Molecular characteristics and genotypic diversity of enterohaemorrhagic *Escherichia coli* O157:H7 isolates in Gauteng region, South Africa

John Y. Bolukaoto[1], Marleen M. Kock[1, 2], Kathy-Anne Strydom[1, 3], Nontombi M Mbelle[1, 2], and Marthie M. Ehlers[1, 2]

[1]Department of Medical Microbiology, Faculty of Health Sciences, University of Pretoria, South Africa

[2]National Health Laboratory Service, Tshwane Academic Division, Pretoria, South Africa

[3]Ampath National Laboratory Service, Pretoria, South Africa

***Corresponding author**:

Marthie M. Ehlers

Department of Medical Microbiology, Faculty of Health Sciences, University of Pretoria; Private Bag X323, Arcadia 0007, South Africa

E-mail: marthie.ehlers@up.ac.za

Telephone: (+27)12 319 2170; Fax: (+27) 12 325-5550

Highlights

• Environmental water samples and clinical stool specimens were analysed for the detection of enterohaemorrhagic *Escherichia coli* (EHEC) O157:H7

• The EHEC O157:H7 isolates were more prevalent in environmental samples than in clinical specimens

• The combination of virulence genes was detected among EHEC O157:H7 isolates

• The EHEC O157:H7 isolates were genetically diverse. Three sequence types (STs) (ST10, ST11 and ST1204) were identified in this study that have never been reported in South Africa

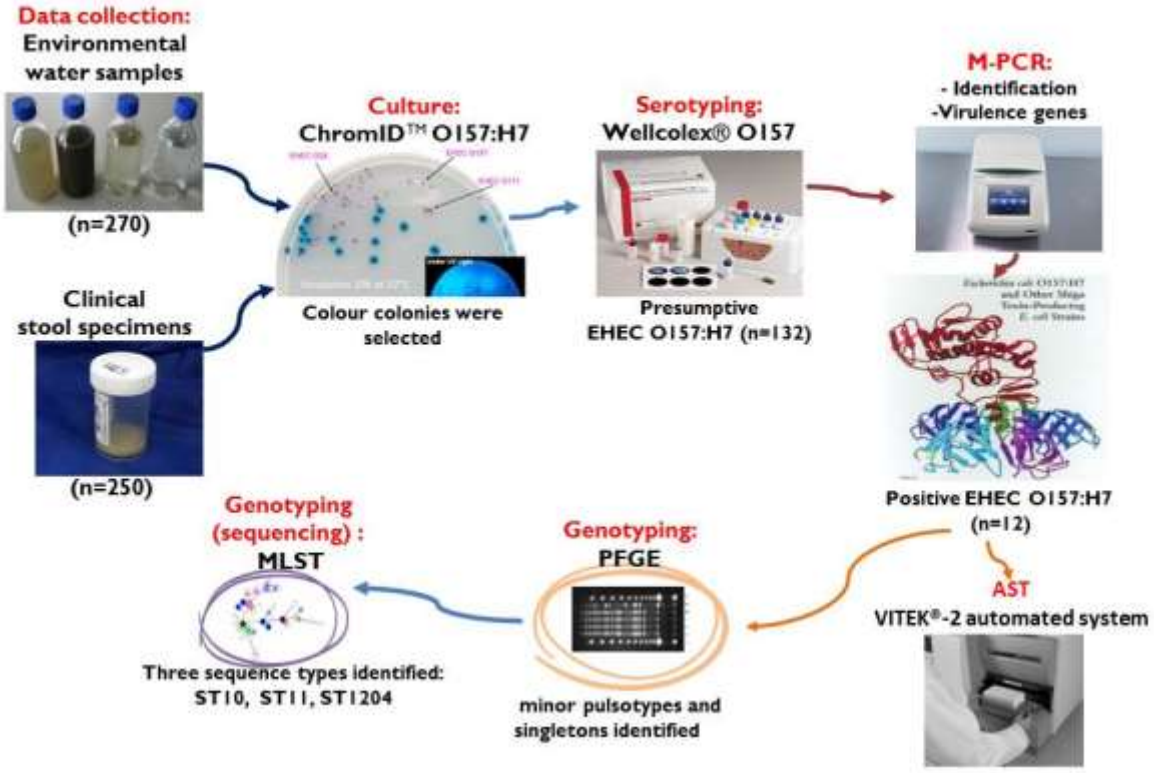
• These STs pose a potential public health risk to consumers of untreated water and closed human contacts

Abstract

Enterohaemorrhagic *Escherichia coli* (EHEC) O157:H7 is one of the major foodborne and waterborne pathogens causing severe diseases and outbreaks worldwide. There is scarcity of EHEC O157:H7 data in South Africa. This study was carried out to determine the molecular characteristics and genotypic diversity of EHEC O157:H7 isolates in the Gauteng region, South Africa. Samples were cultured on selective chromogenic media. Antibiotic susceptibility profile of isolates was determined using the VITEK®-2 automated system. Isolates were characterised using multiplex PCR assays and the genetic diversity was determined using pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST).A total of 520 samples of which 270environmental water samples and 250 stool specimens were collected and analysed.Overall, EHEC O157:H7 was recovered from 2.3% (12/520) of samples collected. Environmental water samples and clinical stool specimens showed a prevalence of 4.07% (11/270) and 0.4% (1/250) respectively. Antibiotic susceptibility profile varied from isolates with full susceptibility to isolates with resistance to multiple antibiotics. Most resistance was detected to the penicillins, specifically ampicillin (7/12),amoxicillin (3/12) and piperacillin/Tazobactam (3/12) followed by one of the folate inhibitors, trimethoprim (3/12)and the carbapenems, imipenem and meropenem (2/12) each. Three isolates harboured a combination of Shiga-toxins (*Stx*)-2, intimin (*eae*) and enterohaemolysin (*hly*A) genes, while two isolates harboured the *Stx*-1, *Stx*-2 and *hly*A genes. The PFGE performed showed that EHEC O157:H7 isolates were genetically diverse, with two minor pulsotypes and eight singletons. The MLST analysis identified three sequence types (STs) (ST10, ST11 and ST1204) that have never been reported in South Africa but have previously been reported worldwide and associated with outbreaks. The STs identified in this study pose a potential public health risk to consumers of untreated environmental water and closed human contacts. There is necessity to enhance surveillance in reducing the propagation of this bacterium which is a public health problem.

Keywords: Enterohaemorrhagic *Escherichia coli* O157:H7; environmental water sample, genetic diversity; virulence gene; antimicrobial resistance; South Africa.

Graphical abstract



1. Introduction

Enterohaemorrhagic *Escherichia coli* (EHEC) serotype O157:H7or Shiga-toxin producing *E. coli* has emerged as an important enteric pathogen causing human gastrointestinal disease (Bai *et al.*, 2018) and a variety of clinical syndromes including bloody diarrhoea, haemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS) (Pennington, 2010). Haemolytic uremic syndrome can be an important cause of acute renal failure in children, the elderly and immunocompromised patients (Iweriebor *et al.*, 2015). Enterohaemorrhagic *E. coli* isolates is associated with different virulence genes of clinical importance such as Shiga toxins (*Stx*1 and *Stx*2), enterohaemolysin (*hly*A) and intimin (Gyles, 2007; Baranzoni *et al.*, 2016). Shiga toxin genes are phage-encoded cytotoxins located on lysogenic lambdoid double-stranded DNA phagesthat inactivate ribosomal RNA, inhibit protein synthesis and cause the death of host cells (Johansen *et al.*, 2001; Müller *et al.*, 2002). Shiga toxins play a major role in the pathogenesis of HC and HUS through cytotoxic effects on cells of the kidneys, intestines, central nervous system and other organs (Ibekwe *et al.*, 2002). Enterohaemolysin gene, located on the large EHEC O157:H7 virulence plasmid is a pore-forming toxin that lyses sheep and human erythrocytes and is commonly associated with diarrhoeal disease and HUS (Croxen *et al.*, 2013). Intimin is an adhesin responsible for intimate attachment of the agent to the intestinal epithelial

cells, characterised by the destruction of host cell microvilli, causing attaching and effacing (*eae*) lesions in the intestinal mucosa (Launders *et al*., 2016).

