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Prevalence of *E. coli* O157:H7 strains in irrigation water and agricultural soil in two district municipalities in South Africa

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ABSTRACT

We assessed the prevalence of *E. coli* O157:H7 in irrigation water and agricultural soil samples in two District Municipalities in South Africa using standard culture-based and molecular techniques. Presumptive *E. coli* O157:H7 counts in irrigation water and agricultural soil samples ranged from 1.00 Log_{10} CFU/100 ml to 4.11 Log_{10} CFU/100 ml and 2.20 Log_{10} CFU/g to 4.21 Log_{10} CFU/g respectively ($P \leq 0.05$). Thirteen (28%) of the confirmed isolates (n = 46) were Shiga toxigenic and 33 (72%) were Shiga toxin negative. The presence of Shiga toxigenic *E. coli* O157:H7 strain in irrigation water and agricultural soil samples reported by this study suggests potential risk to food safety and population health.

KEYWORDS

Prevalence; *E. coli* O157:H7; virulence; soil; water; diarrhoea

Introduction

Escherichia coli O157:H7 serotype is a common Shiga toxigenic *E. coli* (STEC) that causes food-related disease in human beings. It is the most frequently recovered serotype of enterohaemorrhagic *E. coli* (EHEC) from sick individuals. Non-motile strains of the STEC are seldom recovered [1].

E. coli O157:H7 harbours the somatic (O) antigen 157 and flagella (H) antigen 7 [2], which are typically screened for during their molecular confirmation. Shiga-toxin (Stx) is the major virulence determinant of *E. coli* O157:H7 and promotes the extensive damage of the intestinal lining leading to diarrhoea. This toxin is further divided into Stx 1 and Stx 2, coded by the *stx1* and *stx2* genes which causes the haemorrhagic uraemic syndrome (HUS) [3]. STEC O157:H7 poses a great danger to the health of humans owing to its high virulence potential, low infectious dose (10–100 CFUs), persistence in the environment and resistance to treatment options [3]. Clinical syndromes produced by *E. Coli* O157:H7 range from slight intestinal distress to HUS, end-stage renal disease (ESRD), and death [4]. Owing to the high rate of mortality associated with *E. coli* O157, this pathogen is easily

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differentiated from other pathotypes of *E. coli* such as enteroaggregative *E. coli* (AggEC), enteropathogenic *E. coli* (EPEC) and enterotoxigenic *E. coli* (ETEC) [1].

Cattle are the main and usually asymptomatic carriers of *E. coli* O157:H7. Generally, 1–50% of healthy cattle pass this pathogen through their faeces at any given time, hence regarded as the main contamination source [5]. Other animals that harbour this pathogen include goats, pigs, sheep and turkeys and these also pass it via faeces [2].

E. coli O157:H7 is transferred to agricultural soils when the faeces of cattle and other ruminants are used as manure. The pathogen is also transferred to nearby freshwater bodies including rivers and streams through water runoff [6]. *E. coli* O157:H7 is spread to the food chain and eventually to human consumers when farm produce grown on contaminated soil comes in contact with this pathogen or when polluted water is used to irrigate the farm's crops. There is every possibility for vegetables grown on manure amended soil to be contaminated with *E. coli* O157 [7] and the contamination routes are not only restricted to direct contact with the pathogen but also via passive diffusion from the soil, through plant roots into edible parts of the plant [8].

Ready-to-eat (RTE) vegetables and fruits enhance the nutritional and health status of human beings, and the demand for fresh or minimally-processed farm produce has increased in the last few decades [9,10]. The contamination of fresh produce including fruits, leafy and non-leafy vegetables with *E. coli* O157 is well documented [11,12]. But there is limited information on the occurrence and relative abundance of Shiga toxin and non-Shiga toxin-producing *E. coli* O157:H7 in irrigation water and agricultural soil in South Africa, although these are known as important transmission routes of pathogenic bacteria to fresh produce. This study, therefore, aims at assessing the prevalence of Shiga toxin and non-Shiga toxin producing *E. coli* O157:H7 strains in irrigation water and agricultural soil samples collected from two District Municipalities in South Africa.

