Prevalence of enterohaemorrhagic *Escherichia coli* O157:H7 in drinking water and its predicted impact on diarrhoeic HIV/AIDS patients in the Amathole District, Eastern Cape Province, South Africa

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Abstract

Immunosuppressed persons such as HIV/AIDS patients are at risk of acquiring diarrhoeal infections from water-borne *E. coli* O157:H7. In the present study, we investigated the prevalence of *E. coli* O157:H7 in drinking water collected from selected distribution systems within the Amathole District of the Eastern Cape and its predicted impact on diarrhoeic conditions of HIV/AIDS persons living in this area. One hundred and eighty water samples and 360 stool swabs from confirmed and non-confirmed HIV/AIDS diarrhoeic patients were analysed. *Escherichia coli* O157:H7 were isolated using enrichment culture and confirmed using molecular techniques.

Of the 180 drinking water samples, 46 (25.56%) were positive for *E. coli* O157. The prevalence of *E. coli* O157 in the stools was at 36.39% (131/360) of which 56.5% (74/131) and 43.5% (57/131) were from stools of confirmed and non-confirmed HIV/AIDS patients, respectively. Molecular analysis of 27, 25 and 29 representative presumptive *E. coli* O157 from water and stools of confirmed and non-confirmed HIV/AIDS patients, respectively, revealed that 14.81%, 36% and 17.24% of the isolates were *E. coli* O157:H7. The findings predicted a possible link between *E. coli* O157:H7 isolated from drinking water and diarrhoeic conditions of both confirmed and non-confirmed HIV/AIDS patients visiting Frere Hospital for treatment.

Keywords: prevalence, drinking water, HIV/AIDS, stool specimens, *Escherichia coli* O157:H7 and PCR

Introduction

Safe drinking water that complies with the South African National Standards (SANS) 241 Drinking Water Specification (SANS, 2001) does not pose a significant risk to public health (especially for babies, infants, immunosuppressed persons such as HIV/AIDS individuals, and the elderly) over a lifetime of consumption. This is the norm in almost all South African metropolitan areas. However, in many rural and some peri-urban areas, the situation is very different. Prior to 1994, 30 to 40% of the South Africa's population (approximately 14 to 18 m. people) was without adequate water supply. As recently as 2004, some 4 m. people were still obtaining water from rivers, ponds and springs, (Kasrils, 2004), which were usually not treated and were faecally contaminated (Muyima and Ngcakani, 1998; Momba and Kaleni, 2003; Momba and Notshe, 2003). While the present South African Government has implemented many rural water supply schemes under the National Reconstruction and Development Programme, where rural water supplies do exist, drinking water is often of poor quality and considered unsafe (Momba et al., 2003; 2004a;b; 2005a;b; 2006a;b; Obi et al., 2006). This means that consumers are at risk of contracting water-borne diseases even from treated water supplies.

By the year 2002, a large proportion of the Eastern Cape population (68%) still lived below the South African national poverty line (UNDP, 2004) and approximately 11% and 38% of the population lived in informal and traditional structures, respectively. Piped water distribution had reached only 62% of households and 31% of households had no toilet facilities (Statistics South Africa, 2003). The Amathole District Municipality, where this research was conducted, is in the Eastern Cape Province, which is home to 12.1% of HIV positive persons in South Africa (Dorrington et al., 2006). By June 2006, Dorrington et al. (2006) reported that the majority of these HIV-positive individuals in the Eastern Cape Province were youths with an estimated prevalence of 16.4%. 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). However, it is not only HIV/AIDS that has ravaged the Eastern Cape community; other diseases such as strokes, hypertensive heart disease, diarrhoeal diseases, diabetes mellitus and tuberculosis have also been reported (Bradshaw et al., 2000).

The World Health Organization (WHO) estimated that about 88% of diarrhoeal diseases in the world are attributed to unsafe water, sanitation and hygiene. Approximately 3.1% of annual deaths (1.7 m.) and 3.7% of the annual health burden (disability-adjusted life years [DALYSs]) worldwide (54.2 m.) are attrib-

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Received 26 October 2007; accepted in revised form 21 April 2008.

uted to unsafe water, sanitation and hygiene (WHO, 2003). The impact of diarrhoeal diseases is significant in South Africa, with annual estimated deaths of about 50 000. Three million cases of illnesses and treatment cost the state about R3.4 bn. (Pegram et al., 1998; Mackintosh et al., 2002; Sapkota et al., 2004). The most alarming situation is the death of about 20% of all children under 5 years of age living in settlements with access to rudimentary water supply and sanitation (Bourne and Coetzee, 1996).

