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Incidence and antimicrobial susceptibility of *Escherichia coli* O157:H7 isolates recovered from dairy farms in Amathole District Municipality, Eastern Cape, South Africa

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ABSTRACT

Objective: To assess the incidence of *Escherichia coli* (*E. coli*) O157:H7 in water and cattle rectal samples from three commercial dairy farms in Amathole District Municipalities in the Eastern Cape Province of South Africa.

Methods: Samples were collected bimonthly from cattle rectum and dairy water sources including irrigation water, drinking water troughs and wastewater between June and November 2014. Standard culture-based methods were applied for the microbial analyses, the disc diffusion method was employed for the antibiotic susceptibility test and PCR approach was utilized for identification of the isolates.

Results: A total of 252 presumptive *E. coli* O157:H7 were isolated and subjected to molecular confirmation by PCR. About 18.7% (47/252) of these were confirmed as *E. coli* O157:H7. The antimicrobial susceptibility profile of these confirmed isolates revealed high-level resistance against penicillin G (81%), tetracycline (43%), oxytetracycline (62%), erythromycin (68%), sulphamethoxazole (57%), chloramphenicol (55%), doxycycline (51%) and trimethoprim-sulphamethoxazole (45%).

Conclusions: This is the first report of multi-drug resistance *E. coli* O157:H7 in commercial dairy farms in the province and suggests the possibility of same in other provinces of the country, and this is the subject of the intensive investigation in our group.

1. Introduction

Escherichia coli (*E. coli*) O157:H7 is a widespread pathogen which is mainly carried by cattle and other ruminants. This *E. coli* serotype is the major causative agent of water and food-borne diseases such as bloody diarrhoea and haemolytic uremic syndrome (HUS) in humans[1]. Despite the fact that antimicrobials are not prescribed for treatment of *E. coli* O157:H7 infections in individuals, proof exists on the dissemination of antibiotic resistance to certain antimicrobial agents[2], probably because people often have antibiotics readily available to them to treat any illness, and that farm owners also use antibiotics as growth promoters for their livestock[3,4]. *E. coli* O157:H7 serotype is perceived as the utmost abundant Shiga-toxin producing *E. coli* (STEC) serotype connected to diseases in human and other warm-blooded animals[5], however, a few identified illnesses associated with enterohaemorrhagic *E. coli* (EHEC) have been associated with different pathotypes, known as non-O157 EHEC pathotypes[6]. *E. coli* O157:H7 serotype can interchangeably be regarded to as STEC, verocytotoxin-producer (VTEC), or EHEC in nature[7]. STEC has been involved in copious outbreaks linked with foods, water and contact with infected food producing animals[1,4].

This *E. coli* O157:H7 serotype is mainly transmitted into the food production process through faecal contamination of meat, milk or fruit and vegetables when they come into primary or secondary contact with ruminants' faeces[4,8]. The other significant route of infection by *E. coli* O157:H7 in humans and animals is water

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mainly used for irrigation, drinking and sometimes washing of fruits and vegetables[9]. A variety of resources and reservoirs of *E. coli* O157:H7 has been recognized[9-11], with cattle as the most important source of this serotype in outbreaks linked to undercooked ground beef and raw milk consumption[12]. Cattle waste contains a large range of microbial agents[4,11], and most dairy farms mainly depend on surface, stream or river water[9]. This type of water may become polluted with *E. coli* O157:H7 via primarily depositing faeces or animal waste used as manure or for many other agricultural activities[8,13]. The use of this contaminated water may re-introduce the enteric *E. coli* O157:H7 bacteria into farms' water distribution system or infect livestock thus increasing the dissemination of *E. coli* O157:H7 within the farm[9]. Consequently, cattle may become infected through grazing or by principal contact with the polluted water[12].

