Review

Characterisation of *Escherichia coli* O157 strains from humans, cattle and pigs in the North-West Province, South Africa

Collins Njie Ateba [a,*], Cornelius Carlos Bezuidenhout [b,1]

[a] Centre for Animal Health Studies, School of Agricultural Sciences, North-West University — Mafikeng Campus, P. Bag X2046 Mmabatho 2735, South Africa
[b] School of Environmental Science and Development, North-West University — Potchefstroom Campus, South Africa

A B S T R A C T

Escherichia coli O157 strains cause diseases in humans that result from the consumption of food and water contaminated with faeces of infected animals and/or individuals. The objectives of this study were to isolate and characterise *E. coli* O157 strains from humans, cattle and pigs and to determine their antibiotic resistant profiles as well as detection of virulence genes by PCR. Eight hundred faecal samples were analysed for typical *E. coli* O157 and 76 isolates were positively identified as *E. coli* O157 strains. 16S rRNA sequence data were used to confirm the identity of the isolates. Susceptibility profiles to 9 antibiotics were determined and the multiple antibiotic resistant (MAR) patterns were compiled. A large proportion (52.6%–92.1%) of the isolates from pigs, cattle and humans were resistant to tetracycline, sulphamethoxazole and erythromycin. Thus the phenotype Smx–T–E (sulphamethozaxole–tetracycline–erythromycin) was present in most of the predominant MAR phenotypes obtained. Cluster analysis of antibiotic resistances revealed a closer relationship between isolates from pig and human faeces than cattle and humans. PCR were performed to amplify STEC virulence and tetracycline resistance gene fragments. A *tetB* gene fragment was amplified among the isolates. Eighteen (60%) of the isolates possessed the *hlyA* gene and 7(23.3%) the *eae* gene while only 5(16.7%) possessed both genes. Although shiga toxin genes were detected in the *E. coli* O157:H7 positive control strain none of the isolates that were screened possessed these genes. In a related study we reported that the prevalence of *E. coli* O157 was higher in pigs than cattle and humans. A high market demand for pork and beef in South Africa amplifies the risk that diseased animals pose to human health. This highlighted the need for proper hygiene management to reduce the prevalence of *E. coli* O157 in farm animals and prevent the spread from animals to humans.

© 2008 Elsevier B.V. All rights reserved.

Contents

* Corresponding author. Tel.: +27 18 389 2731, +27 78 334 4878 (mobile); fax: +27 18 386 2686.
  E-mail addresses: collins.ateba@nwu.ac.za, atebacollins1@hotmail.com (C.N. Ateba), carlos.bezuidenhout@nwu.ac.za (C.C. Bezuidenhout).
[1] Tel.: +27 18 299 2315, +27 82 494 0221 (mobile); fax: +27 18 299 2330.

1. Introduction

Escherichia coli O157 are the predominant strains of the shiga toxin-producing *E. coli* that cause infections to humans in many parts of the world including the Southern African region (Armstrong et al., 1996; WHO, 1997; Browning et al., 1990; Dunn et al., 2004). These diseases range from simple diarrhoea to the more complicated haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS) (Browning et al., 1990; Armstrong et al., 1996; Smith et al., 2003; Dunn et al., 2004). The pathogenicity of *E. coli* O157 strains result from the production of virulence genes and stx_1, stx_2 and its variants, *eae* and *hlyA* are the most frequently identified (Brunder et al., 1999; Gioffre et al., 2002; Paton and Paton, 2002; Smith et al., 2003).