The intestinal tract of ruminants, particularly cattle, is regarded as the natural reservoir of EHECO157:H7; which are infected asymptomatically and shedding the bacterium in their faeces (Nguyen and Sperandio, 2012). The infectious dose is low, less than 10 bacterial cells can cause disease (Chattaway *et al.,* 2016). Human infection has been associated with various transmission routes, including direct exposure to infected animals faeces, ingestion or consumption of faecally contaminated foods or water or undercooked food or unpasteurized raw milk and also *via* person-to-person contact (Lim *et al.,* 2010; Vasant *et al.,* 2017).In developed countries, most reported EHEC O157:H7 foodborne outbreaks were associated with exposure to or consumption of contaminated food, such as hamburgers and other beef products and consumption of raw cow's milk and cheese (CDC, 2014; Berenger *et al.,* 2015), while waterborne outbreaks were mostly linked to the ingestion of recreational water during swimming, consumption of water from contaminated water sources or originating from leaks in drinking water systems (Ramirez-Castillo *et al.,* 2015). In developing countries and the African continent, only a few EHEC O157:H7 foodborne and waterborne related diarrhoea outbreak reports have been documented (Effler *et al.,* 2001; Smith *et al.,* 2017; Lupindu, 2018). In South Africa, due to its semi-arid climate, the Department of Water and Sanitation (DWS) recommends the re-use of treated wastewater discharged into aquatic environment as one of the strategies to increase the primary source of water (Hamiwe *et al.,* 2018). Some South African rivers flow through rural areas and are accessible to people for drinking and domestic use, as well as for use in abattoirs and agriculture (Abong'O and Momba, 2009). As a consequence, a number of potentially infectious biological water contaminants, including *E. coli* can be introduced and circulated into the water sources leading to potential adverse public health risks (Hamiwe *et al.,* 2018). The EHEC O157:H7 is considered as an important clinical, environmental and veterinarian pathogen due to its ability to harbour antibiotic resistance and virulence genes (Day *et al.,* 2017, Bai *et al.,* 2018). There are only a few reports available on EHECO157:H7 isolates in South Africa. To the best of our knowledge, there is no study on the determination of circulating sequence types of EHEC O157:H7 strains isolated from the environmental water samples and clinical stool specimens in South Africa. This study was carried out to investigate the occurrence, the major virulence genes, antibiotic resistance profiles and molecular epidemiology of EHEC O157:H7 isolates in the Gauteng region, South Africa. This research study followed a "One Health" approach to determine EHEC O157:H7 in environmental, animal and clinical specimens.

2. Material and methods

2.1. Study area and sample collection

A total of 520 samples of which environmental water samples (n=270) and clinical stool specimens (n=250) were collected between August 2016 and December 2017.Environmental water samples were collected from 10 water sources and included: (i) run-off water and sewage from three abattoirs (cattle) located in Pretoria-West (41/270), Hammanskraal area (18/270) and Pretoria-North(20/270) and run-off and sewage water from three farms (cattle, pigs and chicken) located in Pretoria-North (10/270), Pretoria-West (10/270) and Cullinan area (10/270) , (ii) clinical wastewater from an academic hospital located in the Pretoria-Gezina area (75/270) and (iii) surface water and catchment from three rivers located around Vaal Marina (42/270), Orangeville (39/270) and Vanderbijl park (5/270), all in the Gauteng Province, South Africa. These water samples were collected in collaboration with the: (i) Department of Agriculture and Rural Development Gauteng Province (GDARD) and (ii) Rand Water, using their routine monitoring sites in the Gauteng province. Water samples were collected aseptically in sterile 1 litre glass bottles properly labeled and transported in an ice box to the research laboratory of the Department of Medical Microbiology, University of Pretoria for processing within 3 hours.

The remainder of the stool specimens (n=250) of patients presenting with diarrhoea, which were sent to the clinical laboratory of the Department of the Medical Microbiology, University of Pretoria/ National Health Laboratory Service (NHLS) for routine analysis were also collected.The collected stool specimens included loose stool (103/250), mucoid (82/250), watery (60/250) and bloody (5/250). The majority of stool specimens were from adults (154/250) compared to children (96/250). The patients' age ranged from ≤1 to ≥ 65 years. Ethical clearance was obtained from the University of Pretoria Research Ethic committee (219/2016) prior sample collection.

2.2. Isolation of enterohaemorrhagic Escherichia coli O157:H7

Enterohaemorrhagic *Escherichia coli* O157:H7 were recovered from the water samples by a standard membrane filtration procedure as previously described by Müller *et al.* (2003). Briefly, 100 mL of water samples were filtered through 0.45 µm cellulose nitrate filter membranes (Sartorius Biotech, Germany). The membranes filters were aseptically placed directly onto selective chromogenic media, ChromID^{TM} O157:H7 plates (bioMérieux, France) and incubated (Vacutec, South Africa) at 37ºC for 18 to 24 hours. Similarly, the stool specimens were inoculated onto selective chromogenic media, ChromID^{TM} O157:H7 plates (bioMérieux, France) and incubated at 37ºC for 18 to 24 hours. Green or bluish-green colonies were an indication of presumptive EHEC O157:H7 according to the manufacturer's manual and were selected, subcultured onto nutrient agar plates (DMP-NHLS, South Africa) and re-incubated (Vacutec, South Africa) at 37ºC for 24 hours. The colourless colonies on

nutrient agar were Gram stained, serotyped using the Wellcolex™ *E. coli* O157 agglutination test (Remel, UK) following the manufacturer's instructionsand recorded as presumptive EHEC O157:H7 isolates. The presumptive EHEC O157:H7 were inoculated into 5 mL of 3.7% brain heart infusion (BHI) broth (Lab M Limited, UK) and incubated for 24 hours at 37ºC. The cultures were stored long-term at -80ºCfreezer (New Brunswick, USA) in 50% sterile glycerol solution until further analysis.

2.3. Molecular identification of enterohaemorrhagic Escherichia coli O157:H7 isolates

A conventional multiplex-PCR assay was carried out to confirm the identity of all presumptive EHEC O157:H7 isolates using specific primers (Table1) targeting the *uid*A (β-glucuronidase) gene specific for all *E. coli* species (Antikainen *et al.,* 2009), the *rfbE*O157 (somatic-O) and *flicH*$_7$ (flagellar-H) genes specific for EHEC serotype O157:H7 (Kanjhar and Alwan, 2014; Wang *et al.,* 2014). All the primers for the M-PCR assays used in this study were synthesised by Inqaba Biotechnical Industries (Pty) Ltd, South Africa.