Diarrhoea occurs in about 30 to 60 % of HIV/AIDS patients in developed countries and in an estimated 90% of such patients in developing countries (Sapkota et al., 2004). Epidemiological investigations during outbreaks have identified *E. coli* O157:H7 as a pathogenic strain that causes severe and life-threatening diarrhoea (Galane and Le Roux, 2001). *Escherichia coli* O157:H7 infections pose the greatest risk to immunosuppressed individuals because it can easily invade the cells of HIV/AIDS patients due to a suppressed cell-mediated immunity (Morris and Potter, 1997; Hoffman, 2004). 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 supplies to communities (Ashton and Ramasha, 2002).

Although HIV/AIDS and water-borne diarrhoeal diseases are among the leading causes of morbidity and mortality in South Africa, the association of diarrhoea disease-causing agent such as *E. coli* O157:H7 in drinking water and the diarrhoeal conditions of HIV/AIDS individuals still remains unknown in South Africa**.** We, therefore, conducted the present study to ascertain the prevalence of *E. coli* O157:H7 in drinking water collected from selected distribution sources within the Amathole District of the Eastern Cape Province and its predicted impact on diarrhoeic conditions of HIV/AIDS persons living in this area.

Experimental

Study site

The study was conducted between March 2005 and August 2006. The Amathole District was selected based on the researchers' familiarity with the area, HIV prevalence and the presence of a referral hospital, Frere Hospital, which is situated in the City of East London and caters for HIV/AIDS patients of various races, gender and age groups from rural areas of the district. Fort Beaufort, Alice, Dimbaza, Mdantsane, Ngwenya and Kwasaki were chosen because potential pathogenic *E. coli* strains have been reported to be predominant in water sources used by these communities (Momba and Kaleni, 2003; Momba and Notshe, 2003; Momba et al., 2004a;b; 2005a;b).

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 Committees 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.

Collection of samples

In total, 180 water samples (30 for each site) were collected from the standpipes that supplied treated drinking water to the communities of Fort Beaufort, Alice, Dimbaza, Mdantsane, and untreated groundwater to the communities of Ngwenya and Kwasaki, using internationally accepted techniques and principles.

Three hundred and sixty stool swabs were obtained from confirmed HIV/AIDS positive (180 stool swabs) and non-confirmed HIV/AIDS (180 stool swabs) diarrhoeic patients visiting Frere Hospital for treatment, using sterile cotton swabs (Merck, Johannesburg, SA). The stools swabs were dipped in sterile specimen bottles filled with 30 m of 1% (w/v) sterile saline solutions (Merck, SA). 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 patients' locations and their diarrhoeal conditions and HIV/AIDS status were recorded. Anonymity of the patients was protected as much as possible. Diarrhoeic stools in this study were diagnosed in the case of patients experiencing three or more watery stools in 24 h. The water samples and stool swabs were transported on ice in a cooler box and transported to the laboratory for the isolation of *E. coli* O157:H7. Microbiological analyses were performed within 1 to 4 h of their collection.

Isolation of *E. coli* O157:H7

Escherichia coli O157:H7 from water samples and stool swabs of patients was isolated using a pre-enrichment process followed by immunomagnetic separation and spread-plate procedure. For pre-enrichment, 1 mℓ of water sample or saline stool swab suspension was added to 99 mℓ of modified *E. coli* (mEC) broth containing 20 $\mu g \cdot m\ell^{-1}$ of Novobiocin (n) (Merck, SA) (Heuvelink et al., 1998). The samples were incubated for 8 h at 37°C on a rotary shaker (143 × g) (Gallenkamp, Loughborough, England). Thereafter, 1 mℓ of pre-enriched water sample or stool swab suspension was added to an Eppendorf tube(Eppendorf, SA) containing 20 $\mu \cdot m\ell^{-1}$ of magnetic beads coated with antibody to O157.

Immunomagnetic separation (IMS) proceeded as described by the Dynabead manufacturer (Dynal Product Brochure**,** 2006). The bacterial-IMS bead complex were re-suspended into 1% (w/v) sterile Phosphate Buffered Saline (PBS) (Merck, SA) and 50 µℓ of this bacterial-IMS concentrate was spread in duplicate onto Sorbitol-MacConkey agar supplemented with cefixime ($0.05\ mg \cdot m\ell^{-1}$) and potassium tellurite ($2.5\ mg \cdot m\ell^{-1}$) (CT-SMAC) (Merck, SA) agar plates (Müller et al., 2003). The plates were then incubated at 37°C for 24 h. Sorbitol-non-fermenting colonies (up to 5 colourless colonies per plate per sample) were randomly selected and further plated onto Eosin Methylene Blue (EMB) agar (Merck, SA). Forty six water samples and 74 and 57 stools from confirmed and non-confirmed HIV/AIDS patients respectively, had presumptive *E. coli* O157 with greenish blue black metallic sheen on EMB agar.