Most infections caused by *E. coli* O157 result from the consumption of food and water contaminated with faecal matter of infected animals (Riley et al., 1983; Armstrong et al., 1996; Muhldorfer et al., 1996; Müller et al., 2001; Dunn et al., 2004). In South Africa both environmental and foodborne sources of *E. coli* O157 pose threats to human health (Müller et al., 2001; Ateba et al., 2008). Although *E. coli* O157 is frequently isolated from the gastrointestinal tract of several animal species in some European countries (Chapman et al., 1993; Armstrong et al., 1996; Pritchard et al., 2000; Bryan et al., 2004; Mi-Yeong et al., 2004), there is little information available on the prevalence of this pathogen in the faeces of animals in South Africa (WHO, 1997; Müller et al., 2001; Ateba et al., 2008). Furthermore, very few cases of infections caused by this pathogen are documented in South Africa (Browning et al., 1990; WHO, 1997) since patients rarely report their cases to the hospital (Müller et al., 2001). In a related study, the prevalence of *E. coli* O157 in pigs and cattle was higher than that from human stool samples (Ateba et al., 2008). Moreover, the animals that harboured these isolates were asymptomatic while the human subjects presented with cases of diarrhoea. Thus, from a clinical point of view, determining the prevalence of these virulence genes within an isolated *E. coli* O157 population could help in reducing the incidence of diseases in humans.

It is generally discouraged to treat STEC infections with antibiotics (Yoh and Honda, 1997; Igarashi et al., 1999) as they may induce shiga-toxins and increase the chances of the disease to progress to HUS (Wong et al., 2000; Dundas et al., 2001). However, studies have also revealed that *E. coli* O157 isolates are resistant to antibiotics (Galland et al., 2001; Zhao et al., 2001; Schroeder et al., 2002; Bettelheim et al., 2003; Wilkerson et al., 2004). Determining the antibiotic resistant phenotypes of these pathogens may reveal the distribution of antibiotic resistant genes within the population and thus provide suggestions that could help in the control of antibiotic resistance. The aim of the study was to characterise *E. coli* O157 strains isolated from faeces of cattle, pigs and human stool samples using their antibiotic resistance profiles and STEC virulence genes.

2. Materials and methods

2.1. Bacterial strains

In this study, 800 faecal samples were collected from communal cattle and pigs as well as humans. The human stool samples were obtained from the bacteriology laboratory of a local provincial hospital. Samples were collected from patients that visited the hospital for cases of diarrhoea and were provided without indication of patient identity. All samples were handled with care during bacterial isolation and were incinerated immediately after analysis.

Animal faecal samples were collected directly from the rectum of animals using sterile arm-length gloves. Pig faeces samples were collected from animals from commercial and communal farms in Mareetsane and Tlapeng, respectively. Samples from cattle were collected from commercial farms in Lichtenburg and Rustenburg and a communal farm in Mogosane. Animal samples were placed in sterile sample collection bottles, immediately placed on ice and transferred to the laboratory for analysis. Positive and negative controls were *E. coli* O157:H7 (ATCC 43889) and *E. coli* O157:H7 (ATCC 43888), respectively. The former possessed stx_1, stx_2, *eae* and *hlyA* genes while the latter was negative for all these genes.

Enrichment, isolation and identification for *E. coli* O157 were achieved using methods outlined by Ateba et al. (2008). A loopful of the faecal sample was inoculated into MacConkey broth medium (3 ml) [Biolab, Merck (South Africa)] and incubated at 37 °C for 18 to 24 h. After incubation a tenfold serial dilution was performed with sterile distilled water and aliquots (100 µl) of each dilution was plated onto Sorbitol MacConkey agar [Biolab, Merck (South Africa)]. Plates were incubated at 37 °C for 18 to 24 h. Potential *E. coli* isolates were subcultured onto sorbitol MacConkey agar and the plates were incubated at 37 °C for 18 to 24 h. Isolates were Gram stained (Cruikshank et al., 1975) and pure Gram negative rods were retained for biochemical identification. Primary (oxidase test, triple sugar iron agar test) and secondary (API 20E, *E. coli* O157 rapid slide agglutination test) identification tests were utilized. *E. coli* O157 isolates were stored as 30% glycerol stocks at −4 °C for further characterisation.

