the prevalence of presumptive *E. coli* O157 because of a faster growing sexually active but yet immunocompromised individuals due to HIV/AIDS affliction. HIV/AIDS has been reported

to be rife amongst the youths of ages between 15 and 30 years (Dorrington *et al.,* 2006). Nevertheless, the burden of *E. coli* O157 was felt across all the ages for both confirmed and non-confirmed HIV/AIDS patients.

By location, *E. coli* O157 prevalence was higher in East London than it was for other locations. This was probably because of the close proximity of Frere Hospital to East London. Secondly, it could be a reflection of rural-urban migration, which is known to affect most cities universally. The prevalence of presumptive *E. coli* O157 was at 75.7% (56/74) for confirmed HIV/AIDS diarrhoeic patients and 73.6% (42/57) for non-confirmed diarrhoeic HIV/AIDS patients originating from East London. Other locations that had a noticeable prevalence of *E. coli* O157 for the confirmed HIV/AIDS patients were Stutterheim 4.05% (3/74) while for each of the other locations (Butterworth, Duncan Village and Mdantsane) the prevalence was 2.7% (2/74). The rates of *E. coli* O157 prevalence for the locations of the non-confirmed diarrhoeic HIV/AIDS patients was 5.26% (3/57) for Butterworth, 3.51% (2/57) for Duncan Village, King Williams Town and Stutterheim. Localities, which had very low numbers of patients, were categorized as others and prevalence of presumptive *E. coli* O157 for confirmed diarrhoeic HIV/AIDS patients from such locations was 12.6% (9/74) whereas for the non-confirmed diarrhoeic HIV/AIDS patients the prevalence was 10.53% (6/57) (Fig. 3).

The use of PCR to characterize genes associated with *E. coli* O157:H7 has been documented (Gannon *et al.,* 1997; Fratamico *et al.,* 2000; Feng and Monday, 2000; Wang *et al.,* 2002). *Escherichia coli* O157:H7 is often diagnosed based on its virulence factors. Some of the virulence factors are *stx*1, *stx*2, *hly*A, *fliC*$_{H7}$, *rfb*E$_{O157}$, *eae*A and many other genes (Feng and Monday, 2000; Wang *et al.,* 2002). During this study, we employed PCR method to characterize the presumptive *E. coli* O157 isolates from the stool swab suspensions. Subsequent PCR analysis of the isolates using primers specific for *fliC*$_{H7}$, *rfb*E$_{O157}$ and *eaeA* genes indicated the presence of *E. coli* O157:H7. Representative gel electrophoreses profiles for the amplified products for the target genes are shown in Fig. 4.

Approximately 14.86% of the representatives of presumptive *E. coli* O157 isolates from stool swabs of confirmed diarrhoeic HIV/AIDS patients were all positive for *fliC*$_{H7}$ genes. However, only 12.16% of these isolates carried *rfb*E$_{O157}$ and *eaeA* genes. The other 2.70% lacked *rfb*E$_{O157}$ and *eaeA* genes. Such isolates were not considered as *E. coli* O157:H7. On the other hand, 12.28% of the representatives of presumptive *E. coli* O157 isolates from stool swabs of non-confirmed HIV/AIDS
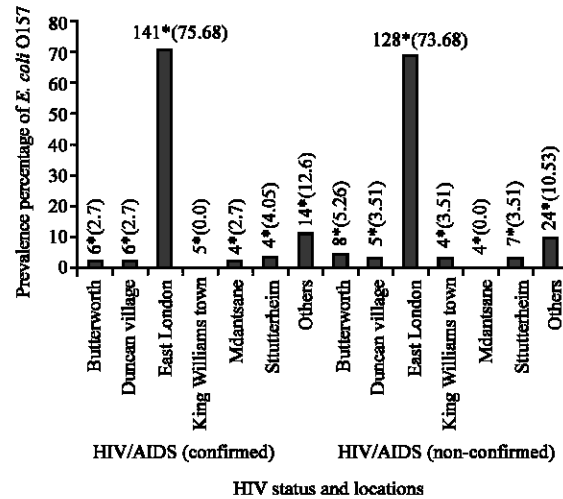

Fig. 3: Prevalence of *E. coli* O157 in diarrhoeic confirmed and non-confirmed HIV/AIDS patients visiting Frere Hospital, East London from various locations. (*) Number of patients diagnosed with diarrhoea per location

patients that were positive for *fliC*$_{H7}$, only 8.77% carried *rfb*E$_{O157}$ and *eaeA* genes. The other 3.51% lacked *rfb*E$_{O157}$ and *eaeA* genes. Only the representatives of presumptive *E. coli* O157 that had the three target genes (*fliC*$_{H7}$, *rfb*E$_{O157}$ and *eaeA*) were considered as *E. coli* O157:H7. This implies that the percentage proportion of confirmed diarrhoeic HIV/AIDS patients who had *E. coli* O157:H7 was higher than that for the non-confirmed diarrhoeic HIV/AIDS patients.