Many farms use antimicrobial agents as growth promoters in animal husbandry. When such agents are used, they tend to act directly on the animals' gut causing it to develop resistance to certain bacteria[2]. This factor contributes to the prevalence of antibiotic-resistant bacteria within the environment, and the development of antimicrobial resistance in enteric bacteria is increasing rapidly around[13,14]. Also, studies from diverse geographical locations in Africa have witnessed global developments in the pervasiveness of antibiotic resistance within and among enteric bacteria[9,13]. In this paper, we report on the incidence and antimicrobial susceptibility profiles of *E. coli* O157:H7 isolates recovered from some commercial dairy farms in Amathole District Municipality, Eastern Cape, South Africa as part of our larger study on the reservoirs of antibiotic resistance determinants in the environment.

2. Materials and methods

2.1. Study area

Three commercial dairy farms located in the Eastern Cape Province of South Africa, under the Amathole District Municipality were used for this study. For confidential reasons the farms were coded F, K and M. Dairy farm F is located on geographical coordinates of 32°47'0" S and 26°50'0" E with a total of 800 cows and has 10 farm workers with milk production of about 10 000 L per day. Dairy farm M is located on geographical coordinates of 32°49'0" S and 26° 59'0" E on 280 hectares of land with 600 cows producing 2 000 L of milk per day and has 16 permanent workers. Dairy farm K, on the other hand, occupies about 700 hectares of land with about 400 cows producing about 2 000 L of milk per day and run by 36 workers.

2.2. Sample collection

Water samples from drinking water troughs, irrigation water systems, and dairy wastewater were collected from the three commercial farms every month in a period of 6 months (June to November 2014). Proceeding to sample, all sample containers were thoroughly washed with detergent, thoroughly rinsed and sterilized by autoclaving. A total of 500 mL samples from drinking water troughs, water for irrigation and dairy wastewater were collected from each dairy farm. A total of 108 samples from diverse water sources (drinking water, dairy wastewater and irrigation water) were collected in all the three dairy farms. Sterile cotton-tipped swabs were used to collect rectal samples from each cattle. A total of 60 rectal samples were collected from each farm throughout the sampling period, which amounts to a total of 180 rectal swab samples collected from all the three farms. All water and rectal samples were transported on ice to the laboratory of the Applied and Environmental Microbiology Research Group (AEMREG), University of Fort Hare, Alice, South Africa and analysed within 6 h of collection.

2.3. Enumeration and isolation of E. coli O157:H7 from water samples and cattle faecal samples

Water samples were analysed for the enumeration and isolation of *E. coli* O157:H7 using membrane filtration technique. Water samples were serially diluted and filtered through a sterile membrane filter of pore size 0.45 μm and 37 mm diameter. The membrane filters were carefully transferred onto Sorbitol-MacConkey agar augmented with tellurite (25 mg/L) and cefixime (50 μg/L) and incubated aerobically at 37 ℃ for 24 h. Colourless colonies were presumptively identified as *E. coli* O157:H7 and enumerated as such and expressed as CFU/mL and several distinct colonies were isolated and purified for further analysis.

Serial dilutions (10^{-1}–10^{-6}) were prepared from the rectal swabs by transferring 1.0 mL from a raw rectal sample suspended in 9.0 mL of sterile physiological saline contained in 10.0 mL tubes (10^{-1} dilution). The prepared diluents were directly cultivated on Sorbitol-MacConkey agar plates augmented with tellurite (25 mg/L) and cefixime (50 μg/L). Colourless colonies were presumptively identified as *E. coli* O157:H7 and several distinct colonies were isolated and purified for further analysis.

2.4. Molecular detection of presumptive E. coli O157:H7

2.4.1. DNA extraction

DNA extraction from the presumptively identified *E. coli* O157:H7 isolates was conducted using boiling method[15]. Briefly, pure isolates were obtained from the sorbitol-MacConkey agar, and these colonies were inoculated on sorbitol-MacConkey agar to ensure purity, from which the resulting pure colonies were further grown on nutrient agar prior to DNA extraction. A loopful of the resulting colonies was picked and suspended in 200 μL nuclease-free water contained in 2 mL Eppendorf tube aseptically. Cells were lysed by boiling for 10 min at 100 ℃, using AcuuBLOCK Digital Dry Bath and centrifuged for 10 min at 15 000 r/min using PRISMR

Centrifuge (Labnet International, Inc). The cell debris was discarded while the supernatant was stored at –80 ˚C.