2.2. Antibiotic susceptibility test

Antibiotic susceptibility tests were performed on all *E. coli* O157 isolates to determine their antibiotic resistant profiles using the paper disc diffusion method (Kirby et al., 1966). Briefly isolates were grown on sorbitol MacConkey agar at 37 °C for 18 to 24 h. Bacterial suspensions were prepared and aliquots of 100 µl were spread over Mueller Hinton agar. Antimicrobial disks impregnated with streptomycin (10 µg), erythromycin (15 µg), tetracycline (30 µg), ampicillin (10 µg), neomycin (30 µg), norfloxacin (10 µg), kanamycin (30 µg), sulphamethoxazole (10 µg) and chloramphenicol (30 µg) were obtained from Mast Diagnostics (United Kingdom). These disks (6 µm in diameter) were placed on the surface of the inoculated agar plates and the plates were incubated at 37 °C for 18 to 24 h. After incubation, the antibiotic inhibition zone diameters (IZD) were measured. Results obtained were used to classify isolates as being resistant, intermediate resistant or susceptible to a particular antibiotic using standard reference values (NCCLS, 1999). Isolates that were resistant to tetracycline were screened for the presence of *tet* resistant genes. MAR phenotypes were generated for isolates that showed resistance to 3 and more antibiotics (Rota et al., 1996).

2.3. Haemolysis on blood agar

Haemolysis, on blood agar (Biolab, Merck, S. A.) supplemented with 5% (v/v) sheep blood, was determined for *E. coli* O157 isolates (Beutin et al., 1998). The genotype was confirmed by PCR using *hlyA* specific primers (Paton and Paton, 1998).

2.4. Extraction of genomic DNA and PCR

Genomic DNA was extracted from *E. coli* O157 isolates using the hot (65 °C) CTAB (cetyltrimethyl-ammonium bromide) – PVP

Table 1
Oligonucleotide primers used in the study

Primer	Sequence (5′–3′)	Specificity	Amplicon size (bp)
GM5F[c]	TACGGGAGGCAGCAG	16S bacterial ribosomal genes	550
907R[c]	CCGTCAATTCCTTTGAGTTT		
stx 1F[a]	ATAAATCGCCATTCGTTGACTAC	A subunit coding region of stx_1	180
stx 1R[a]	AGAACGCCCACTGAGATCATC		
stx 2F[a]	GGCACTGTCTGAAACTGCTCC	A subunit coding region of stx_2	255
stx 2R[a]	TCGCCAGTTATCTGACATTCT		
eae F[a]	GACCCGGCACAAGCATAAGC	eae virulence gene (eae)	384
eae R[a]	CCACCTGCAGCAACAAGAGG		
hlyAF[a]	GCATCATCAAGCGTACGTTCC	Hemolysin A virulence gene (hlyA)	534
hlyAR[a]	AATGAGCCAAGCTGGTTAAGCT		
TetA1[b]	CGA GCC ATT CGC GAG AGC	tetA	500
TetA3[b]	GCC TCC TGC GCG ATC TGG		
TetB BF[b]	CAG TGC TGT TGT TGT CAT TAA	tetB	500
TetB BR[b]	GCT TGG ATT ACT GAG TGT AA		
TetC Cl[b]	CTT GAG AGC CTT CAA CCC AG	tetC	500
TetC CR[b]	TGG TCG TCA TCT ACC TGC C		
TetD DF[b]	GGA TAT CTC ACC GCA TCT GC	tetD	500
TetD DR[b]	CAT CCA TCC GGA AGT GAT AGC		

[a] Paton and Paton, 1998.
[b] Miranda et al., 2003.
[c] Muyzer et al., 1995.

(polyvinylpyrrolidone) DNA extraction procedure of Doyle and Doyle (1990). The DNA concentration was determined using an aquamate spectrophotometer (Thermo Spectronic, Mercers Row, Cambridge CB58HY UK) by measuring the absorbance at 260 nm.

PCR reactions were performed to amplify bacterial 16S rRNA gene fragments (Muyzer et al., 1995) as well as detection of STEC virulence genes (Paton and Paton 1998) and tetracycline resistant genes (Miranda et al., 2003) using oligonucleotide primer combinations indicated in Table 1. A Peltier Thermal Cycler (model-PTC-220 DYAD™ DNA ENGINE) was used for the PCR amplifications. The reactions were performed in 25 µl volumes comprising of 1 µM of the template DNA, 50 pmol of each oligonucleotide primer set, 1X PCR master mix, 50 ng bovine serum albumen, 1 U Taq DNA polymerase and RNase free distilled water. All the PCR reagents were Fermentas, USA products. The amplifications were performed at 95 °C for 300 s; 45 cycles of 95 °C for 30 s; 62 °C for 60 s; 72 °C for 60 s and a final elongation step at 72 °C for 300 s. Both positive and negative control reactions were included in each PCR amplification experiment.