Out of the nine patients whose presumptive *E. coli* O157 isolates were positive for genes specific for *E. coli* O157:H7, five were from East London, two from Stutterheim and one each from Chulumna and Duncan Village. On the other hand only 5 of the 57 (8.77%) of the presumptive *E. coli* O157 isolates from stool swabs of non-confirmed diarrhoeic HIV/AIDS patients carried *fliC*$_{H7}$, *rfb*E$_{O157}$ and the attaching and effacing (A/E) (*eaeA*) genes. Out of these 5 patients, 4 of them were from East London and 1 was from Butterworth.

The PCR detection of *fliC*$_{H7}$, *rfb*E$_{O157}$ and *eaeA* genes of *E. coli* O157:H7 is an excellent indication of the presence of *E. coli* O157:H7 (Wang *et al.,* 2002; Cagney *et al.,* 2004). The PCR analysis using *fliC*$_{H7}$, rfbE$_{O157}$ and *eaeA*-specific primers confirmed that a genetic region homologous in size to the *E. coli* O157:H7 *fliC*$_{H7}$, *rfb*E$_{O157}$ and *eaeA* structural gene was present in the *E. coli* O157 isolates. It is therefore interesting to note that the PCR assay was successful in amplifying the 247bp *fliC*$_{H7}$, 327bp *rfb*E$_{O157}$ and 248bp *eaeA* fragments that were present in the genomic DNA of the isolates from
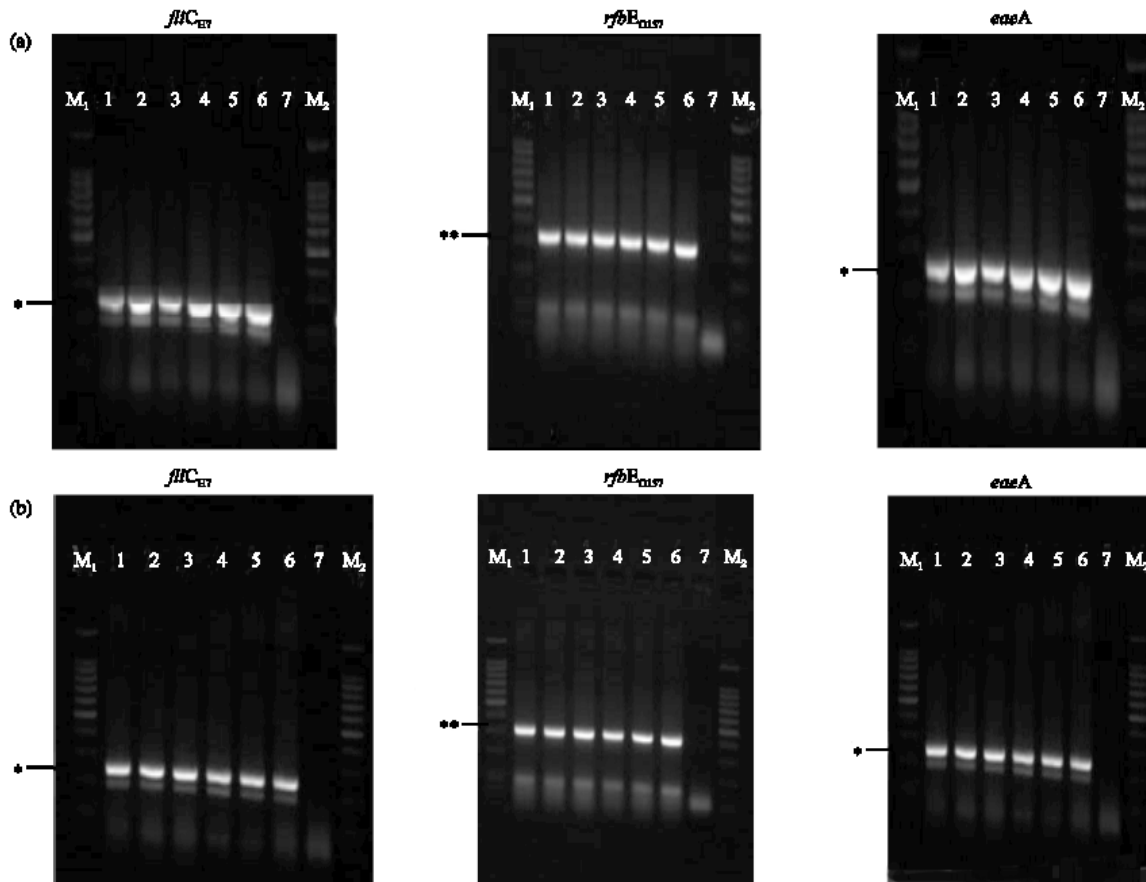
Fig. 4: The amplified $fliC_{H7}$, $rfbE_{O157}$ and $eaeA$ gene of *E. coli* O157:H7 isolated from stool swabs of (a) confirmed diarrhoeic HIV/AIDS (b) non-confirmed diarrhoeic HIV/AIDS patients. Lanes M_1 and M_2: 100 bp DNA ladder marker (Promega, USA). All lanes 1 and 7: Positive (*E. coli* O157:H7, ATCC 43895) and negative control, respectively. In (a) lanes 2: s_{235}, lanes 3: s_{237}, lanes 4: s_{242}, lanes 5: s_{247}, lanes 6: s_{323} whereas in (b) lanes 2: s_7, lanes 3: s_{116}, lanes 4: s_{117}, lanes 5: s_{125} and lanes 6: s_{355}. Expected amplifications were at 247, 327 and 248 bp as indicated by *, ** and *, respectively