Identification of *E. coli* O157

Escherichia coli O157 colonies were identified as described by Cagney et al. (2004). The Oxidase test was performed on the colonies that were Gram negative prior to the conventional indole-methyl red-Voges-Proskauer-citrate (IMViC) tests (Heuvelink et al., 1998; Müller et al., 2003). Colonies of presumptive *E. coli* O157 each representing the 46 water samples and 74 and 57 stool samples from confirmed and non-confirmed HIV/AIDS patients respectively, were subjected to IMViC test and further confirmed as *E. coli* with API 20E kits.

The strips were read and final identification was secured using API LAB PLUS computer software (BioMérieux, Marcy-

Available on website http://www.wrc.org.za
ISSN 0378-4738 = Water SA Vol. 34 No. 3 July 2008
ISSN 1816-7950 = Water SA (on-line)

TABLE 1
Base sequences, locations and predicted sizes of amplified products for *fliC*$_{H7,}$ *rfbE*$_{O157}$ and *eaeA* specific oligonucleotide primers (Wang et al., 2002)

Primer Set	Primer	Primer Sequence (5′ to 3′)	Target gene	Location within gene	PCR amplicon (bp)
A	FliC-a	TAC CAT CGC AAA AGC AAC TCC	*fliC*$_{H7}$	1068–1088	247
	FliC-b	GTC GGC AAC GTT AGT GAT ACC		1314–1294	
B	RfbE-a	CTA CAG GTG AAG GTG GAA TGG	*rfbE*$_{O157}$	673–693	327
	RfbE-b	AATT CCT CTC TTT CCT CTG CGG		999–979	
C	EAE-a	ATG CTT AGT GCT GGT TTA GG	*eaeA*	132–151	248
	EAE-b	GCC TTC ATC ATT TCG CTT TC		379–360	

Etoile, France) (Momba et al., 2006b). Of the 46 water samples, 74 and 57 stool swabs of confirmed and non-confirmed HIV/AIDS patients, respectively, only 27, 25 and 29 representative *E. coli* O157 from these respective samples had 99.9% profile for *E. coli* with API PLUS computer software program, thus these were used for molecular characterisation.

Molecular characterisation of *E. coli* O157:H7 using polymerase chain reaction (PCR)

Bacterial DNA extraction

DNA was extracted from colonies identified as *E. coli* O157 and also from a positive control strain (*E. coli* O157:H7, ATCC 43895) purchased from the Microbiology Department of the National Health Laboratory Services (NHLS), Johannesburg, South Africa. The extraction was performed according to the method previously used by Torres et al. (2003). Briefly, 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 20 000 × g for 2 min using a MiniSpin micro-centrifuge (Merck, SA). The lysate supernatant was placed on ice for 5 min. Sterile Milli-Q PCR grade water (Merck, SA) was included in each PCR assay as a negative control.

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) (Table 1). Three sets of primer mixtures were prepared according to the method used by Wang et al. (2002). A total volume of 10 µℓ genomic DNA was used in each PCR reaction. The PCR assays for *fliC*$_{H7,}$ *rfbE*$_{O157}$ and *eaeA* genes were carried out in a 50 µℓ reaction volume containing 10× SuperTherm GOLD Buffer, 1.5 mM $MgCl_2$, each of the four deoxynucleoside triphosphates (dNTPs) (Southern Cross Biotechnology, SA) at a concentration of 0.25 mM, 100 pmol for each of *fliC*$_{H7,}$ *rfbE*$_{O157}$ and *eaeA* specific primers, and 5 U of *Taq* DNA polymerase (Southern Cross Biotechnology, SA).

The reaction was carried out in the Eppendorf model AG 22331 Thermocycler (Merck, SA). The following PCR conditions for *fliC*$_{H7,}$ *rfbE*$_{O157}$ and *eaeA* genes optimised in our laboratory were similar to those previously used by Wang et al. (2002). Initial denaturation at 95°C for 8 min; followed by 30 cycles of amplification, denaturation at 95°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s. The final extension cycle was followed by incubation at 72°C for 7 min and cooling to 4°C.