PCR products were cooled at 4 °C and resolved by electrophoresis on a 1% (wt/v) agarose gel in a horizontal Pharmacia biotech equipment system (model Hoefer HE 99X). Each run contained a DNA molecular weight marker (Lambda DNA — Hind III digest) obtained from Roche Biochemicals, Germany. The gels were stained with ethidium bromide (0.001 µg/ml) for 15 min and observed under UV light at a wavelength of 420 nm (Sambrook et al., 1989). A Gene Genius Bio Imaging System (Syngene, Synoptics UK) was used to capture the image using GeneSnap (version 6.00.22) software. Images were analysed using GeneTools (version 3.00.22) software (Syngene, Synoptics, UK) to determine the relative sizes of the bands in each lane.

2.5. Sequence analysis

Amplified 16S rRNA gene fragments were sequenced by Inqaba Biotec, Pretoria — South Africa. Blast searches (http://www.ncbi.nlm.nih.gov/BLAST) were used to confirm the identity of the amplified sequences.

2.6. Statistical analysis

Statistical analysis was performed using the Minitab Release software (version 13.31). Correlations between antibiotic resistant E. coli O157 isolates from the various species were determined using the Pearson's product of moment and were scored as significant if $P < 0.05$. Furthermore, cluster analysis of antibiotic susceptibility data for E. coli O157 isolated from the different stations was determined using Wards algorithm and Euclidean distances on Statistica version 7.0 (Statsoft, US).

3. Results

### 3.1. Antibiotic resistant data of E. coli O157 isolated from the different species at various sampling stations

A total of 76 E. coli O157 isolates were positively identified from the different host species. These comprised 37 isolates from commercial (Mareetsane) pig faeces and 23 from pig faeces obtained from the communal (Tlapeng) farm. Furthermore, 4, 5 and 4 isolates were obtained from cattle faeces obtained from the commercial farms in Lichtenburg, Rustenburg and a communal farm at Mogosane, respectively. Only 3 E. coli O157 isolates were obtained from human stool samples. All the E. coli O157 isolated as well as E. coli control strains were tested to evaluate their susceptibilities to 9 different

Table 2
Percentage antibiotic resistance of E. coli O157 isolated from the different species at the various sampling stations

Specie	Sampling site	K	S	T	E	Ne	Nor	C	Ap	Smx
Pigs	Tlapeng N=23	8.7	52.2	100	69.6	4.3	0	8.7	34.8	95.7
	Mareetsane N=37	13.5	70.3	94.6	83.8	43.2	5.4	13.5	37.8	94.6
Cattle	Rustenburg N=4	0	0	0	100	0	0	0	0	100
	Mogosane N=10	25	50	100	100	0	0	25	25	100
	Lichtenburg N=5	0	0	100	100	0	0	0	0	100
Humans	Mafikeng Provincial Hospital	0	0	100	100	0	0	33.3	100	100

Ap (ampicillin), C (chloramphenicol), T (tetracycline), Nor (norfloxacin), S (streptomycin), E (erythromycin), Ne (neomycin), K (kanamycin), Smx (sulphamethoxazole).