2.4.2. Molecular confirmation of E. coli O157:H7 isolates

Molecular confirmation of the presumptive *E. coli* O157:H7 isolates was carried out using PCR technique. PCR amplification was performed in a reaction mixture (25.0 μL) with the master mix (12.5 μL), forward (0.25 μL) and reverse (0.25 μL) primers, nuclease-free water (2.0 μL) and template DNA (10 μL). Two specific primer pairs (rfbEO157 and fliCH7) were used (Table 1)[16]. The thermal cycling protocol was adopted as follows: denaturation at 95 ˚C for 5 min, denaturation at 94 ˚C for 30 s, annealing at 60 ˚C for 90 s, extension at 72 ˚C for 90 s, with final extension at 72 ˚C for 5 min and the amplicons were held at 4 ˚C until removed[17]. For further analysis, 5.0 μL aliquot of each resultant PCR amplified product was further analyzed by 2% agarose, stained with 5.0 μL of ethidium bromide at 100 V for 1 h and imagined using UV trans-illuminator (Alliance 4.7).

Table 1
Primer used in this study.

Target gene	Primer	Primer sequence (5'→3')	Amplicon size (bp)	Reference
$rfbE_{O157}$	RfbE-a	F-CTACAGGTGAAGGTGGAATGG	327 bp	Wang *et al.*[16]
	RfbE-b	R-ATTCCTCTCTTTCCTCTGCGG		
$fliC_{H7}$	FliC-a	F-TACCATCGCAAAAGCAACTCC	247 bp	
	FliC-b	R-GTCGGCAACGTTAGTGATACC		

2.6. Antibiotic susceptibility testing

Antibiotic susceptibility testing was carried out on Mueller-Hinton agar using the standard Kirby Bauer disc diffusion method[18] against a panel of antibiotics including amikacin (30 μg), ampicillin (10 μg), cefoperazone (75 μg), cefotaxime (30 μg), cephalothin (30 μg), chloramphenicol (30 μg), doxycycline (30 μg), erythromycin (15 μg), gentamicin (10 μg), oxytetracycline (30 μg), penicillin G (10 μg), polymyxin B (300 μg), sulphamethoxazole (30 μg), trimethoprim (25 μg), trimethoprim-sulfamethoxazole (25 μg), streptomycin (25 μg), kanamycin (30 μg) and tetracycline (30 μg). These antibiotics were selected on the basis that they are frequently applied in the therapy of *E. coli* related diseases. Briefly, fresh isolates from sorbitol-MacConkey agar plates were cultivated on nutrient agar and incubated at 37 ˚C for 18–24 h. These fresh colonies were inoculated on sterile physiological saline and standardized to 0.5 McFarland standards. One hundred microliters of the bacterial suspension were spread evenly on the entire surface of Mueller-Hinton agar plates using a sterile swab, after which the antibiotic discs were aseptically placed on the bacterial lawn, and the plates incubated at 37 ˚C for 18–24 h. At the end of the incubation period, the plates were examined for zones of inhibition and interpreted based on minimal inhibition concentrations (MIC) break-point from the Clinical Laboratory Standards Institute (2014) and interpreted as Resistant, Intermediate or Sensitive.