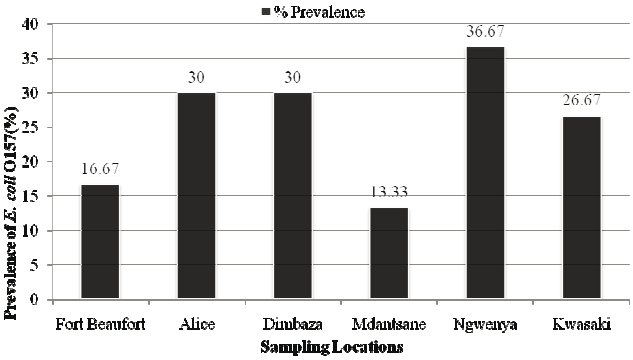

Figure 1
Prevalence of presumptive E. coli *O157 in drinking water from various distribution points in Amathole district. The 'n' sample size was 30 for all the sampling locations.*

DNA electrophoresis

The amplicons (10 µℓ aliquots) were visualised 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). The amplified products were photographed under the BioDoc-It System (UVP Upland, CA 91786, USA). A 100-bp DNA ladder (Promega, Madison, USA) was included on each gel as a molecular size standard. The electrophoresis was carried out at 76 V for 1 h.

Statistical analysis

The statistical analysis was done by use of SAS program (SAS Institute, Cary, NC). The *Chi* square test was used to establish the significance difference between the prevalence rates of *E coli* O157:H7 in stool samples of confirmed HIV/AIDS patients and those of non-confirmed HIV/AIDS patients. The *Chi* square test was run at a statistical significance level of ($P \leq 0.05$).

Results

Prevalence of *E. coli* O157 in water samples

Of the 180 drinking water samples, 46 (25.56 %) were positive for *E. coli* O157. Water samples collected from Fort Beaufort, Alice and Dimbaza, Mdantsane, Ngwenya and Kwasaki were positive at prevalence rates of 16.67% (5/30), 30% (9/30), 30% (9/30), 13.33% (4/30), 36.67% (11/30) and 26.67% (8/30) respectively (Fig. 1).

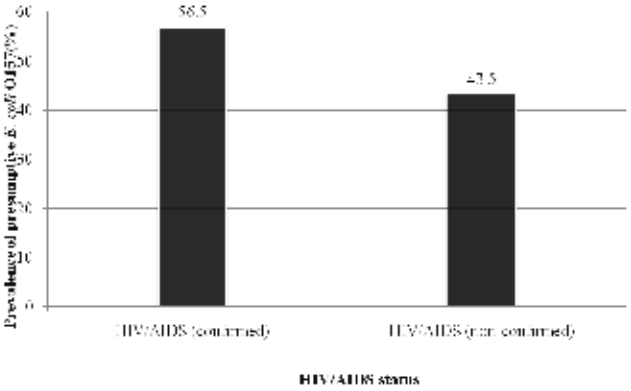

Figure 2
Prevalence of presumptive E. coli *O157 in diarrhoea confirmed and non-confirmed HIV/AIDS patients visiting Frere Hospital, East London. 'n' sample size was 131 for patients presumptively positive for* E. coli *O157:H7.*

Prevalence of *E. coli* O157 in stool specimens of diarrhoeic patients

Of the 360 stool swabs of diarrhoeic patients, 131 (36.39%) were positive for *E. coli* O157. About 56.5% (74/131) were from confirmed HIV/AIDS diarrhoeic patients and 43.5% (57/131) were from non-confirmed HIV/AIDS diarrhoeic patients (Fig. 2). By location, the prevalence of *E. coli* O157 was at 75.7% (56/74) for confirmed HIV/AIDS patients and 73.6% (42/57) for non-confirmed HIV/AIDS patients originating from East London.

Another location that had a noticeable prevalence of *E. coli* O157 for the confirmed HIV/AIDS patients was Stutterheim 4.05% (3/74) whereas locations such as Duncan Village and Mdantsane had a prevalence of 2.7% (2/74). The rates of *E. coli* O157 prevalence for the other locations of the non-confirmed HIV/AIDS patients were 5.26% (3/57) for Butterworth, 3.51% (2/57) for Duncan Village, King Williams Town and Stutterheim whereas Mdantsane had no *E. coli* O157. Localities, which had very low numbers of patients, were categorised as 'Others' and *E. coli* O157 prevalence for confirmed HIV/AIDS patients from such locations was 9.5% (7/74) whereas for the non-confirmed HIV/AIDS patients the prevalence was 8.77% (5/57). Although the study focused on the Amathole District Municipality and the majority of the patients who visited Frere Hospital originated from this district, some of the patients originated from other districts (Table 2). The χ^2 value of 0.058 predicted that HIV/AIDS status was a significant variant for *E. coli* O157 infection. The arguments were based on the statistical significance level at which the χ^2 test was run ($P \leq 0.05$).