stools of confirmed and non-confirmed HIV/AIDS patients. The χ^2-value of 0.034 predicted that gender was a significant variant for *E. coli* O157 infection and that females were more likely to be infected with *E. coli* O157:H7 than males. However, the χ^2-values of 0.058, 0.57 and 0.58 for HIV status, age and race respectively were insignificant in determining the prevalence of *E. coli* O157 in confirmed and non-confirmed HIV/AIDS patients. The arguments for the significance were based on a statistical significance level ($p \leq 0.05$).

Formerly curable bacterial diseases have developed antibiotic resistance and new bacterial pathogens have emerged. This problem has been compounded by the global spread of HIV and AIDS as reported by Paine and Flower (2002). The duo also blames this phenomenon primarily on antibiotic mishandling by both health practitioners and patients (Paine and Flower, 2002).

Resistance to antibiotics by bacterial pathogens infecting HIV/AIDS patients, just as in non-HIV/AIDS patients, may spread through the resident bacteria developing resistant genes against drugs that are being used on these patients. The pathogenic bacteria then acquire the resistance genes through lateral or horizontal gene transfer (Ochman *et al.*, 2000; Paine and Flower, 2002).

The present study equally investigated anti-microbial susceptibilities of the *E. coli* O157:H7 isolates towards some commonly used antibiotics. This test was conducted on the isolates confirmed by PCR as being *E. coli* O157:H7. All the fourteen (100%) *E. coli* O157:H7 isolates from the stools of confirmed and non-confirmed HIV/AIDS patients were susceptible to amikacin. Susceptibility (S) to ampicillin was amongst 62.97% of the *E. coli* O157:H7 isolates from the stools of confirmed HIV/AIDS patients and to 80% of *E. coli* O157: H7

isolates from the stools of non-confirmed HIV/AIDS patients were susceptible to ceftriaxone. All the nine out of nine (9/9) *E. coli* O157:H7 isolates from stools of confirmed HIV/AIDS patients were resistant to gentamyacin, erythromycin and tetracycline whereas *E. coli* O157:H7 isolates from stools of non-confirmed HIV/AIDS patients had 100% resistance against gentamyacin and erythromycin only. Approximately 33.33 and 40% of *E. coli* O157:H7 isolates from stools of confirmed and non-confirmed HIV/AIDS patients, respectively were resistant to ampicillin. An intermediate susceptibility to chloramphenicol was amongst 88.88% of the *E. coli* O157:H7 isolates from stool swabs of confirmed HIV/AIDS patients whereas 77.8% of the isolates were intermediately susceptible (I) to both nalidixic acid and ceftriaxone (Table 1).

The high rate of resistance against the antibiotics by *E. coli* O157:H7 isolates from stools of confirmed HIV/AIDS and non-confirmed diarrhoeic HIV/AIDS patients could be due to the use of antibiotics by these patients to control their diarrhoeal conditions (Kelly *et al.*, 1996). However, the type of antibiotics, which were prescribed to the patients, was not established, even though, most HIV/AIDS patients are often assumed registered for antiretroviral drugs. The present study could assist medical practitioners in the Eastern Cape in the administration of antibiotics to patients, taking into account their HIV/AIDS status. During a diarrhoea outbreak in Cameroon, the *E. coli* O157:H7 that were isolated from the stool specimen were found to be resistant to amoxicillin and chloramphenicol, but were sensitive to tetracycline and nalidixic acid (Cunin *et al.*, 1999). The antibiotic profile of the present study and the Cameroon one; show how antibiotic profile of organisms can vary from country to country.

Escherichia coli O157:H7 was more prevalent in the stools of confirmed HIV/AIDS patients than in those of non-confirmed HIV/AIDS patients. However, the prevalence was much more dependent on gender than the age, race and HIV/AIDS status of the patients. The distribution of the *E. coli* O157 strains with regard to the age of the patients showed that *E. coli* O157:H7 infects all ages. The prevalence of *E. coli* O157:H7 was ubiquitous with respect to patients' locations; however, it was noted mostly in urban patients. Although some of the *E. coli* O157:H7 isolates were sensitive to some of the commonly used antibiotics, previous studies have opposed the use of antibiotics in the treatment of diarrhoea linked to *E. coli* O157:H7 (Wong *et al.*, 2000). It is recommended that HIV/AIDS patients with prolonged diarrhoea should be routinely screened for this bacterium. The Eastern Cape Department of Health should implement strategies to curb spread of *E. coli* O157:H7 outbreaks.

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