Materials and methods

Study sites

This study was carried out in Chris Hani and Amathole District Municipalities (DMs), Eastern Cape Province of South Africa. Agricultural production of fresh produce is one of the major activities within the Province. These DMs enjoy the benefit of proximity to seaports of Port Elizabeth and East London, attracting investment in agro-processing. Agricultural soil and irrigation water samples were retrieved from 19 sampling sites located within these two DMs (Table 1). We could not collect soil samples from S1, S4, S6, S10, S15 and S19 because the farms were not accessible.

Sample collection

A grab sampling of 13 agricultural soil and 19 irrigation water samples was carried out, after the research team obtained permission from farm owners. During the sampling regimen, irrigation water samples (1 litre approximately) were collected in triplicates in 1 L sterile sample bottles and agricultural soil samples (30 g approximately) were collected in triplicates in sterile plastic bags. All the samples were placed on ice and transported to the laboratory for processing.

Table 1. Description of sampling points.

District Municipality	Sample sites	Coordinates	Samples collected
Amathole	S1	S32°46.922 E026°50.799	Irrigation water
	S2	S32°46.857 E026°51.373	Irrigation water and soil
	S3	S32°43.48 E027°1.32	Irrigation water and soil
	S4	S32°45.2 E27°19.07	Irrigation water
	S5	S32°48.45 E26°59.27	Irrigation water and soil
	S6	S32°35.412 E026°57.793	Irrigation water
	S7	S32°38.157 E026°55.954	Irrigation water and soil
	S8	S32°39.202 E026°55.301	Irrigation water and soil
	S9	S32°40.998 E026°54.062	Irrigation water
		S32°43.259 E026°52.707	Soil
	S10	S22°45.617 E026°51.450	Irrigation water
	S11	S32°45.618 E026°51.428	Irrigation water and soil
	S12	S32°46.616 E026°50.203	Irrigation water and soil
	S13	S32°35.783 E027°26.880	Irrigation water and soil
	S14	S32°37.165 E027°26.418	Irrigation water and soil
Chris Hanni	S15	S32°19.375 E027°08.543	Irrigation water
	S16	S32°01.927 E027°04.619	Irrigation water and soil
	S17	S31°55.297 E026°49.882	Irrigation water and soil
	S18	S31°53.203 E026°47.764	Irrigation water and soil
	S19	S32°00.855 E027°35.597	Irrigation water

Note: For confidentiality sake, sampling sites are coded with S1-S19. Coordinates for each sampling site were retrieved using the 'etrex-LEGENDH' GPS equipment.

Processing of samples and enumeration of E. coli O157:H7 *isolates*

Irrigation water samples were serially diluted as described by Adefisoye et al. [13]. One hundred millilitre aliquot of the dilutions was filtered via 0.45 µm pore size and 37 mm diameter membrane filters (Merck, SA) in triplicates aided with a vacuum pump. After filtration, the filters were aseptically transferred to plates containing Sorbitol-MacConkey agar (SMA) (Merck, SA) augmented with cefixime (50 µg/L) and tellurite (25 mg/L) for the enumeration of *E. coli* O157:H7. One gramme of each soil sample was vortexed with sterile 10 ml distilled water. The soil suspension was then serially diluted as described by Ogunmwonyi et al. [14]. Thereafter, 100 µL of each dilution were inoculated on already prepared SMA (Merck, SA) augmented with cefixime (50 µg/L) and tellurite (25 mg/L) and spread evenly on the medium using a sterile glass spreader as described by Cheesbrough [15] in triplicates for enumeration purposes. All the plates were then incubated for 24 hours at 37°C. After incubation, the target isolates were identified by morphology, counted and recorded. Colourless colonies were counted as presumptive *E. coli* O157:H7. The data were presented as 'Log (x + 1) CFU/100 ml and Log (x + 1) CFU/g' for water and soil samples respectively.

Isolation of E. coli O157:H7

Ten millilitres of each water sample and 10 g of each soil sample were transferred to 90 ml of Trypticase soy broth (TSB) (Merck, SA) for enrichment purposes, and incubated at 37° C for 24 hours, after which a loop full of the broth was streaked on SMA augmented with cefixime (50 µg/L) and tellurite (25 mg/L) and incubated at the same conditions. After incubation, pure colonies considered presumptive were stored in 25% glycerol at −80 °C for subsequent investigations. A total number of 202 presumptive isolates were stored in glycerol stocks.