Table 1: Primer sequences used for multiplex PCR in the study

Target genes	Primers name	Primers sequence (5'- 3')	Product size (bp)	Reference
uidA	*uidA*-1	(F)-ATGCCAGTCCAGCGTTTTTGC	1187	Antikainen*et*
	uidA-2	(R)-AAAGTGTGGGTCAATAATCAGGAAGTG		*al.,* 2009
***rfbE*O157**	*rfbEO157*-1	(F)-AAGATTGCGCTGAAGCCTTTG	296	Wang *et al.,*
	rfbEO157-2	(R)-CATTGGCATCGTGTGGACAG		2014
***fliC*$_{H7}$**	*fliC*$_{H7}$-1	(F)-GCGCTGTCGAGTTCTATCGAG	625	Kanjhar*et al.,*
	fliC$_{H7}$-2	(R)-CAACGGTGACTTTATCGCCATTCC		2014
eae	*eae*-1	(F)- TTAACCACACCCACGGCAGT	106	Ibekwe*et al.,*
	eae-2	(R)-TCAGCGTGGTTGGATCAACCT		2015
hlyA	*hlyA*-1	(F)-GCATCATCAAGCGTACGTTCC	532	Paton *et al.,*
	hlyA-2	(R)- AATGAGCCAAGCTGGTTAAGCT		2002
Stx1	*Stx-1-A*	(F)-ACACTGGATGATCTCAGTGG	614	Kanjhar*et al.,*
	Stx-1-B	(R)-CTGAATCCCCCTCCATTATG		2014
Stx2	*Stx-2-A*	(F)-TTAACCACACCCACGGCAGT	346	Müller *et al.,*
	Stx-2-B	(R)-GCTCTGGATGCATCTCTGGT		2003

The total genomic DNA was extracted from presumptive EHEC O157:H7 (grown overnight in 2 mL BHI), using the ZR Fungal/Bacterial DNA Miniprep™ commercial kit (Zymogen, Thermo Scientific, USA) according to the manufacturer's instructions with the modification of adding β-mercaptoethanol to the Bacterial DNA binding buffer to a final dilution of 0.5% (v/v) i. e 500µL per 100 mL. The 25 µL multiplex PCR reaction consisted of 2X PCR MyTaqHS Red Mix (Bioline®, UK), 0.2 µM for each primer and<1 µg DNA. The PCR amplification was performed in a G-storm thermocycler

(Vacutec, UK) using the following conditions: an initial denaturation step of 95ºC for 5 min, followed by 35 cycles of denaturation at 94ºC for 1.5 min, annealing at 60ºC for 1.5 min and an extension step at 72ºC for 1.5 min, with a final extension at 72ºC for 5 min as previously described (Smith *et al.,* 2011; Tau *et al.,* 2012).The PCR amplification products were separated on a 2% (m/v) agarose gel (SeaKem® Lonza, USA), stained with 5 µL of a 10 mg/mL stock solution of ethidium bromide (Merck, USA). Gel electrophoresis was performed at 90 volts for 90 minutes. A 100 bp molecular weight marker (Fermentas, Thermo Scientific, USA) was included as reference in all gels. The amplicons were visualised and images were captured using a Gel Doc™ EZ system (Bio-Rad, USA). *Escherichia coli* ATCC 25923 and sterile nuclease free water were used as negative controls in this study. A previously characterised and published EHEC O157:H7 strain that was positive for the genes that encode for *uid*A, *rfbE*O157 and $fliC_{H7}$ genes (Smith *et al.,* 2011; Tau *et al.,* 2012) was used in this study as a positive control.

2.4. Antimicrobial Susceptibility Testing

The confirmed EHEC O157:H7 isolates were tested for antibiotic susceptibility using the VITEK®2 automated system (bioMérieux, France). Briefly, a bacterial suspension with an optical density (turbidity) of 0.5 McFarland was prepared in saline (0.85%) (DMP-NHLS, South Africa) from an overnight bacterial culture incubated (Vacutec, South Africa) for 16 hours to 18 hours. The minimum inhibitory concentration (MIC) for each isolate tested was interpreted according to the Clinical and Laboratory Standards Institute (CLSI) 2018 guidelines.

2.5. Detection of the virulence genes using multiplex PCR assay

Multiplex PCR assays were performed using primer sequences targeting the EHEC O157:H7 virulence genes (*eae*, *hly*A, *Stx*-1 and *Stx*-2) as previously described (Paton and Paton, 1998; Müller *et al.,* 2003; Khanjar and Alwan, 2014). The primer sequences used for the amplification ofEHEC O157:H7 virulence genes are shown in Table 1. The 25 µL multiplex PCR reaction consisted of 2X PCR MyTaq HS Red Mix (Bioline®, UK), 0.2 µM for each primer and <1 µg DNA. Previously described PCR conditions were used (Smith *et al.,* 2011; Tau *et al.,* 2012). A characterised EHEC O157:H7 strain positive for the virulence genes: *Stx*-1, *Stx*-2, *hly*A and *eae* genes (Smith *et al.,* 2011; Tau *et al.,* 2012) was used in this study as a positive control.

2.6. Molecular typing of enterohaemorrhagic Escherichia coli O157:H7isolates

Enterohaemorrhagic *Escherichia coli* O157:H7 isolates were genotyped using the PFGE technique as previously described (Greenquist *et al.,* 2005; CDC-PulseNet, 2017) with modifications. The chromosomal DNA of the EHEC O157:H7 isolates was digested by *Xba*1 (New England Biolabs, USA) restriction enzymes. Electrophoresis was performed using the Rotaphor system (Biometra, Germany). The gel was viewed and captured using the UV Transilluminator (DigiDoc-It, UVP, LCC, USA). The resultant PFGE banding patterns were analysed using the GelCompar II software (Applied Maths, Belgium) and clustered using the Dice coefficient. A dendrogram was constructed using the unweighted pair group method with arithmetic mean (UPGMA). Pulsotypes were defined as isolates sharing ≥80% similarities. Major pulsotypes included five or more isolates, while minor pulsotypes included less than five isolates. Representative EHEC O157:H7 isolates were chosen following the analysis of the PFGE for the MLST based on the criteria described by Wirth *et al.* (2006). Four isolates were selected and typed by MLST following a scheme based on the internal sequences of seven housekeeping genes previously described (Wirth *et al.,* 2006; Wang *et al.* 2014) and according to their pulsotypes and sites of collection (abattoir, farms, hospital and river). Singleplex PCR assays were performed for each isolate based on the methods described by Wirth *et al.* (2006) using primers and conditions shown in Table 2.The PCR assay was done in a 25 µL volume consisting of 2X PCR MyTaq HS Red Mix (Bioline®, UK), 0.5 µM for each primer and <1 µg DNA. The PCR conditions consisted of an initial denaturation step of 95ºC for 2 min, followed by 30 cycles of denaturing at 94ºC for 1 min, annealing was different for each gene (Table 2) for 2 min and extension step at 72ºC for 2 min, with a final extension at 72ºC for 5 min as previously described (Wirth *et al.,* 2006; Wang *et al.* 2014).

The PCR products were detected by gel electrophoresis and purified using a PCR purification kit (Qiagen, Germany). The purified PCR amplicons were sequenced by Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa using forward and reverse primers. The resulting sequences were analysed using CLC-Bio main workbench version 6.0 (Qiagen, Denmark).The sequencesobtained were uploaded to the *E. coli* MLST database (https://enterobase.warwick.ac.uk/) for analysis and comparison. The allelic profile (which included the allele number for each of the seven loci) was compared to other profiles in the database and a sequence type (ST) was generated.