Table 3
Predominant multiple antibiotic resistant (MAR) phenotypes for E. coli O157 isolated from the different sampling sites

Communal pigs (Tlapeng) isolates (N=23)			Commercial pigs (Mareetsane) isolates (N=37)		
Phenotype	Number observed	Percentage	Phenotype	Number observed	Percentage
Smx–T–E–Ap	2	8.7	Smx–T–E	6	16.2
Smx–T–Ap	5	21.7	S–Smx–T–E–Ne	4	10.8
Smx–T–E	4	17.4	S–Smx–T–E	3	8.1
S–Smx–T–E	7	30.4	K–S–Smx–T–E–Ap–Ne	2	5.4
			S–Smx–T–E–Ap–Ne	2	5.4
			S–Smx–T	2	5.4
			S–Smx–T–E–Ap	2	5.4
			S–Smx–T–Ap	2	5.4

Commercial cattle (Lichtenburg) isolates (N=5)			Communal cattle (Mogosane) isolates (N=4)		
Phenotype	Number observed	Percentage	Phenotype	Number observed	Percentage
Smx–T–E	5	100	Smx–T–E–C	1	25.0
			K–S–Smx–T–E	1	25.0
			S–Smx–T–E	1	25.0
			Smx–T–E–Ap	1	25.0

Human isolates (N=3)		
Phenotype	Number observed	Percentage
Smx–T–E–Ap	2	66.7

MAR phenotypes (MAR) were expressed using the abbreviation letters as they appear on the antibiotic paper discs with a modification of small letters.
Ap (ampicillin), C (chloramphenicol), T (tetracycline), Nor (norfloxacin), S (streptomycin), E (erythromycin), Ne (neomycin), K (kanamycin), Smx (sulphamethoxazole).
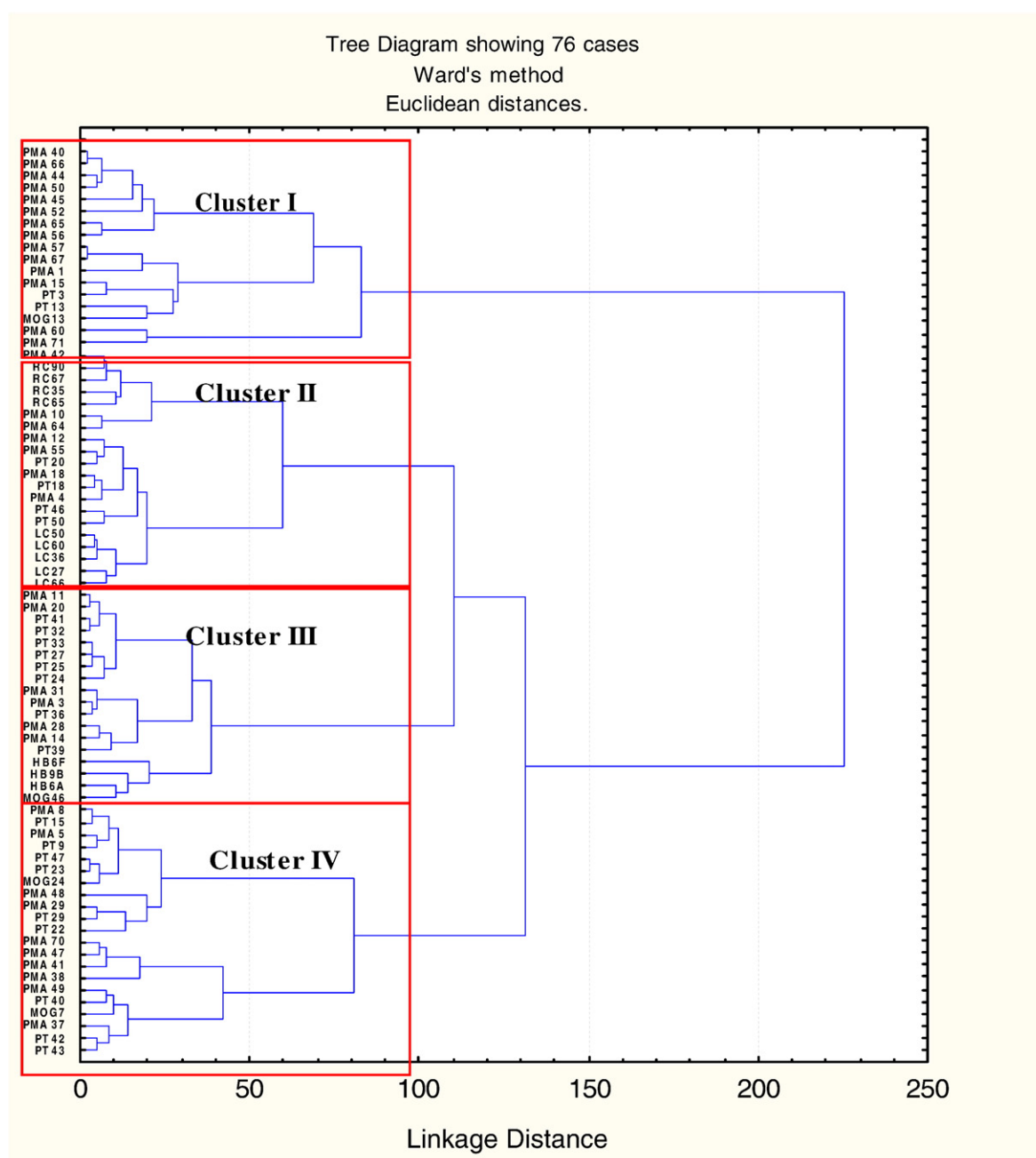
Fig. 1. Dendogram showing the relationship between *E. coli* O157 isolated from faeces samples of pigs and cattle and human stool samples based on inhibition zone diameter (IZD) data. Bacterial designation prefixes are based on host and sample station origin. The tree was constructed using Ward's method and Euclidean distances in Statistica, version 7 (Statsoft, US). Designation: PT (Pig Tlapeng), PMA (Pig Mareetsane), HB (Human — Bophelong), RC (Cattle Rustenburg), LC (Cattle Lichtenburg) and MOG (Cattle Mogosane).