Molecular identification and virulence typing

Prior to molecular identification, the DNA of the presumptive *E. coli* O157:H7 isolates was extracted using the boiling technique described by Maugeri et al. [16] and Lopez-Saucedo et al. [17]. Thereafter, the confirmation of the isolates was done using PCR as described by Wang et al. [18]. Supplementary Table 1 details the primer sequence and conditions used for the identification of the target genes ($rfbE_{O157}$ and $fliC_{H7}$). For reference purposes, '*E. coli* O157:H7 ATCC 35,150' was included as a control strain. PCR amplicons (5.0 µL aliquot each) were electrophoresed in 2% (w/v) agarose gel (Merck, SA) as described by Wang et al. [18] and photographed using a UV transilluminator. A 50-bp DNA ladder (Inqaba Biotec, SA) was included in each reaction to serve as a DNA size marker.

Virulence genes including *stx1* and *stx2* that code for Shiga toxins were screened for using PCR as described previously [19]. The extraction of the DNA template from confirmed isolates was done using the boiling technique as described by Maugeri et al. [16] and Lopez-Saucedo et al. [17]. Supplementary Table 2 details the specific primers and cycling conditions used for the screening of Shiga toxins. The electrophoresis of the PCR amplicons was done as described previously [19] and photographed using a UV transilluminator. A 100 bp DNA ladder (Inqaba Biotec, SA) was included on each gel as a DNA size marker.

Data analysis

The 'IBM Statistical Package for Social Sciences' (SPSS version 21) was used to analyse the data obtained from the present study. The one-way analysis of variance was used to compare the counts of *E. coli* O157:H7 in irrigation water and agricultural soil samples obtained from various sampling sites. Mean was significant at *P*-values ≤ 0.05.

Results and discussion

The distribution of presumptive *E. coli* O157:H7

Figure 1 shows the distribution of presumptive *E. coli* O157:H7 in the samples collected from the study sites. In irrigation water samples, presumptive *E. coli* O157:H7 counts ranged from 1.00 Log_{10} CFU/100 ml in S12 and S17 to 4.11 Log_{10} CFU/100 ml in S18. In agricultural soil samples, presumptive *E. coli* O157:H7 counts ranged from 2.20 Log_{10} CFU/g in S12 to 4.21 Log_{10} CFU/g in S9. The distribution of the isolates in the irrigation water and agricultural soil samples with respect to each sampling site was statistically significant ($P \leq 0.05$).

There are no available guidelines set for the counts of *E. coli* O157:H7 in irrigation water and agricultural soil in South Africa. The guidelines set for faecal coliforms (0.0 CFU/100 ml) in domestic water in South Africa [20] and the standard (≤100 CFU/100 ml coliforms) set by the World Health Organisation on the use of wastewater in agriculture and aquaculture [21] were therefore used to assess the quality of the irrigation water samples collected in this study. Accordingly, all the water samples, except those collected from S12 and S17 were unfit for the primary cultivation of farm produce, and consequently potential transmission routes of *E. coli* O157:H7 to fresh produce, corroborating previous

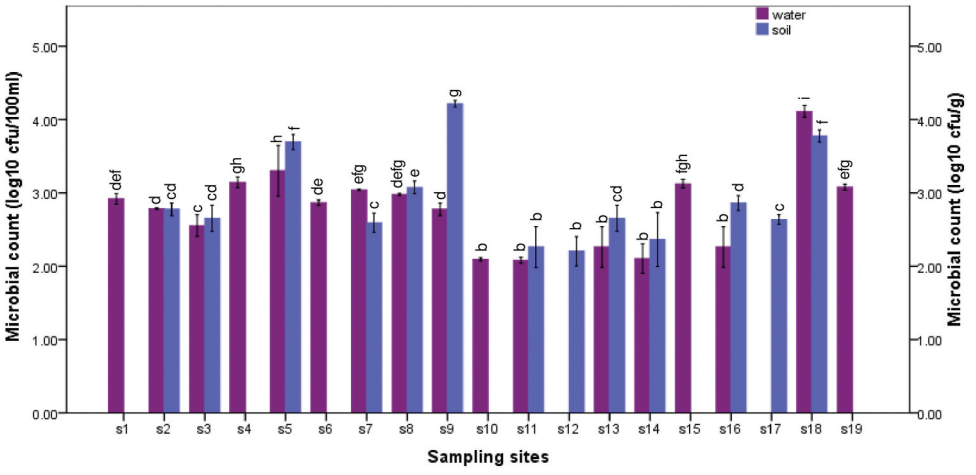

Figure 1. The distribution of *E. coli* O157:H7 in irrigation water samples (purple bars) and agricultural soil samples (blue bars) retrieved from the study sites. Mean of *E. coli* O157:H7 counts within the study sites are statistically significant at $P \leq 0.05$, $F = 211.790$ for water samples and $P \leq 0.05$, $F = 437.191$ for soil samples. The bars with the same colour which have the same letter(s) are not significantly different ($P \geq 0.05$, Duncan) across the sampling sites.