3. Results

3.1. Distribution of E. coli O157:H7

The distribution of presumptive *E. coli* O157:H7 from the water samples is presented in Table 2. In the water samples, presumptive *E. coli* O157:H7 counts varied as follows: DW (1.0×10^2–3.0×10^3 CFU/100 mL), IW (0.0–8.0×10^3 CFU/100 mL), WW (3.0×10^3–1.0×10^6 CFU/100 mL) in farm F; DW (0.0–3.0×10^3 CFU/100 mL), IW (0.0–4.0×10^3 CFU/100 mL), WW (3.5×10^1–4.3×10^6 CFU/100 mL) in farm K; and DW (0.0–1.0×10^3 CFU/100 mL), IW (0.0–2.2×10^3 CFU/100 mL), WW (0.0–2.9×10^6 CFU/100 mL) in farm M (Table 2). *E. coli* O157:H7 presumptive isolates recovered from rectal swabs ranged across all farms between $1.0 \times 10^0 \pm 0.0 \times 10^0$ and $9.4 \times 10^2 \pm 1.8 \times 10^3$ CFU/mL (Table 3). No counts were observed in September in farm F and K.

3.2. Molecular confirmation of presumptively identified E. coli O157:H7 isolates by PCR

A total of 47 (18.7%) out of 252 presumptive isolates were further confirmed as *E. coli* O157:H7 using primers $rfbE_{O157}$ and $fliC_{H7}$ genes, respectively. Figures 1 and 2 represent a gel picture for some of the positive *E. coli* O157:H7 isolates of expected amplicon sizes.

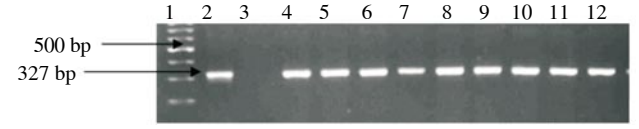

Figure 1. The amplified $rfbE_{O157}$ gene of *E. coli* O157:H7 recovered from water and cattle rectal samples.
Lane 1: Molecular marker; Lane 2: Positive control; Lane 3: Negative control; Lanes 4–12: Some of the positive isolates.

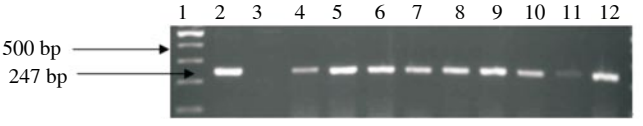

Figure 2. The amplified $fliC_{H7}$ gene of *E. coli* O157:H7 recovered from water and cattle rectal sample.
Lane 1: Molecular marker; Lane 2: Positive control; Lane 3: Negative control; Lanes 4–12: Some of the positive isolates.

3.3. Antibiotics susceptibility profile of E. coli O157:H7

The antibiotic susceptibility patterns observed from *E. coli* O157:H7 isolates recovered from dairy water systems and cattle faecal samples are presented in Table 4. The susceptibility of the isolates to the antibiotics tested were observed to be in the following proportions: penicillins [ampicillin 24/47 (51%) and penicillin G 9/47 (19%)]; cephems [cephalotin 41/47 (87%), cefotaxime 43/47 (91%) and cefoperazone 30/47 (64%)]; aminoglycosides [gentamycin 33/47 (70%), amikacin 38/47 (81%), kanamycin 32/47 (68%) and streptomycin 34/47 (72%)]; tetracyclines [tetracycline 27/47 (57%), doxycycline 19/47 (40%) and oxytetracycline

Table 2

Enumeration of *E. coli* O157:H7 in the water samples of the three dairy farms between June and November 2014 (CFU/100 mL).