Molecular characterisation of isolates

Water samples isolates identified by biochemical profiles for *E coli* O157 that were positive by PCR for $fliC_{H7,}$ $rfbE_{O157,}$ and *eaeA* genes characteristics of *E. coli* O157:H7 are summarised in Table 3. Of the 27 water samples positive for *E. coli* O157 isolates, 4 (14.81%) carried $fliC_{H7,}$ $rfbE_{O157}$ and *eaeA* genes whereas 3.70% (1/27) was only positive for $fliC_{H7}$ gene. The three target genes of *E. coli* O157:H7 ($fliC_{H7,}$ $rfbE_{O157,}$ and *eaeA)* under the present study were noticed in isolates obtained from Dimbaza, Fort Beaufort, Ngwenya and Mdantsane water samples. Representative gel electrophoretic profiles of amplified products of target genes for *E. coli* O157:H7 are illustrated in Fig. 3.

TABLE 2
Patients visiting Frere Hospital in East London for the treatment of diarrhoea (March 2005 and August 2006)

District	Location	Number of patients	
		Confirmed HIV/AIDS	Non-confirmed HIV/AIDS
Amathole	Berlin	1(3)	1(8)
	Duncan Village	2(4)	2(3)
	East London	56(80)	42(86)
	King Williams Town	0(5)	2(2)
	Mdantsane	2(2)	0(4)
	Stutterheim	3(1)	2(5)
	Butterworth	2(4)	3(5)
	[a]Others	7(7)	5(8)
OR Tambo	Umtata	0(0)	0(1)
Chris Hani	Queenstown	0(0)	0(1)
Ethekwini	Durban	1(0)	0(0)
Total number of patients		74(106)	57(123) = N= 360

[a]Represents locations where only one patient who was either confirmed or non-confirmed HIV/AIDS with diarrhoea came from while visiting Frere Hospital for treatment during the study period. These locations included Cathcart, Chulumra, Idutywa, Kentani, Peddie and Tshomo. Others were Elliot, Engobo, Mooiplaas, Peddie and Southern Wood for the confirmed HIV/AIDS patients. On the other hand, locations for the non-confirmed patients categorised as others also included Alice, Bedford, Willowvale, Kentani and Mooiplaas, Cala, Cathcart, Chulumra and Gombia, Idutywa, Jongolanya, Komga and McClean Town. The numbers in parenthesis are patients whose stool swabs were negative for E. coli *O157:H7 even though they had diarrhoea.*

TABLE 3
Summarised PCR amplified genes of *E. coli* O157:H7 isolated from drinking water from various distribution points in Amathole district

Water source	Amplified genes			Water samples positive for *E. coli* O157:H7
	$fliC_{H7}$	$rfbE_{O157}$	eaeA	
[a]Alice	+	–	–	0
Dimbaza	+	+	+	1
Fort Beaufort	+	+	+	1
Ngwenya	+	+	+	1
Mdantsane	+	+	+	1
Total number of water samples positive with *E. coli* O157:H7				4([a]1)

'+' target gene present, '–' target gene absent. n = 27 Representative number of presumptive E. coli O157 characterised by PCR. [a] Represents water samples whose E. coli O157 only had either 1 or 2 of the targeted genes. These water samples were not considered as being positive for E. coli *O157:H7 due to the absence of the other genes.*

Available on website http://www.wrc.org.za
ISSN 0378-4738 = Water SA Vol. 34 No. 3 July 2008
ISSN 1816-7950 = Water SA (on-line)

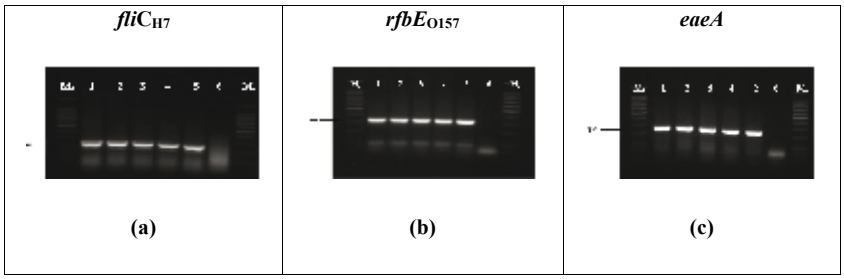

Figure 3
The amplified fliC$_{H7}$ rfbE$_{O157}$ and eaeA gene of E. coli O157:H7 isolated from drinking waters from Ngwenya, Dimbaza, Mdantsane and Fort Beaufort. Lanes M$_1$ & M$_2$: 100 bp DNA ladder marker (Promega, USA), Lane 1: Positive control (E. coli O157:H7, ATCC 43895), Lane 2:NgweW$_4$, Lane 3: DimW$_9$, Lane 4: MdaW$_{27}$, Lane 5: FbW$_{27}$, Lane 6: Negative control. The expected molecular sizes of fliC$_{H7,}$ rfbE$_{O157}$ and eaeA fragments were at 247 bp, 327 bp and 248 bp respectively.