reports [6,22]. This is probably because most irrigation water sources sampled in the present study were surface waters which were vulnerable to various contamination sources including runoff, animal intrusion, discharge of farm and WWTPs effluents. This suggests that these water sources are unfit for use to irrigate fresh produce unless adequately treated. Interestingly, no count was observed in water samples collected from S12 and S17, probably because, water used in S12 and S17 came from pipe borne and borehole water respectively, with little human interference. These water sources are recommended as the most reliable water sources for the irrigation of farm produce and other agricultural practices. Owing to their high cost, however, most farmers revert to surface water and sometimes, wastewater for irrigation purposes which is detrimental to human health unless adequately treated.

There is little information on counts of *E. coli* O157:H7 in agricultural soil. Regrettably, it is almost inevitable for agricultural soil to contain pathogens such as *E. coli* O157:H7 because it is naturally open to direct and indirect sources of microbial contaminants such as animal dung, runoff, contaminated irrigation water, free-ranging animals, municipal sewage and effluents [23,24]. Implementing good agricultural practices such as steady cleaning of farm tools, erecting of fences around the farm to avoid unwanted animal intrusion, efficient treatment of organic fertilisers before application to the soil, use of potable water for irrigation purposes and appropriate irrigation method such as surface irrigation rather than spray irrigation method, will help to prevent the incidence and transmission of pathogenic bacteria such as *E. coli* O157:H7 to fresh produce [25,26].

The prevalence of confirmed E. coli O157:H7 *isolates*

Presumptive *E. coli* O157:H7 was confirmed in 37% (7/19) of the irrigation water samples and 38% (5/13) of the agricultural soil samples.

The prevalence of *E. coli* O157:H7 in irrigation water samples in this study is much higher than the 2.1% reported by Chigor et al. [27] in river water used for the irrigation of fresh produce. The prevalence is also higher than the 18.7% reported by Myataza et al. [6] in samples collected from portable water channels, irrigation water systems and dairy wastewater in commercial dairy farms. This finding is not entirely surprising as herds of feral cattle, considered as the chief source of *E. coli* O157:H7 were seen flocking around most of the irrigation water sources. The high prevalence of *E. coli* O157:H7 in irrigation water samples reported in this study is also attributed to the vulnerability of most of the irrigation water sources to several anthropogenic activities: dumping of refuse, discharge of WWTP effluents, discharge of swine effluents, etc. These are indeed potential sources of *E. coli* O157:H7, which consequently impairs the safety of the fresh produce irrigated by the irrigation water sources.

The prevalence of *E. coli* O157:H7 in agricultural soil samples in the present study is also high compared to the study of Khandaghi et al. [28], where only 1.77% of soil samples fertilised with manure in agricultural farms were positive for *E. coli* O157:H7. In the present study, however, the prevalence of *E. coli* O157:H7 in agricultural soil was much lower than 52.7% reported for manure obtained from organic and low input dairy farms [29]. Again, our finding is not entirely surprising as improperly composted manure of animal origin was used to amend the soil in most of the sampling sites during the primary production of fresh produce. This shows that the survival and persistence of *E. coli* O157:H7 in the soil is favoured by the presence of organic materials. This is evident in the study of Gagliardi and Karns [30], where they observed that *E. coli* O157:H7 survived between 21–45 days in fallow soil following the application of dairy manure. Proper treatment of animal-based manure before being applied to the soil is necessary.