Months	Farm F			Farm K			Farm M		
	DW	IW	WW	DW	IW	WW	DW	IW	WW
June	$6.0 \times 10^2 \pm 3.0 \times 10^0$	0	$3.5 \times 10^5 \pm 7.0 \times 10^2$	$2.0 \times 10^2 \pm 2.0 \times 10^0$	$12.0 \times 10^2 \pm 3.0 \times 10^2$	$8.2 \times 10^4 \pm 3.0 \times 10^1$	0	$6.0 \times 10^1 \pm 8.0 \times 10^3$	$8.9 \times 10^3 \pm 1.0 \times 10^0$
July	$9.5 \times 10^2 \pm 1.0 \times 10^0$	$9.0 \times 10^1 \pm 3.0 \times 10^2$	$3.0 \times 10^3 \pm 1.0 \times 10^0$	$7.8 \times 10^1 \pm 3.0 \times 10^1$	0	$9.0 \times 10^4 \pm 7.0 \times 10^3$	$7.8 \times 10^1 \pm 2.0 \times 10^1$	0	0
August	$7.0 \times 10^1 \pm 5.0 \times 10^0$	$8.0 \times 10^3 \pm 1.0 \times 10^0$	$3.0 \times 10^3 \pm 2.0 \times 10^2$	0	$3.5 \times 10^1 \pm 8.0 \times 10^3$	$3.5 \times 10^1 \pm 1.0 \times 10^0$	0	0	$3.6 \times 10^3 \pm 7.0 \times 10^1$
September	$3.0 \times 10^3 \pm 2.0 \times 10^2$	$6.0 \times 10^2 \pm 3.0 \times 10^0$	$2.6 \times 10^5 \pm 8.0 \times 10^2$	$2.0 \times 10^2 \pm 5.0 \times 10^3$	$4.0 \times 10^2 \pm 1.0 \times 10^0$	$7.9 \times 10^5 \pm 7.0 \times 10^3$	$1.0 \times 10^2 \pm 9.0 \times 10^1$	$5.7 \times 10^2 \pm 1.0 \times 10^0$	$2.6 \times 10^5 \pm 4.0 \times 10^0$
October	$7.0 \times 10^2 \pm 1.0 \times 10^0$	$3.8 \times 10^2 \pm 2.0 \times 10^0$	$7.0 \times 10^5 \pm 4.0 \times 10^0$	$2.5 \times 10^3 \pm 1.0 \times 10^0$	$1.2 \times 10^2 \pm 4.0 \times 10^2$	$9.0 \times 10^4 \pm 4.0 \times 10^0$	$2.7 \times 10^2 \pm 7.0 \times 10^1$	$9.3 \times 10^2 \pm 3.0 \times 10^0$	$2.9 \times 10^6 \pm 1.0 \times 10^0$
November	$1.0 \times 10^2 \pm 3.0 \times 10^2$	$5.0 \times 10^2 \pm 4.0 \times 10^0$	$1.0 \times 10^6 \pm 1.0 \times 10^0$	$3.0 \times 10^3 \pm 1.0 \times 10^0$	$4.0 \times 10^3 \pm 1.0 \times 10^0$	$4.3 \times 10^6 \pm 1.0 \times 10^0$	$1.0 \times 10^3 \pm 4.0 \times 10^0$	$2.2 \times 10^3 \pm 1.0 \times 10^0$	$2.8 \times 10^6 \pm 1.0 \times 10^0$

DW: Drinking water; IW: Irrigation water; WW: Wastewater.

Table 3

Population cell density of *E. coli* O157:H7 of rectal samples recovered from the three dairy farms between June and November 2014 (CFU/mL).