Table 2: Primer sequences used for the amplification of the seven housekeeping genes (Wirth et *al.*, 2006)

Genes	Primer sequences (5'-3')	Product size (bp)	Annealing t°
adk (adenylate kinase)	**F**-ATTCTGCTTGGCGCTCCGGG	583	54°C
	R-CCGTCAACTTTCGCGTATTT		
fumC (fumaratehydratase)	**F**-TCACAGGTCGCCAGCGCTTC-	806	54°C
	R-GTACGCAGCGAAAAAGATTC-		
gyrB (DNA gyrase)	**F**-TCGGCGACACGGATGACGGC-	911	60°C
	R-ATCAGGCCTTCACGCGCATC-		
icd (isocitrate/isopropylmalate dehydrogenase)	**F**-ATGGAAAGTAAAGTAGTTGTTCCGGCACA	878	54°C
	R-GGACGCAGCAGGATCTGTT		
mdh (malate dehydrogenase)	**F**-ATGAAAGTCGCAGTCCTCGGCGCTGCTGGCGG	932	60°C
	R-TTAACGAACTCCTGCCCCAGAGCGATATCTTTCTT		
purA (adenylosuccinate dehydrogenase)	**F**-CGCGCTGATGAAAGAGATGA'	816	54°C
	R-CATACGGTAAGCCACGCAGA		
recA (ATP/GTP binding motif)	**F**-CGCATTCGCTTTACCCTGACC	780	58°C
	R-TCGTCGAAATCTACGGACCGGA		

3. RESULTS

3.1. Isolation and detection of EHEC O157:H7 from water samples and stool specimens

Of the 520 samples collected (270 water samples and 250 stool specimens), the selective chromogenic agar (ChromID™ O157:H7, bioMérieux, France) permitted the growth of 165 colonies, which appeared green or bluish green, of which 154/270 were obtained from environmental water samples and 11/250 from stool specimens. Overall, 132/520 presumptive EHEC O157:H7 were identified using Wellcolex™ *E. coli* O157 (Remel, UK) agglutination test of which 121 from water samples and 11from clinical stool specimens. The distribution of collected environmental water samples and the presumptive EHEC O157:H7 is shown in Table 3.

Table 3: The distribution of collected water samples, stool specimens and presumptive enterohaemorrhagic *Escherichia coli* O157:H7 isolates

Sample source	Location	Type of samples collected	No of sample collected	Presumptive EHECO157:H7
Abattoir 1 (cattle)	Pretoria West	Run-off water	41	33
Abattoir 2 (cattle)	Hammanskraal	Sewage water	18	8
Abattoir 3 (cattle)	Pretoria North	Run-off water	20	9
Farm 1 (Cattle)	Pretoria North	Sewage water	10	5
Farm 2 (Cattle + pig)	Cullinan	Sewage + run-off water	10	6
Farm 3 (chicken)	Pretoria West	Run-off water	10	0
River 1 (K-19)	Vaal Marina	Catchment, Surface water	42	27
River 2&3 (C&P-RV2)	Orangeville	Catchment, Surface water	39	18
River 4 (Lochvaal)	Vanderbijl park	Drainage, Surface water	5	5
Clinic (Hospital)	Pretoria-Gezina	Wastewater	75	10
Stool specimens	NHLS/ TAD	Bloody, loose, mucoid, watery	250	11
	Total		**520**	**132**

*** NHLS/TAD: National Health Laboratory Service/ Tshwane Academic Division**

MultiplexPCR assay confirmation indicated that 2.3% (12/520) of the isolates were EHEC O157:H7 [isolates harbouring the *uid*A, $fliC_{H7}$ and *rfbEO*157 genes]. Run-off water showed an EHEC O157:H7 prevalence of 4.07% (11/270) of which 1.48% (4/270) isolates were from abattoir-1, 1.11% (3/270) from river-1 (K-19), 0.74% (2/270) from river-4 (Lochvaal), 0.37% (1/270) from river-3 (C-RV2) and 0.37% (1/270) from the clinical setting (hospital). The prevalence of EHEC O157:H7 in the diarrhoeagenic patients was 0.4% (1/250).

3.2. Antimicrobial susceptibility testing results

The antibiotic susceptibility profile of EHEC O157:H7 isolates (n=12) was determined using the VITEK®2 automated system (bioMérieux, France). AllEHEC O157:H7 isolates were 100% (12/12) susceptible to amikacin, cefepime, cefotaxime, ceftazidime, ciprofloxacin, ertapenem, gentamicin, nitrofurantoin and tigecycline. A total of 7/12 of isolates was resistant to ampicillin; 3/12 resistant to amoxicillin, piperacillin/Tazobactam and trimethoprim each; 2/12 resistant to cefoxitin, imipenem and meropenem each and 1/12 was resistant to cefuroxime (Figure 1). No antibiotic colistin resistance was reported among EHEC O157:H7 isolates.

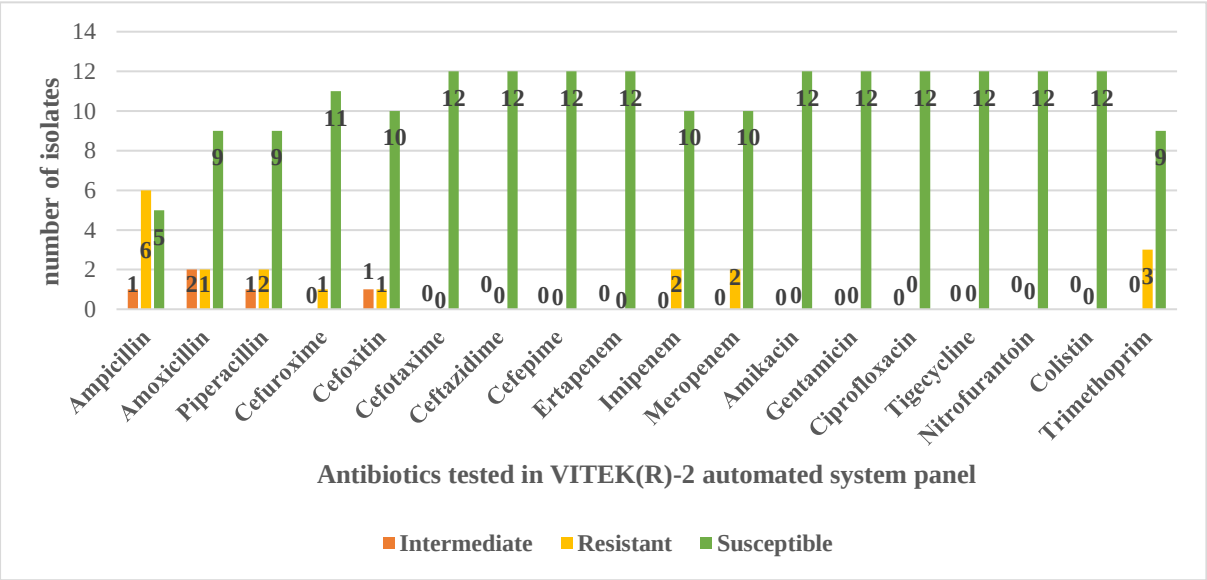


Figure 1: Antibiotic resistance profiles of EHEC O157:H7 isolatesin Gauteng region using the VITEK®-2 automated system (bioMérieux, France).

3.3. Detection of virulence genesamong EHEC O157:H7 isolates using multiplex PCR

Multiplex PCR assays performed on the EHEC O157:H7 isolates detected a combination of virulence genes (*stx*1, *stx*2, *eae*, *hly*A) among the isolates. The combination of virulence genes associated with the antibiotic resistance profile of isolates is indicated in Table 4. (See Table 5.)