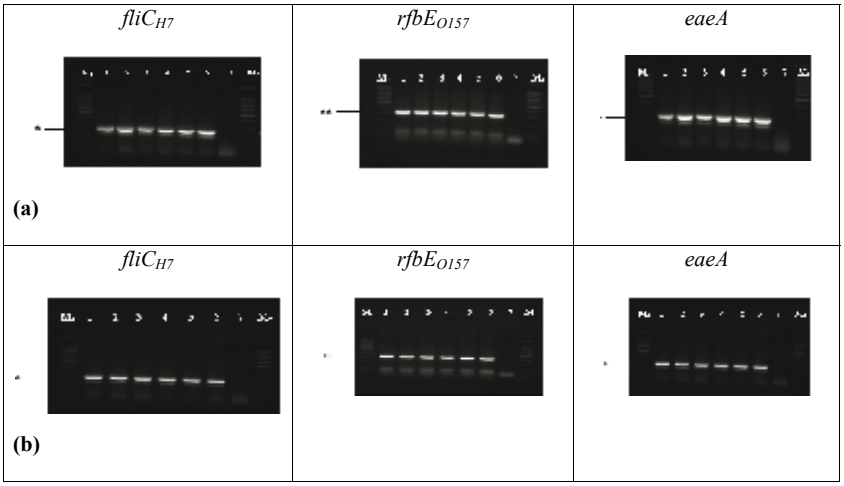

Figure 4
The amplified fliC$_{H7}$ rfbE$_{O157}$ and eaeA gene of E. coli O157:H7 isolated from stool swabs of (a) confirmed HIV/AIDS (b) non-confirmed HIV/AIDS patients all with diarrhoea. Lanes M$_1$ & M$_2$: 100 bp DNA ladder marker (Promega, USA). All Lanes 1 and 7: Positive (E. coli O157:H7, ATCC 43895) and negative controls, respectively. In (a) Lanes 2: F$_{235}$, Lanes 3: F$_{237}$, Lanes 4: F$_{242}$, Lanes 5: F$_{247}$, Lanes 6: F$_{323}$ whereas in (b) Lanes 2: F$_7$, Lanes 3: F$_{116}$, Lanes 4: F$_{117}$, Lanes 5: F$_{125}$ and Lanes 6: F$_{355}$. The expected molecular sizes of fliC$_{H7,}$ rfbE$_{O157}$ and eaeA fragments were at 247 bp, 327 bp and 248 bp respectively.

Molecular analysis of isolates obtained from stool specimens of diarrhoeic patients revealed that *fliC$_{H7}$*, *rfbE$_{O157}$* and *eaeA* genes were present in 9 of the 25 (36%) *E. coli* O157 isolates from stool swabs of confirmed HIV/AIDS diarrhoeic patients (Table 4). On the other hand, only 5 of the 29 (17.24%) of *E. coli* isolates from stool swabs of non-confirmed HIV/AIDS diarrhoeic patients carried *fliC$_{H7}$*, *rfbE$_{O157}$* and the attaching and effacing (*eaeA*) genes (Table 5). Representative gel electrophoretic profiles of amplified products of target genes for *E. coli* O157:H7 are illustrated in Fig. 4.

TABLE 4
Summarised PCR amplified genes of *E. coli* O157:H7 isolated from stool swabs of diarrhoeic confirmed HIV/AIDS patients

Patient location	Amplified genes			Patients positive for *E. coli* O157:H7
	fliC$_{H7}$	rfbE$_{O157}$	eaeA	
Cathcart	+	+	+	1
Duncan Village	+	+	+	1
East London	+	+	+	6
[a]East London	+	–	–	0
[a]East London	+	+	–	0
[a]Gompo	+	–	–	0
Stutterheim	+	+	+	1
Total number of stool specimens positive with *E. coli* O157:H7				9([a]3)

'+' target gene present, '–' target gene absent. n = 25 Representative number of presumptive E. coli *O157 characterised by PCR. [a] Represents patients whose* E. coli *O157 only had either 1 or 2 of the targeted genes. These patients were not considered as being positive for* E. coli *O157:H7 due to the absence of the other genes.*

Discussion and conclusion

The present study revealed important findings on the prevalence of *E. coli* O157 in the drinking water supplied to the communities of Fort Beaufort, Alice, Dimbaza, Mdantsane, Ngwenya and Kwasaki. A higher *E. coli* O157 prevalence rate was noted in water samples collected from Ngwenya groundwater samples (36.66%) while a lower prevalence rate was recorded in Fort Beaufort treated drinking water samples (16.66%) (Fig. 1). With the exception of the water samples collected from Alice, which showed only one target gene (*fliC$_{H7}$*) (Table 3), the PCR successfully amplified the three target genes (*fliC$_{H7,}$ rfbE$_{O157}$* and *eaeA*) of the *E. coli* O157:H7 from the isolates obtained from the drinking water samples (Table 3, Fig. 3).