Detection of virulence markers

The confirmed isolates (n = 46) were screened for Shiga toxin genes. Thirteen (28%) were Shiga toxigenic *E. coli* O157:H7 (STEC O157:H7), out of which 6 (13%) harboured the *stx1* gene and 7 (15%) harboured the *stx2* gene. Thirty-three (72%) of the isolates did not harbour either of the *stx* genes. They are hence referred to as non-Shiga toxigenic *E. coli* O157:H7 (NSTEC O157:H7). All *stx2* positive isolates were recovered from irrigation water samples. Two (33%) of the *stx1* positive isolates were recovered from irrigation water and the remaining 4 (67%) were recovered from agricultural soil samples. Nine (27%) of the NSTEC O157:H7 were recovered from irrigation water samples and 24 (73%) NSTEC O157:H7 were recovered from agricultural soil samples as shown in Table 2.

The prevalence of STEC O157:H7 in the present study is high when compared to the prevalence observed in sediment and water samples (4.4% and 3.3%, respectively) collected from a leafy greens production region [31]. This is attributed to the

Table 2. The prevalence of STEC and NSTEC O157:H7 isolates.

	Number of isolates	prevalence in irrigation water samples	Prevalence in agricultural soil samples
STEC *(stx1)*	6 (13%)	2 (33%)	4 (67%)
STEC *(stx2)*	7 (15%)	7 (100%)	0
NSTEC	33 (72%)	9 (27%)	24 (73%)

high level of intrusion by free-ranging cattle to most of the irrigation water sources as well as the use of cow dung as manure in some of the sampling sites. This, in turn, could lead to food safety problems when the pathogens are disseminated to the fresh produce grown on the contaminated soil or irrigated by the contaminated water.

In the present study, a higher prevalence of *stx*2 was observed than of *stx*1, contrary to a previous report [32]. All the STEC O157:H7 isolated from irrigation water samples in this study harboured the *stx*2 genes. Epidemiologically, Stx2 is usually more implicated in human infections especially HC or HUS than the Stx1 [33]. This implies that some of the irrigation water sources in this study might have been contaminated with faeces containing STEC O157:H7, coming from either surface runoff or from improperly treated municipal WWTPs effluents. For instance, the irrigation water source in sampling site S18 is a receiving shed for the effluents of a WWTP. This is detrimental to food safety since irrigation water is almost always in direct contact with farm produce. Conversely, most of *stx1* positive variants were isolated from agricultural soil samples. Since *stx1* genes are less implicated in human infections, it is hypothesised that the agricultural soil in this study was mostly contaminated by the faeces of ruminants, in the form of manure.

The overall prevalence of NSTEC O157:H7 was higher than STEC O157:H7 in this study. They have also been implicated in severe diseases, even though their impact as pathogens is unknown. It has been shown that Stx does not need to be involved in all manifestations of diseases associated with *E. coli* O157:H7 [34]. Interestingly, *Shigella dysenteriae* serotype 1, which lacks the ability to secret Stx, causes non-bloody diarrhoea in monkeys [35]. Li et al. demonstrated that NSTEC O157:H7 induced structural abnormalities in the colon of white rabbits in New Zealand [36]. *Streptococcus pneumoniae* and *Neisseria meningitides* are examples of other bacteria that occasionally cause HUS without secreting Stx [37]. Although Schmidt et al. identified *stx*-deficient *E. coli* O157 strains from human beings infected with diarrhoea and HUS, they speculated that the isolates had other characteristics that are closely similar to the toxigenic strains [34]. Since the diagnosis of STEC infections is usually based on the detection of *stx* genes, there may be numerous misdiagnoses of NSTEC associated infections in laboratories [38]. There is a need to improve procedures in testing for these emerging pathogenic strains [38].

Conclusion

The irrigation water and agricultural soil samples collected from Amathole and Chris Hani District Municipalities harbour *E. coli* O157:H7, which could be transferred to fresh produce intended for human consumers. Some of the isolates harboured either the *stx*1 or *stx*2 gene making them Shiga toxigenic and highly virulent, thus capable of causing severe forms of infection in human beings. We, therefore, recommend the control of the incidence of *E. coli* O157:H7 on the farm via improved cattle management practices, fencing of farms and irrigation water sources to prevent the encroachment of ruminants, besides proper treatment of ruminant-derived manure and irrigation water before application to the soil.

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Author Contributions

CDI and AIO contributed to the conception and design of the study. CDI collected the data and prepared the draft manuscript. EDP, LK, AIO and CDI revised the sections of the manuscript. All authors contributed to revision of the manuscript, and read and approved the submitted version.

Disclosure statement

No potential conflict of interest was reported by the authors.

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