Dairy farm	Sampling months range					
	June	July	August	September	October	November
F	1.1×10^1–1.0×10^4 ($9.2 \times 10^2 \pm 3.0 \times 10^3$)	1.6×10^0–2.9×10^2 ($9.8 \times 10^1 \pm 1.5 \times 10^2$)	3.0×10^2–3.7×10^3 ($9.4 \times 10^1 \pm 1.8 \times 10^3$)	0	3.0×10^2–3.7×10^3 ($9.4 \times 10^2 \pm 1.8 \times 10^3$)	6.0×10^2–6.0×10^4 ($3.0 \times 10^4 \pm 4.2 \times 10^4$)
K	4.0×10^1–2.0×10^2 ($4.5 \times 10^1 \pm 7.7 \times 10^1$)	1.0×10^0–1.0×10^0 ($1.0 \times 10^0 \pm 0.0 \times 10^0$)	1.0×10^0–6.0×10^0 ($4.3 \times 10^0 \pm 2.9 \times 10^0$)	0	2.9×10^1–2.8×10^4 ($5.1 \times 10^3 \pm 1.1 \times 10^4$)	1.0×10^1–9.0×10^3 ($1.7 \times 10^3 \pm 3.6 \times 10^3$)
M	2.0×10^0–3.0×10^1 ($1.6 \times 10^1 \pm 1.1 \times 10^1$)	3.0×10^2–3.0×10^2 ($3.0 \times 10^2 \pm 0.0 \times 10^0$)	1.0×10^2–6.5×10^1 ($2.3 \times 10^1 \pm 3.3 \times 10^1$)	1.0×10^0–1.9×10^2 ($5.3 \times 10^1 \pm 7.9 \times 10^1$)	3.0×10^0–9.7×10^4 ($1.5 \times 10^4 \pm 3.6 \times 10^4$)	4.0×10^0–1.0×10^3 ($2.9 \times 10^2 \pm 3.9 \times 10^2$)

Values in parenthesis represent mean ± SD.

18/47 (38%)]; folate pathway inhibitors [trimethoprim 24/47 (51%), trimethoprim-sulphamethoxazole 20/47 (43%) and sulphamethoxazole 32/47 (68%)]; lipopeptides [polymyxin B 40/47 (85%)]; and phenicols [chloramphenicol 21/47 (45%)].

The resistances profile to the antibiotics were as follows: penicillins [penicillin G 38/47 (81%) and ampicillin 19/47 (40%)]; cephems [cephalotin 5/47 (11%) and cefoperazone 10/47 (21%)]; aminoglycosides [gentamycin 14/47 (30%), streptomycin 12/47 (26%), kanamycin 12/47 (26%) and amikacin 7/47 (15%)]; tetracyclines [tetracycline 20/47 (43%), oxytetracycline 29/47 (62%), and doxycline 24/47 (51%)]; macrolides [erythromycin 32/47 (68%)]; folate pathway inhibitors [trimethoprim-sulphamethaxozole 27/47 (57%), trimethoprim 21/47 (45%), and sulphamethaxozole 9/47 (19%)]; lipopeptides [polymyxin B 4/47 (9%)]; phenicols [chloramphenicol 26/47 (55%)].

Table 4

Trends on antibiotic susceptibility of *E. coli* O157:H7 isolates [*n* (%)].

Antibiotic group	Antimicrobial agents	Percentage profile ($n = 47$)		
		R	I	S
Penicillins	Penicillin G (10 μnits)	38 (81)	0 (0)	9 (19)
	Ampicillin (10 μg)	19 (40)	4 (9)	24 (51)
Cephems	Cephalothin (30 μg)	5 (11)	1 (2)	41 (87)
	Cefotaxime (30 μg)	0 (0)	4 (9)	43 (91)
	Cefoperazone (75 μg)	10 (21)	7 (15)	30 (64)
Aminoglycosides	Gentamycin (10 μg)	14 (30)	0 (0)	33 (70)
	Amikacin (30 μg)	7 (15)	2 (4)	38 (81)
	Kanamycin (30 μg)	12 (26)	3 (6)	32 (68)
	Streptomycin (25 μg)	12 (26)	1 (2)	34 (72)
Tetracyclines	Doxycycline (30 μg)	24 (51)	4 (9)	19 (40)
	Tetracycline (30 μg)	20 (43)	0 (0)	27 (57)
	Oxytetracycline (30 μg)	29 (62)	0 (0)	18 (38)
Macrolides	Erythromycin (15 μg)	32 (68)	15 (32)	0 (0)
Folate pathway inhibitors	Trimethoprim (25 μg)	21 (45)	2 (4)	24 (51)
	Sulfamethoxazole (30 μg)	9 (19)	6 (13)	32 (68)
	Trimethoprim-Sulphamethoxazole (25 μg)	27 (57)	0 (0)	20 (43)
Lipopeptides	Polymyxin B (300 units)	4 (9)	3 (6)	40 (85)
Phenicols	Chloramphenicol (30 μg)	26 (55)	0 (0)	21 (45)

R: Resistant; I: Intermediate; S: Sensitive.