Table 4. Detection of virulence genes and antimicrobial resistance profile of EHEC O157:H7 isolates

No	Isolate no	Sample type	Collection site	Identification genes	Virulence genes	Antibiotic resistance
1	WS1	Run-off water	Abattoir-1	*uid*A, *rfbEO*157, *flicH*$_7$	*stx*1, *stx*2, *hly*A	AMP, AMX, CXM, FOX, TMP
2	WS3	Wastewater	Hospital	*uid*A, *rfbEO*157, *flicH*$_7$	*stx*1, *stx*2, *hly*A	AMP, AMX, FOX
3	WS95	Surface water	River-1	*uid*A, *rfbEO*157, *flicH*$_7$	*stx*1, *hly*A	AMP, AMX, TMP
4	WS108	Catchment	River-3	*uid*A, *rfbEO*157, *flicH*$_7$	*stx*2, *eae*, *hly*A	N/A*
5	WS114	Drainage	River-4	*uid*A, *rfbEO*157, *flicH*$_7$	*stx*2, *eae*, *hly*A	AMP, TAZ, IMP, MERO
6	WS115	Surface water	River-1	*uid*A, *rfbEO*157, *flicH*$_7$	*eae*, *hly*A	AMP, AMX, TAZ
7	WS116	Surface water	River-1	*uid*A, *rfbEO*157, *flicH*$_7$	*eae*, *hly*A	AMP, TMP,
8	WS120	Drainage	River-4	*uid*A, *rfbEO*157, *flicH*$_7$	*eae*, *hly*A	AMP, TAZ, IMP, MERO
9	WS124	Run-off water	Abattoir-1	*uid*A, *rfbEO*157, *flicH*$_7$	*stx*1, *eae*, *hly*A	N/A*
10	WS125	Run-off water	Abattoir-1	*uid*A, *rfbEO*157, *flicH*$_7$	*stx*2, *eae*, *hly*A	N/A*
11	WS126	Run-off water	Abattoir-1	*uid*A, *rfbEO*157, *flicH*$_7$	*stx*1, *eae*, *hly*A	N/A*
12	St5	Stool specimen	NHLS/TAD	*uid*A, *rfbEO*157, *flicH*$_7$	*stx*1, *eae*	N/A*

*** WS: water samples* st: stool specimens**

*** NHLS/TAD: National Health Laboratory Service/ Tshwane Academic Division**

*** (R): resistance to Ampicillin (AMP), Amoxicillin (AMX), Piperacillin-tazobactam (TZP), Cefuroxime (CMX), Cefoxitin (FOX), Imipenem (IMP), Meropenem (MERO), Trimethoprim (TMP).**

***N/A: no antibiotic resistance observed**

3.4. Molecular typing of EHEC O157:H7 isolates

The genetic relatedness of EHEC O157:H7 isolates was determined by using PFGE. A total of 12 confirmed EHEC O157:H7 isolates were successfully digested with *Xba*1 restriction enzyme. The dendrogram revealed genetically diverse nature of the EHECO157:H7 isolates which belonged to two minor pulsotypes having two isolates each and eight singletons (Figure 2). Isolate WS1 collected from abattoir-1 and WS3 recovered from hospital wastewater shared a high level of similarity.

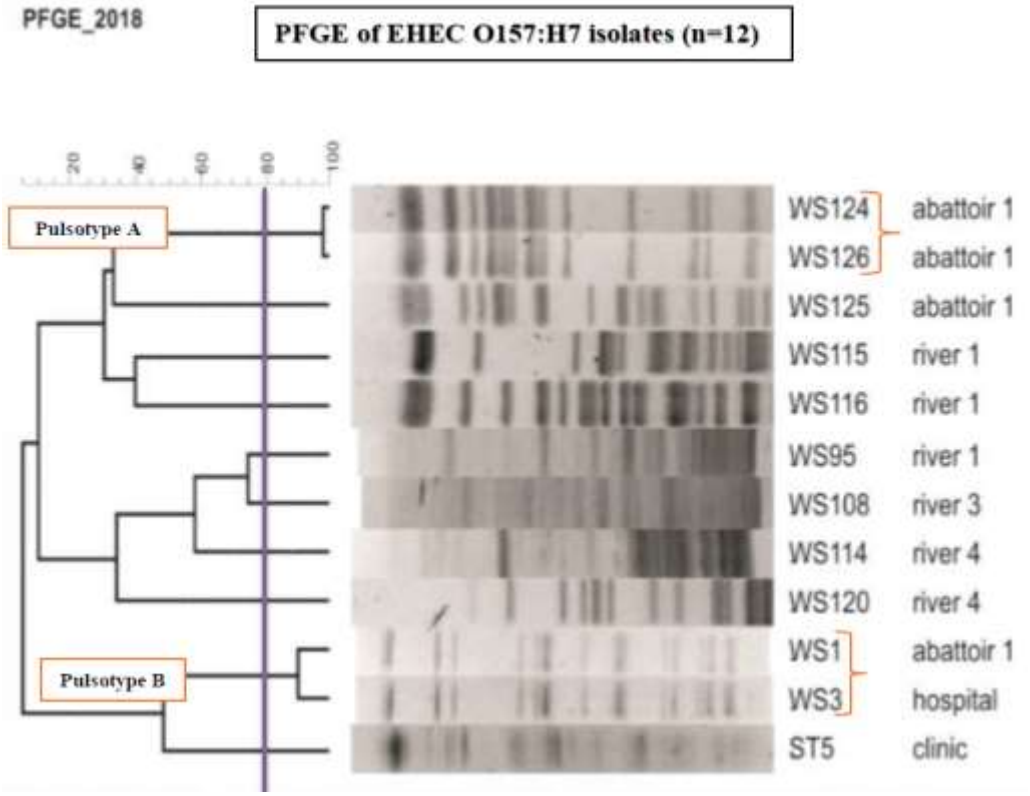


Figure 2: **The dendrogram of PFGE analysis showing genetic diversity among EHEC O157:H7 isolates**

*** WS: water samples**

*** ST: stool specimens**

Four EHEC O157:H7 isolates were selected for MLST and were assigned STs (ST10, ST11 and ST1204) as shown in Table 5. Isolates WS95 obtained from river-1 and WS114 from river-4 shared the same ST (ST1204) with allelic profile 83-230-120-161-159-2-2. Isolate WS108 recovered from river 3 belonged to ST11 with allelic profile 12-12-8-12-15-2-2 and isolate WS126 obtained from abattoir-1 with allelic profile 6-11-4-8-8-8-2 belonged to ST10.

Table 5: The sequence types (STs) and allelic profile of EHEC O157:H7 isolates

Isolate no	Sample type	Site of collection	Sequence type	Allelic profile						
				adk	***fumC***	***gyrB***	***icd***	***mdh***	***purA***	***recA***
WS95	Surface water	River-1	**ST1204**	83	230	120	161	159	2	2
WS108	Catchment	River-3	**ST11**	12	12	8	12	15	2	2
WS114	Drainage	River-4	**ST1204**	83	230	120	161	159	2	2
WS126	Run-off water	Abattoir-1	**ST10**	6	11	4	8	8	8	2

*** WS: water samples**

*** ST: sequence types**

4. DISCUSSION

The zoonotic transfer of resistant and virulent bacteria amongst animals, environment and humans leads to an increase burden, which results in fewer treatment options and increased morbidity and mortality rates around the world (WHO, 2011). Potential infectious strains can be found circulating in both clinical and environmental samples. This emphasises the importance of the One Health approach to investigate the complexity surrounding the relationship between animal, environmental and human health and the transmission of zoonotic pathogens (Mendelson *et al*., 2018). The present study was conducted to characterise and determine the genetic diversity ofEHEC O157:H7 isolatesfrom clinical stool specimens and environmental water samples in the Gauteng region, South Africa.