antibiotics. Results obtained are depicted in Table 2. A large proportion (83.8% to 100%) of *E. coli* O157 isolated from pig faeces from both the communal and commercial farms were resistant to tetracycline, erythromycin and sulphamethoxazole. None and 4.3% of *E. coli* O157 isolated from pig faeces from the communal farm were resistant to norfloxacin and neomycin, respectively. Despite the fact that 5.4% of the isolates from pig faeces obtained from the commercial farm were resistant to norfloxacin a large proportion (43.2%) of these were resistant to neomycin.

All the *E. coli* O157 isolated from cattle faeces from the commercial and communal farms from Lichtenburg and Mogosane were resistant to erythromycin, tetracycline and sulphamethoxazole (Table 2). On the other hand, none of the isolates from cattle from the Rustenburg commercial farm were resistant to tetracycline. A low percentage (25% to 50%) of *E. coli* O157 isolated from communal cattle from Mogosane

were resistant to chloramphenicol, kanamycin, ampicillin and streptomycin. None of the isolates from cattle faeces obtained from any of the farms were resistant to either neomycin or norfloxacin.

Table 4
The percentage representation of *E. coli* O157 isolated from different species within the various clusters

Specie	Sample type/site name	Cluster I N=18	Cluster II N=19	Cluster III N=18	Cluster IV N=21
Pigs	Communal (Tlapeng)	2 (11.1%)	4 (21.1%)	8 (44.4%)	9 (42.9%)
Cattle	Commercial (Mareetsane)	15 (83.3%)	6 (31.5%)	6 (33.3%)	10 (47.6%)
	Communal (Mogosane)	1 (5.6%)	0 (0%)	1 (5.6%)	2.(9.5%)
	Commercial (Lichtenburg)	0 (0%)	5 (26.1%)	0 (0%)	0 (0%)
	Commercial (Rustenberg)	0 (0%)	4 (21.1%)	0 (0%)	0 (0%)
Human	Mafikeng Provincial Hospital	0 (0%)	0 (0%)	3 (16.7%)	0 (0%)

Table 5
Prevalence of the STEC virulence genes identified in *E. coli* O157 isolated from the different species

Virulence gene(s)	*E. coli* O157 (ATCC)43889	*E. coli* O157 (ATCC)43888	No. of isolates with indicated virulence gene(s).			
			Humans (3)	Cattle (7)	Pigs (20)	Total (%)
stx₁	+	−	0	0	0	0
stx₂	+	−	0	0	0	0
eae	+	−	1	1	5	7 (23.7%)
hlyA	+	−	2	5	11	18 (60.0%)
eae and *hlyA*	+	−	0	1	4	5 (16.7%)

+=Gene was present in the Control strains; −=Gene was absent in the Control strains.