4. Discussion

Several *E. coli* O157:H7 sources and reservoirs have been identified with cattle being the main carriers[5,17]. A study conducted by Ateba *et al.*[19] described the prevalence of *E. coli* O157:H7 in pigs (44%–50%) compared to cattle (5.4%–20%). The study also showed that the predominance of *E. coli* O157:H7 was comparatively greater in faecal samples collected from commercially farmed ruminants than communally[19]. On the other hand, Ateba and Bezuidenhout[20] documented that a number of *E. coli* O157:H7 linked cases of infection have been linked with the intake of polluted water and/or animal products. The current study, therefore, seeks to bring insight on the distribution of *E. coli* O157:H7 in dairy farms in the Eastern Cape Province, where farming remains one of the major drivers of economic growth.

The obtained and *E. coli* O157:H7 counts from water samples and cattle faecal sample in this study suggest that the three dairy farms under study may aid as significant sources of re-infection or dissemination of *E. coli* O157:H7. The observed cell densities fell short of recommended standards for faecal coliforms and suggested that the dairy water was not suitable for use. This observation is similar to the findings of Van Donkersgoed *et al.*[21] reported that *E. coli* O157:H7 subtypes from the collected feed, faeces and water in the lot indicated possible dissemination of *E. coli* O157:H7 organism among the investigated sources. Findings from this present investigation have also recognized that the isolated *E. coli* O157:H7 from water sources were of cattle faecal origin, as cattle were found grazing alongside the streams used for irrigation, and drinking on the drinking water troughs throughout the study period. Also, the wastewater of these farms is mainly from water used to the clean milking equipment during the milking process.

A faecal examination is the widely used specimen for detecting or estimating *E. coli* O157:H7[17]. The current study indicated that the distribution of *E. coli* O157:H7 has been established from water and faecal specimens of all the three farms. Generally, low distribution

rates of *E. coli* O157:H7 were recorded in cattle faecal sample when paralleled to water samples, except for farm F. A higher distribution rate of *E. coli* O157:H7 was observed in water samples collected from farm K (11.9%), while a lower distribution rate was noted in water samples collected from farm F (5.1%). Many other studies have reported *E. coli* O157:H7 emanating from drinking water troughs in the animal husbandry[4]. Van Donkersgoed *et al*.[21] documented *E. coli* O157: H7 as having been recovered from 12% of investigated water troughs. While LeJeune and Wetzel[22] recovered *E. coli* O157:H7 from 6 out of 473 investigated water troughs situated at 99 various cattle operations. Another study performed in the Eastern Cape Province of South Africa, documented that 54% of raw cattle milk specimens were positive for *E. coli* O157:H7, further implying that cattle are foremost reservoirs of this organism[23]. *E. coli* O157:H7 thrives in water and animal faeces for longer time frames, and these may be implicated as foremost carriers or origin of infection[23]. The above-mentioned studies and the current investigation highlight the need for proper handling of drinking water for livestock and other water systems including good animal waste management to avert the widespread of *E. coli* O157:H7 to the environment, food products, vegetables, crops and back to animals.

According to a study conducted by Abong'o and Momba[24] vegetables, meat, meat products and water serve as possible transmission routes of *E. coli* O157:H7. Several other studies including Igbinosa and Okoh[25] as well as Osode and Okoh[26] reported that wastewater treatment plants within the Eastern Cape region are ineffective in reducing enteric microorganisms. These insufficiently treated effluents may further contaminate surface, stream or river waters which are mainly used by most dairy farms for irrigation and drinking for cattle[27,28]. Food-producing animals can transfer *E. coli* O157: H7 between each other by consumption of water or feeds that have been contaminated with the pathogen and thus could serve as an important pathway for transmission[23]. Pollution of these water sources poses risks to animal and vegetation and consequently humans[9,13]. This study suggests that the dairy farms around the Eastern Cape regions are potential sources of *E. coli* O157:H7 isolates and the associated diseases, and consequently a threat to public health.