In total, 520 samples were included in this study from which 165 characteristics colonies were identified using the selective chromogenic agar (ChromIDTM O157:H7, bioMérieux, France). However, this media was not selective enough, since it accommodated the growth of other species and lead to the misidentification of colonies especially in environmental samples. The selective media, therefore, required confirmatory tests to be performed. The latex agglutination test (Wellcolex^{TM}*E. coli* O157, Remel, UK) was used as the confirmatory test and allowed the identification of 132 presumptive EHEC O157:H7 from both clinical and environmental samples.Molecular testing was done and confirmed 12/132 EHEC O157:H7 isolates. Overall, 2.3% (12/520) positive isolates were identified as EHEC O157:H7. This observation was supported by a study conducted in the Cape Town metropolitan area, South Africa in which Kaluleand colleagues (2018) screened 507 stool specimens directly on CHROMagar^{TM} STEC (Microbiology, Paris, France) and the media permitted the growth of 204/507 isolates of which only 12/204 diarrhoeic*E. coli* were confirmed with molecular test. The authors concluded that chromogenic agar cannot be relied on solely to screen specimens for EHEC O157:H7 (Kalule *et al.,* 2018). In the USA, Cooley and colleagues (2013)during the development of a robust method for isolation of EHEC O157:H7 from faecal, plant, soil and water samples observed that chromogenic agar O157 could lead to false positive results, even though the

selective medium was supplemented with cefixime tellurite. The colony colour for the O-type might not be displayed uniformly due to colony density on the plates (Cooley *et al.*, 2013).

The present study observed that EHEC O157:H7 was mostly detected in the environmental samples (11/520) as compared to stool specimens (1/520). The prevalence of 2.3% (12/520) EHEC O157:H7 is in agreement with the study conducted in the North West province of South Africa by Ateba and Mbewe (2011), whom reported a EHEC O157:H7 prevalence of 2.3% (130/5600) of which 0.8% (1/130) from human stool specimens, 1.5% (2/130) from tap water, 2.3% (3/130) from river catchment, 28% (36/130) from cattle and a large proportion of 68% (88/130) from pigs. However, the current prevalence was lower when compared to the 26% (46/180) of EHEC O157:H7 isolates reported by Abong'O and Momba (2008) in the rural area of Amathole district, in the Eastern Cape province of South Africa. This is not surprising as the Eastern Cape province is one of the South African provinces where many farms are found and people live in these rural areas with rural and traditional life style (Abong'O and Momba, 2009). In the USA, Cooley and colleagues (2013) conducted a 2.5 year survey of 33 farms and tested water samples, livestock, wildlife and soil for EHEC O157:H7 isolates and reported 2.6% (357/13 650) of EHEC O157:H7 of which water samples were 3.3%, sediment (4.4%) and cattle 7.1%. The differences in EHEC O157:H7 prevalence might be due to differences in sampling and culturing techniques, geographic location, to the environment and to the characteristics and genetics of the population investigated (Cooley *et al.*, 2013).Studies have shown that diet can affect the carriage and shedding of *E. coli* in cattle (Smith *et al.*, 2011). The low prevalence of EHEC O157:H7 detected in this study especially in clinical stool specimens supported the WHO statements saying that the incidence of EHEC O157:H7 in developing countries including South Africa is low where people tend to rely mostly on traditional farming practices where cattle (reservoir) are free-roaming with a diet consisting mainly of naturally growing grass compared to the Northern Hemisphere, where there is an increased practice of rearing cattle (reservoir) in feedlots (factory farming) with specialised diets including grain, hay, corn, sorghum and other food processing (Müller *et al.*, 2002; Smith *et al.*, 2011).

There is no empirical treatment available for EHEC O157:H7 infections (Amirlak and Amirlak, 2006). Antibiotics can promote the expression of Shiga toxin genes and increase the severity of the disease (Amirlak and Amirlak, 2006). Although antibiotics are not recommended for the treatment of EHEC O157:H7, many isolates carry resistance to various antimicrobials (Day *et al.*, 2017; Um *et al.*, 2018). The presence of multidrug resistance in food producing animals, such as cattle and pigs and in environmental run-off and wastewater samples is alarming as these pathogens may be transferred to closed human contacts and consumers of untreated water and contaminated food products (Bai *et al.*, 2018; Um *et al.*, 2018). In the current study, the confirmed EHEC O157:H7 isolates were subjected to antibiotic susceptibility testing using the VITEK®2 automated system (bioMérieux, France). The current study is one of the few studies, which reported the antibiotic

resistance profiles of the EHEC O157:H7 in South Africa. In 2009, Abong'O and Momba, reported EHEC O157:H7 isolates resistant to five of the eight antibiotics tested including ampicillin. Similar results were reported in the United Kingdom by Day *et al.* (2017) whom reported ampicillin and trimethoprim/ sulphonamide resistance occurring in 5.8% of EHEC O157:H7 isolates. Combined antibiotic resistance of EHEC O157:H7 isolates has also been reported in France (Um *et al.,* 2018). In this study, no colistin resistance was reported among the EHEC O157:H7 isolates.

In the present study, a combination of virulence genes (*stx1*, *stx2*, *eae*, and *hly*A*)* was detected among the EHEC 0157:H7 isolates as shown in Table 4. This observation was in contrast to the findings in China by Wang and colleagues (2014) who reported the combination of virulence genes and that the *stx1*, *stx2*, *eae*, and *hlyA* genes were detected in 27% (8/30), 40% (12/30), 63% (19/30) and 50% (15/30) of the EHEC O157 isolates respectively. The presence of virulence genes in EHEC O157:H7 obtained from stool specimens and water samples from abattoir, a clinical setting (hospital), farm and river water samples means that there is potential risk for the consumer of untreated water and consumption of undercooked food products.

A high genetic diversity among the EHEC O157:H7 isolates was observed. This is not surprising as the environmental water samples were from a wide variety of sources including, abattoirs, farms, rivers and clinical setting (hospital). Similar findings have been reported in the USA by Probert and colleagues (2017). No close genetic similarity of EHEC O157:H7 strains from the environment and clinical stool isolates was observed in this study. However, isolate WS124 and WS126, both collected from the same abattoir, (abattoir-1) showed a similarity of 90% indicating high similarity between the two isolates. Interestingly, isolate WS1 from abattoir-1 and WS3 (wastewater)from the hospital showed ≥80% of similarity. This is worrying, since closely related isolates are circulating between humans and animals.

Three STs, ST10, ST11 and ST1204, were identified among the EHEC O157:H7 isolates. These STs have never been reported in South Africa but have previously been reported worldwide and associated with livestock animal, water and human outbreaks (Mellmann *et al.*, 2008; http://www.enterobase.warwick.ac.uk/). The ST10 strain has been reported in Spain producing extended-spectrum β-lactamases (ESBLs) (Oteo *et al.,* 2009) and has also been reported in Egypt (Shabana *et al,* 2013). It has been reported that EHEC O157:H7 ST11 is one of the most virulent strains of EHEC O157:H7 (Laing *at al.,* 2009; Underwood *et al.,* 2012). In the UK, Underwood and colleagues (2012) investigated the genome sequences during an outbreak and reported that EHEC O157:H7 belonging to ST11 was a virulent strain, which harboured two *stx*-2 verotoxins and other putative virulence factors (Underwood *et al.,* 2012). In China, Wang *et al.* (2014) reported nine STs of which ST11 uncounted for a third (10/30) of the EHEC O157 isolates. The ST1204 has previously been reported in Egypt. Shabana and colleagues (2013) used PCR, PFGE and MLST to analyse the

genetic relationships of 48 diarrhoea-associated *E. coli* strains isolated from sporadic diarrhoeal cases from humans, calves and camels. The results showed that ST10 was among the major sequence types detected 27% (13/48) in human and calf and that 17 new STs and 12 new alleles were identified of which variant ST1204 was among them (Shabana *et al.*, 2013).The presence of the STs linked with outbreaks circulating in our setting means there is potential public health risk mostly for abattoir and farm workers and the consumer of untreated water and of undercooked food products.