As indicated in Table 2, all three of *E. coli* O157 isolated from human stool samples were resistant to erythromycin, tetracycline, ampicillin and sulphamethoxazole. Similarly, the two *E. coli* control strains were resistant to erythromycin and sulphamethoxazole while one was resistant to tetracycline. Neither the control strains nor the *E. coli* O157 isolated from human stool samples were resistant to norfloxacin, kanamycin and streptomycin.

3.2. MAR phenotypes of E. coli O157 isolated from pigs, cattle and humans

The predominant antibiotic resistant phenotypes that were obtained for the *E. coli* O157 isolated from cattle, pig and human faeces respectively, are shown in Table 3. The MAR phenotypes S–Smx–T–E and Smx–T–E were dominant among 30.4% and 16.2% of the communal (Tlapeng) and commercial (Mareetsane) pig isolates, respectively.

The dominating MAR phenotype amongst *E. coli* O157 isolated from cattle faeces was Smx–T–E in 100% of the isolates from the commercial farm in Lichtenburg. However, the MAR phenotypes Smx–T–E–C, K–S–Smx–T–E, S–Smx–T–E and Smx–T–E–Ap were observed in 25% of the communal (Mogosane) isolates. The MAR phenotypes for *E. coli* O157 isolated from commercial cattle in Rustenburg were not included as resistance was shown only to two antibiotics (Table 3).

The MAR phenotypes of two of the three *E. coli* O157 isolated from human stool samples were Smx–T–E–Ap while one of the isolates presented with Smx–T–E. MAR phenotypes for control strains were not included since resistance was observed to less than three antibiotics. The phenotype Smx–T–E was present in all the predominant MAR phenotypes for *E. coli* O157 isolated from pig and cattle faeces and human stool samples (Table 3).

3.3. Cluster analysis of E. coli O157 for multiple antibiotic resistance (MAR) relationship on a dendogram

A total of 76 *E. coli* O157 isolated from pigs, cattle and humans were subjected to cluster analysis using their IZD data and a dendogram was generated. This approach was used as a tool in determining the commonness and in resolving differences between the MAR phenotypes of *E. coli* O157 isolates. The dendogram in Fig. 1 indicates four major clusters (I–IV) that were further analysed for patterns of associations of isolates from the different species (Table 4). Of the *E. coli* O157 isolated from pigs, 19 (90.5%) were present in the largest cluster (cluster IV) along with 2 (9.5%) of the *E. coli* O157 isolated from communal cattle from Mogosane. The second largest cluster (cluster II) contained 10 (52.6%) of the *E. coli* O157 isolated from pigs and included the *E. coli* O157 isolates from faeces of pigs and cattle from both commercial farms. Cluster III was a mixed cluster containing *E. coli* isolated from all three species. All the three *E. coli* O157 isolated from human stool samples were present in this cluster including a large proportion (77.8% or 14) of isolates from pig faeces. The smallest cluster (cluster I) was represented mostly by *E. coli* O157

isolated from pigs. The cluster analysis indicated that *E. coli* O157 isolated from faeces samples of pig and cattle potentially had similar antibiotic resistance histories as those of the human *E. coli* O157 isolates. *E. coli* O157 isolated from pigs faeces from Tlapeng and Mareetsane, cattle faeces from Mogosane and human stool samples clustered together (Cluster III). These villages are close to the Mafikeng Provincial hospital that serves the area and could possibly indicate that the individuals sampled might have come from these villages. However, information on the residence of the individuals from which the samples were obtained was not available.