The PCR assay used in this study only amplified two genes ($rfbE_{O157}$ and $fliC_{H7}$). *E. coli* O157:H7 harbour other putative virulence genes which might be adopted in the identification of *E. coli* O157:H7. These include *stx1*, *stx2* and *hlyA*[16]. According to Iweriebor *et al*.[29] on the prevalence and antibiogram of some swine associated serogroups of Shiga toxin producing *E. coli* in the Nkonkobe Municipality, 88.45% of the isolates were detected as O157 isolates targeting virulence determinants (*stx1* and *stx2*). In this study, *E. coli* O157:H7 isolates possess the $rfbE_{O157}$ and $fliC_{H7}$ genes. The gene $rfbE_{O157}$ encodes the *E. coli* somatic antigen O157; $fliC_{H7}$, which encodes for *E. coli* structural flagella antigen H7. The two genes $rfbE_{O157}$ and $fliC_{H7}$ provide genotypic identification of the O157:H7 serotype most connected with disease or infection outbreaks. The occurrence of $rfbE_{O157}$ and $fliC_{H7}$ genes in *E. coli* O157:H7 has been used to identify *E. coli* O157:H7 isolates[16].

The *E. coli* O157:H7 isolates showed increased susceptibilities to most antibiotics, with the highest susceptibility pattern observed against cefotaxime (91%). This is in contrast with the findings obtained by Iwu *et al*.[30], which was most isolated *E. coli* O157:H7 from a faecal specimen of dairy cattle in Eastern Cape of South Africa expressed reduced susceptibility to most tested antimicrobial agents. The highest resistance observed was by 81% of the isolates against penicillin G followed by erythromycin (68%). Ateba and Bezuidenhout[20] also carried out an investigation on characteristics of *E. coli* O157:H7 in the North-West Province, South Africa, where all the isolates of *E. coli* O157:H7 recovered from cattle were resistant to erythromycin which was in contrast to this present study.

Studies carried out in the last 15 years show expanding resistance to ordinarily utilized antimicrobials, for example, trimethoprim-sulphamethoxazole (TMP-SMX, otherwise called cotrimoxazole), chloramphenicol, ampicillin, and tetracycline[9]. Most developing countries such as South Africa tend to use any readily available medication such as antibiotics without first identifying the cause of sickness. This might be one of the factors which have so much contributed to increased bacterial resistance against most antimicrobial agents because resistance in bacteria may be conveyed via bacterial gene transfer[31]. The bacteria that have ultimately acquired resistance to certain antimicrobial agents might be washed down into municipal wastewater treatment plants and further spread to receiving surface water bodies, causing re-infection of farms. These might later be passed on to dairy water systems as the animals' gut develops resistance to specific bacteria and subsequently introduced into surrounding water systems through direct or indirect contact with animal faeces.

The present study demonstrated that isolated *E. coli* O157:H7 recovered from the three dairy farms were resistant to a panel of antibiotics which are commonly recommended by clinicians for *E. coli* O157:H7 related diseases and illnesses. The observation suggests that the dissemination and perseverance of multidrug-resistant bacteria to antimicrobial and resistance genes cassette in human, animals and the environment may become increased. It further demonstrates that dairy homesteads are sources of antimicrobial resistant microbes and this may be reinforced by the way most animal farms used antimicrobial agents for growth development and disease prevention in animals. It, therefore, suggests that the dairy farms around Eastern Cape regions are a potential reservoir of *E. coli* O157:H7 and antimicrobial resistance determinants within the environment, consequently posing threats to public health.

Conflict of interest statement

We declare that we have no conflict of interest.

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