5. Conclusion

The present study has detected EHEC O157:H7 isolates from the abattoirs, farms and hospital run-off water carrying virulent genes. The EHEC O157:H7 isolates obtained from environmental water samples and clinical stool specimens were genetically diverse and were antibiotic resistant. The antibiotic colistin resistance was not present in the isolates. The sequence types isolated in this study are found to be associated with outbreaks. The propagation of theses STs in the environment pose a potential public health risk to close human contacts as well as to consumers of untreated water. This study emphasised the importance of implementation of environmental surveillance and monitoringof this important pathogen, in reducing the potential public health risk and preventing of human diseases.

Acknowledgements

The authors thank Professor Maureen B. Taylor from Rand Water South Africa and Mr GA van der Zel from GDARD, South Africa for collection and transport of water samples and for permission to publish the data. The authors thank also Dr Karen Keddy from National Institute for Communicable Diseases (NICD) for providing with control strains. The authors finally thank National Health Laboratory Service (NHLS), National Research Foundation (NRF), Rand Water and University of Pretoria, South Africa for financial support. The authors have no conflict of interest to declare.

References

Abong'O, B.O., Momba, M.N.B., 2008. Prevalence and potential link between *Escherichia coli* O157:H7 isolated from drinking water, meat and vegetables and stools of diarrhoeic confirmed and non-confirmed HIV/AIDS patients in the Amathole District - South Africa. *Journal of Applied Microbiology* 105:424-431

Abong'O, B.O., Momba, M.N.B., 2009. Prevalence and characterisation of *Escherichia coli* O157:H7 isolates from meat and meat products sold in Amathole District, Eastern Cape Province of South Africa. *Food Microbiology* 26:173-176

Amirlak I and Amirlak B (2006) Haemolytic ureamic syndrome (HUS): An overview. *Journal of Nephrology* 11:213-218

Antikainen, J., Tarkka, E., Haukka, K., Siitonen, A., Vaara, M., Kirveskari, J., 2009. The new16-plex PCR method for rapid detection of diarrhoeagenic *Escherichia coli* directly from stool samples. *European Journal of Clinical Microbiology and Infectious Diseases* 28:899-908

Ateba, C.N., Mbewe, M., 2011. Detection of *Escherichia coli* O157:H7 virulence genes in isolates from beef, pork, water, human and animal species in the North West Province, South Africa: public health implications.*Research in Microbiology* 162:240-248

Bai, X., Mernelius, S., Jernberg, C., Einemo, I.M., Monecke, S., Ehricht, R., Matussek, A., 2018. Shiga toxin-producing *Escherichia coli* infection in Jönköping County, Sweden: Occurrence and molecular characteristics in correlation with clinical symptoms.*Front Cellular and Infection Microbiology* 8:1-8

Baranzoni, G.M., Fratamico P.M., Gangiredla, J., Patel, I., Bagi, L.K., Delannoy, S., Fach, P., Boccia, F., Anastasio, A., Pepe, T., 2016. Characterisation of Shiga toxin subtypes and virulence genes in porcine Shiga toxin-producing *Escherichia coli*. *Frontiers in Microbiology* **7**:1-10

Berenger, B.M., Berry, C., Peterson, T., Fach, P., Delannoy, S., Li, V., Tschetter, L., Nadon, C., Honish, L., Louie, M., Chui, L., 2015. The utility of multiple molecular methods including whole genome sequencing as tools to differentiate *Escherichia coli* O157:H7 outbreaks. *Europeansurveillance* 20:1560-1579

Centers for Disease Control and Prevention, 2014. Surveillance for foodborne disease outbreaks in United States, the 2014 annual report. http://www.cdc.gov/foodsafety/fdoss/. [Accessed 12 Feb 2018]

Centers for Disease Control and Prevention, 2017.Standard operating procedure for PulseNet PFGE of *Escherichia coli* O157:H7, *Escherichia coli* non-O157, *Salmonella serotypes*, *Shigella species*. *PNL05 Updated December* 2017. http://www.cdc.gov/pulsenet/pdf [accessed 20 January 2018]

Chattaway, M.A., Dallman, T.J., Gentle, A., Wright, M.J., Long, S.E., Ashton, P.M., Perry, N.T., Jenkins, C., 2016.Whole genome sequencing for public health surveillance of Shiga toxin-producing *Escherichia coli* other than serogroup O157. *Frontiers in Microbiology* 7:1-4

Clinical and Laboratory Standards Institute, 2018. Performance standards for antimicrobial susceptibility testing. CLSI document M100-S19

Cooley, M.B., Jay-Russell, M., Atwill, E.R., Carychao, D., Nguyen, K., Quinones, B., Patel, R., Walker, S., Swimley, M., Pierre-Jerome, E., Gordus, A.G., Mandrell, R.E., 2013 Development of a robust method for isolation of Shiga toxin-positive *Escherichia coli* from faecal, plant, soil and water samples from a leafy greens production region in California. *PLoS ONE* 8:1-22

Croxen, M.A., Law, R.J., Scholz, R., Keeney, K.M., Wlodarska, M., Finlay, B.B.,2013. Recent Advances in understanding enteric pathogenic *Escherichia coli*. *Clinical Microbiology Reviews* 26:822-880

Day, M., Doumith, M., Jenkins, C., Dallman, T.J., Hopkins, K.L., Elson, R., Godbole, G., Woodford, N., 2017. Antimicrobial resistance in Shiga toxin-producing *Escherichia coli* serogroups O157 and O26 isolated from human cases of diarrhoeal disease in England, 2015. *Journal of Antimicrobial Chemotherapy* 72:145-152

Effler, P., Isaäcson, M., Arntzen, L., Heenan, R., Canter, P., Barrett, T., Lee, L., Mambo, C., Levine, W., Zaidi, A., Griffin, P.M. 2001. Factors contributing to the emergence of *Escherichia coli* O157 in Africa. *Emerging Infectious Diseases* 7:812-819

Enterobase,https://enterobase.warwick.ac.uk/species/ecoli/[acceded date: 20-October-2018]

Greenquist, M.A., Drouillard, J.S., Sargeant, J.M., Depenbusch, B.E., Shi, X., Lechtenberg, K.F., Nagaraja, T.G., 2005. Comparison of recto-anal mucosal swab cultures and faecal cultures for determining prevalence of *Escherichia coli* O157 in feedlot cattle. *Applied and Environmental Microbiology* 71:6031-33

Gyles CL (2007) Shiga toxin-producing *Escherichia coli*: an overview. *Journal of Animal Science* 85:45-62.