The results of this study confirm the poor microbiological quality of the drinking water that is produced by many water

TABLE 5
Summarised PCR amplified genes of *E. coli* O157:H7 isolated from stool swabs of diarrhoeic non-confirmed HIV/AIDS patients

Patient location	Amplified genes			Patients positive for *E. coli* O157:H7
	fliC$_{H7}$	*rfbE$_{O157}$*	*eaeA*	
East London	+	+	+	5
[a]East London	+	+	–	0
[a]East London	–	+	–	0
Total number of stool specimens positive with *E. coli* O157:H7				5([a]2)

'+' target gene present, '–' target gene absent. n = 29 Representative number of presumptive E. coli *O157 characterised by PCR. [a] Represents patients whose* E. coli *O157 only had either 1 or 2 of the targeted genes. These patients were not considered as being positive for* E. coli *O157:H7 due to the absence of the other genes.*

treatment plants in the Eastern Cape Province. From October 2004 to November 2004 and from July to September 2005, a survey of 55 water treatment plants was conducted in 5 district municipalities (Cacadu, Chris Hani, Amathole, Ukhahlamba and O.R. Tambo) of the Eastern Cape Province by Momba et al. (2006b). Of these 55 water treatment plants, only 18% complied with the South African National Standards 241 Drinking Water Specification (SANS, 2001). Total and faecal coliforms were recorded at the points of treatment as well as at the consumers' taps. Among 26 bacterial species identified during the survey, *E. coli* was predominant in treated drinking water supplied to the communities of the above-mentioned 5 district municipalities (Momba et al., 2006a). In another study conducted between 2001 and 2002, the polymerase chain reaction analysis using *UidaA*-specific primers revealed that a genetic region homologous in size to the *E. coli UidaA* structural gene was present in Ngwenya and other groundwater sources used by the communities for domestic consumption (Momba et al., 2006b). These studies and the present investigation gave conclusive evidence that the microbiological quality of drinking water supplied to the Eastern Cape communities poses a high risk to the health of these communities, especially to immunosuppressed individuals such as those infected by HIV/AIDS.

Cultural methods using IMS and selective media followed by biochemical tests indicated that the stool samples collected from diarrhoeic confirmed and non-confirmed HIV/AIDS patients who visited Frere Hospitals in the Amathole District Municipality had *E. coli* O157 at prevalence rates of 56.5% (74/131) and 43.5% (57/131) respectively. Moreover, the burden of *E. coli* O157 in both confirmed and non-confirmed HIV/AIDS individuals was not limited only to the localities within the Amathole District, but also felt across a large number of the localities in the Eastern Cape Province (Table 2). Representative stool isolates identified by biochemical profiles for *E. coli* O157 and subjected to PCR for the amplification of the three target genes ($fliC_{H7,}$ $rfbE_{O157}$ and *eaeA*) unravelled *E. coli* O157:H7 at prevalence rates of 36% (9/25) and 17.24% (5/29) for confirmed and non-confirmed diarrhoeic HIV/AIDS patients, respectively (Fig. 4).

The χ^2 value of 0.058 predicted that HIV/AIDS status was a significant variant for *E. coli* O157 infection compared to non-confirmed HIV/AIDS individuals ($P \leq 0.05$). The molecular profile of results also indicated that HIV/AIDS patients were more susceptible to *E. coli* O157:H7 than non-confirmed HIV/AIDS patients (Tables 3 and 4). Studies of diarrhoeal cases have been reported in both immunocompetent and immunosuppressed persons such as those suffering from HIV/AIDS (Obi and Bessong, 2002; Obi et al., 2003; 2006; 2007). However, it is important to recognise that *E. coli* O157:H7 infections pose the greatest risk to immunosuppressed individuals (Morris and Potter, 1997; Hoffman, 2004).