Hamiwe, T., Kock, M.M., Magwira, C.A., Antiabong, J.F., Ehlers, M.M., 2018. Occurrence of enterococci harbouring clinically important antibiotic resistance genes in the aquatic environment in Gauteng, South Africa. *Environmental Pollution* 245:1041-1049

Ibekwe, A.M., Watt, P.M., Grieve, C.M., Sharma, V.K., Lyons, S.R., 2002. Multiplex fluorogenic real-time PCR for detection and quantification of *Escherichia coli* O157:H7 in dairy wastewater Wetlands. *Applied and Environmental Microbiology* 68:4853-4862

Iweriebor, B.C., Iwu, C.J., Obi, L.C., Nwodo, U.U., Okoh, A.I., 2015. Multiple antibiotic resistances among Shiga toxin producing *Escherichia coli* O157 in faeces of dairy cattle farms in Eastern Cape of South Africa. *BMC Microbiology* 15:1-9

Johansen, B.K., Wasteson, Y., Granum, P.E., Brynestad, S., 2001. Mosaic structure of Shiga-toxin-2-encoding phages isolated from *Escherichia coli* O157:H7 indicates frequent gene exchange between lambdoid phage genomes. *Microbiology,* 147:1929-1936

Kalule, J.B., Keddy, K.H., Nicoll, M.P., 2018. Characterisation of STEC and other diarrhoeic *Escherichia coli* isolated on CHROMagar™STEC at a tertiary referral hospital, Cape Town. *BMC Microbiology* 18:1-8

Khanjar, A.F., Alwan, M.J., 2014. Genotypic study of *Escherichia coli* O157:H7 isolated from stool samples of humans and cattle. *International Journal of Advanced Research* 2:204-212

Laing, C.R., Buchanan, C., Taboada, E.N., Zhang, Y., Karmali, M.A., Thomas, J.E., Gannon, V.P., 2009.*in silico* genomic analyses reveal three distinct lineages of *Escherichia coli* O157:H7, one of which is associated with hyper-virulence *BMC Genomics* 10:1-11

Launders, N., Byrne, L., Jenkins, C., Harker, K., Charlett, A., Adak, G.K., 2016. Disease severity of Shiga toxin producing *Escherichia coli* O157 and factors influencing the development of typical haemolytic uraemic syndrome: a retrospective cohort study, 2009–2012. *BMJdoi:10.1136/bmjopen-2015-009933*

Lim, J.Y., Yoon, J.W., Hovde, C.J., 2010. A brief overview of *Escherichia coli* O157:H7 and its plasmid O157. National Institute of Health (NIH) *Journal of Microbiology and Biotechnology* 20:5-14

Lupindu, A.M., 2018. Epidemiology of Shiga toxin-producing *Escherichia coli* O157:H7 in Africa in review *Southern African Journal of Infectious Disease* 31:24-30

Mellmann, A., Bielaszewska, M., Köck, R., Friedrich, A.W., Fruth, A., Middendorf, B., Harmsen, D., Schmidt, M.A., Karch, H., 2008. Analysis of collection of haemolytic uremic syndrome-associated enterohaemorrhagic *Escherichia coli*. *Emerging Infectious Diseases* 14:1287-1290

Mendelson, M., Brink, A., Gouws, J., Mbelle, N., Naidoo, V., Pople, T., Schellack, N., van Vuuren, M., Rees, H., 2018. The "One Health" stewardship of colistin as an antibiotic of last resort for human health in South Africa. *The Lancet Infectious Disease*: 18:288-294

Müller, E.E., Taylor, M.B., Grabow, W.O.K., Ehlers, M.M., 2002. Isolation and characterisation of *Escherichia coli* O157:H7 and shiga toxin-converting bacteriophages from strains of human, bovine and porcine origin. *Water Science and Technology: Water Supply*2:29-38

Müller, E.E., Grabow, W.O.K., Ehlers, M.M., 2003. Immunomagnetic separation of *Escherichia coli* O157:H7 from environmental and wastewater in South Africa. *Water SA* 29:427-432

Nguyen, Y., Sperandio, V. 2012. Enterohaemorrhagic *Escherichia coli* pathogenesis. *Frontiers in Cellular and Infection Microbiology* 2:1-7

Oteo, J., Diestra, K., Juan, C., Bautista, V., Novais, A., Pérez, M., Moyá, B., Miró, E., Coque, T.M., Antonio, A., Cantón, R., Navarro, F., Campos, J., 2009. Extended-spectrum-ß-lactamase-producing *Escherichia coli* in Spain belong to a large variety of multilocus sequence typing types, including ST10 complex/A, ST23 complex/A and ST131/B2. Spanish Network in Infectious Pathology Project. *International Journal of Antimicrobial Agents* 34:173-176

Paton, A.W., Paton, J.C., 1998. Detection and characterisation of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for *stx*1, *stx*2, *eae*A, *hlyA*, *rfb*O111 and *rfb*O157*Journal of ClinicalMicrobiology* 36:598-602

Pennington, H., 2010.*Escherichia coli* O157. *The Lancet Infectious Disease* 376:1428-1435

Probert, W.S., Miller, G.M., Ledin, K.E., 2017. Contaminated stream water as source for *Escherichia coli* O157 illness in children *Emerging Infectious Diseases* 23:1216-1218

Ramírez-Castillo, F.Y., Loera-Muro, A., Jacques, M., Garneau, P., Harel J., Guerrero-Barrera, A.L., 2015. Waterborne pathogens: Detection methods and challenges. *Pathogens* 4**:**307-334

Shabana, I.I., Zaraket, H., Suzuki, H., 2013. Molecular studies on diarrhoea-associated *Escherichia coli* isolated from humans and animals in Egypt. *Veterinary Microbiology* 167: 532-539

Smith, A.M., Tau, N.P., Sooka, A., Keddy, K.H., 2011. Surveillance for enterohaemorrhagic *Escherichia coli* associated with human diarrhoea in South Africa, 2006-2009. Group for Enteric

Respiratory Meningeal Disease Surveillance in South Africa. *Journal of Medical Microbiology* 60:681-683

Smith, A.M., Kalule J.B., Nicol, M.P., Kleynhans, J., McCulloch, M., Duz, S.T., Ismail, A., Allam, M., Tau, N.P., Keddy, K.H., 2017. Genome Sequence for Shiga toxin producing *Escherichia coli* O26:H11, associated with a cluster of haemolytic uremic syndrome cases in South Africa. *ASM Genome announcements* 5:1-2

Tau, N.P., Meidany, P., Smith, A.M., Sooka, A., Keddy, K.H., 2012.*Escherichia coli* O104 associated with human diarrhoea, South Africa, 2004-2011. *Emerging Infectious Diseases* 18:1-4

Um, M.M., Brugère, H., Kérourédan, M., Oswald, E., Bibbal, D., 2018. Antimicrobial resistance profiles of enterohaemorrhagic of Serotypes O157:H7, O26:H11, O103:H2, O111:H8, O145:H28 Compared to *Escherichia coli* isolated from the same adult cattle. *Microbial Drug Resistance* 4:1-8.

Underwood, A.P., Dallman, T., Thomson, N.R., Williams, M., Harker, K., Perry, N., Adak, B., Willshaw, G., Cheasty, T., Green, J., Dougan, G., Parkhill, J., Waina, J., 2012. Public health value of next-generation DNA sequencing of enterohaemorrhagic *Escherichia coli* isolates from an outbreak. *Journal of Clinical Microbiology* 51:232–237

Vasant, B.R., Stafford, R.J., Jennison, A.V., Bennett, S.M., Bell, R.J., Doyle, C.J., Vlack, S.A., Titmus,H.V., Lambert, S.B., 2017. Mild illness during outbreak of Shiga toxin–producing *Escherichia coli* O157 infections associated with agricultural show, Australia. *Emerging Infectious Diseases* 23:1686-1689

Wang, S., Zhang, S., Liu, Z., Liu, P., Shi, Z., Wei, J., Shao, D., Li, G., Ma, Z., 2014. Molecular Characterisation of enterohaemorrhagic *Escherichia coli* O157 Isolated from Animal Faecal and Food Samples in Eastern China.*The Scientific World Journal* 946394:1-7

Wirth, T., Falush, D., Lan, R., Colles, F., Mensa, P., Wieler, L.H., Karch, H., Reeves, P.R., Maiden, M.C.J., Ochman, H., Achtman, M., 2006.Sex and virulence in *Escherichia coli*: an evolutionary perspective. *Molecular Microbiology Journal* 60:1136-1151

World Health Organisation, 2011. Removing obstacles to health development. Available from http://www.who.int/infectious-disease_report/pages/textonly.html/ [Accessed 28-01-2019]