Although only representative *E. coli* O157 isolates identified by culture-based methods and biochemical tests were subjected to PCR assays, low prevalence rates of *E coli* O157:H7 from both water and stool samples were noted (Table 3 and 4). It is interesting to note that the PCR assays were limited only to the amplification of the three target genes ($fliC_{H7,}$ $rfbE_{O157}$ and *eaeA*) characteristics of the enterohaemorrhagic *E. coli* O157:H7 serotype under this investigation. Most important is the fact that there are other verotoxigenic *E. coli* (VTEC) such as *E. coli* O157: H^- (Hussein and Stanley, 2003). Other virulence as well as putative genes that have been used in the characterisation of *E. coli* O157:H7 include but are not limited to EHEC *hlyA,* stx_1 *and* stx_2, which in some instances may also be referred to as vt_1 *and* vt_2 and *E. coli 16s rRNA* (Wang et al., 2002; Cagney et al., 2004). Different variants of stx_2 such as stx_{2c}, stx_{2d}, stx_{2e} *and* $stx_{2f,}$ have also been reported (Wang et al., 2002). In addition, other virulence factors as well may be involved in *E. coli* O157 pathogenesis (Dean-Nystrom et al., 1998). Consequently, the high prevalence of *E. coli* O157 could be linked to the presence of other serotypes of *E. coli* O157 in water samples and in stools of diarrhoeic patients. No amplified product could then be expected from isolates that did not have the target genes of *E. coli* O157:H7 under this investigation.

Water-borne *E. coli* O157:H7 transmissions have been attributed to the ingestion of contaminated drinking water or recreational waters (Keen et al., 1994; Armstrong et al., 1996; Friedman et al., 1999). Epidemiological studies have identified that only small numbers of *E. coli* O157:H7 (e.g. 10-200 of organisms) are needed to cause diarrhoeal infections (Wilshaw et al., 1994). Considering the microbiological quality of drinking water in the Eastern Cape in general and that of the Amathole District in particular and the profile of the molecular results of the present study, drinking water might be the source of *E. coli* O157:H7 in stool samples of confirmed and non-confirmed HIV/AIDS patients. This study therefore predicts a possible epidemiological link between the *E. coli* O157:H7 isolated from the drinking water and the diarrhoeic conditions of both confirmed and non-confirmed HIV/AIDS patients visiting Frere Hospital for treatment.

Although the PCR assays were able to amplify the *E. coli* O157:H7 genes ($fliC_{H7,}$ $rfbE_{O157}$ and *eaeA)* from water and stool of both confirmed and non-confirmed HIV/AIDS patients, more concern has been raised for confirmed HIV/AIDS patients as the results of this study revealed a significantly higher prevalence rate of *E. coli* O157:H7 in the stools of these patients. Furthermore, the results clearly indicated, especially in East London, which is one of the big cities in the Eastern Cape Province, the highest rate of *E. coli* O157:H7 in the stool specimens of confirmed HIV/AIDS patients in the Amathole district, which includes Alice, Dimbaza, Fort Beaufort, Ngwenya, Kwasaki and Mdantsane locations. With the migration of people from one location to another or from the locations to the city, consumption of drinking water from these locations by HIV/AIDS patients might result in diarrhoeal infections.

A study by Obi et al. (2005) in the Limpopo Province of South Africa actually confirmed the presence of enteric pathogens (such as *Salmonella enteritidis, Salmonella typhimurium, Shigella dysenteriae*, and verotoxigenic *E. coli* (different from *E. coli* O157:H7) in the waters consumed by diarrhoeic and non-diarrhoeic HIV/AIDS positive individuals. The authors concluded that there was a possibility of HIV/AIDS patients being infected by these pathogens due to their suppressed immune system (Obi et al., 2005).

The poor microbiological quality of drinking water and especially the presence of pathogenic *E. coli* strains, including enterohaemorrhagic *E. coli* O157:H7 in drinking water could clearly explain how diarrhoeal diseases become endemic in the region and continue to ravage the Eastern Cape communities (Bradshaw et al., 2000). In addition to bloody diarrhoea, *E. coli* O157:H7 causes hemorrhagic colitis (HC) and haemolytic-uraemia syndrome (HUS) in humans (Dean-Nystrom et al., 1998; Ritchie et al., 2003). The provision of safe drinking water is a basic human right and essential to the health of rural communities of the Eastern Cape in general and the Amathole District in particular. This study and other studies conducted in the province (Momba et al., 2006a; 2006b) therefore, alert the Eastern

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ISSN 0378-4738 = Water SA Vol. 34 No. 3 July 2008
ISSN 1816-7950 = Water SA (on-line)

Cape Local Government to address the safety of drinking water supplied to rural communities in order to protect the health of their populations.

Acknowledgements

Thanks are due National Research Foundation (NRF) of South Africa for their financial support. The technical assistance of Ms VK Malakate in molecular analysis is highly appreciated.

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Available on website http://www.wrc.org.za
ISSN 0378-4738 = Water SA Vol. 34 No. 3 July 2008
ISSN 1816-7950 = Water SA (on-line)