3.4. Detection of E. coli O157 virulence genes and tetracycline resistant genes

A total of thirty *E. coli* O157 consisting of 20 isolated from the faeces samples of pigs, 7 from cattle and 3 from human stool samples were randomly selected and characterised by PCR analysis for the presence of shiga toxin virulence genes and tetracycline resistant genes. Specific PCR analysis for the detection of *E. coli* O157 virulence genes and tetracycline resistant genes were performed using specific primers for the targeted genes (Table 1). Table 5 depicts a summary of the genotypic characteristics of *E. coli* O157 isolated from pigs, cattle, humans and *E. coli* control strains. As indicated in Table 5, a large proportion (60%) of the *E. coli* O157 isolates possessed the enterohaemolysin A (*hlyA*) gene while only a small proportion (23.3%) possessed the *eae* gene. Furthermore, only 5(16.7%) of the isolates possessed both genes. A cause for concern was the fact that not all the *E. coli* O157 isolates that were haemolytic on blood agar possessed the *hlyA* gene. There was a higher prevalence of the *hlyA* gene in *E. coli* O157 isolated from pigs than in cattle and humans. Moreover, none of the *E. coli* O157 isolated from human stool samples possessed both genes.

The *tetB* gene fragment was detected in a small proportion (3.3%) of the isolates. Despite this none of the other tetracycline resistant genes tested was detected. Studies carried out to determine the cause of resistance to tetracycline have identified various tetracycline genes but in different proportions (Bryan et al., 2004). In a related study involving *E. coli* isolated from humans and animals, resistance was found to result from the presence of 14 tetracycline resistant genes and a large proportion (63%) of these isolates harboured the *tetB* resistant determinant (Bryan et al., 2004).

4. Discussion

One of the objectives of this study was to determine to what extent *E. coli* O157 isolated from cattle and pigs, which may enter the food chain and be transmitted to humans, are resistant to antibiotics. A motivation to this was the fact that there has been increasing reports on the isolation and identification of antibiotic resistant *E. coli* O157 strains (Meng et al., 1998; Galland et al., 2001; Schroeder et al., 2002; Vali et al., 2004; Ahmed et al., 2005; Vidovic and Korber, 2006). In the present study multiple antibiotic resistance (MAR), defined as resistance to three or more different classes of antibiotics, was observed in 71(93.4%) of the *E. coli* O157 isolated. The detection of a large proportion of MAR *E. coli* O157 isolates in the present study was a cause for concern as these could pose health risks to humans. Resistant genetic elements from these isolates could also be transferred to other enteric bacteria of clinical importance. This amplified the importance to characterise these isolates using molecular methods.

The frequencies of resistance to tetracycline, sulphamethoxazole and erythromycin were higher among *E. coli* O157 isolated from pigs than cattle and humans. However, a cause for concern was the fact that all three *E. coli* O157 isolated from human stool samples were resistant to these antibiotics. This may have resulted through the consumption of contaminated food products. In a previous study,

(Schroeder et al., 2002) *E. coli* O157 isolates obtained revealed similarly marked resistance to these drugs. Tetracycline is generally used in animal medicine and as a growth promoter but less frequently used to treat infections in humans. On the contrary resistance to sulphamethoxazole and erythromycin was a cause for concern since these drugs are not used on animals in the sampled area.

In South Africa, a large proportion of the population relies on pork and beef as a source of food. A majority of the cases of infections caused by *E. coli* O157 have been found to result from the consumption of undercooked contaminated animal food products and/or contaminated water (Isaäcson et al., 1993; Paquet et al., 1993; Armstrong et al., 1996; Anonymous, 2000; Müller et al., 2001; Olsen et al., 2002). This amplifies the importance of reducing the prevalence of these pathogens and their associated resistant genes in animals, their food products and/or drinking water especially as they may cause disease at a very low infectious dose. However, in a previous study (Ateba et al., 2008) we reported that the prevalence of *E. coli* O157 was high in the animal species sampled. Our findings in the present documentation indicate that a large proportion of those isolates were resistant to most of the antibiotics tested. The situation was even worse in subsistence settings where animals were seen drinking water from rainfall runoffs and pigs sometimes feed on human excreta. Rainfall runoffs may have increased the chances of transmitting antibiotic resistant determinants to *E. coli* O157 strains isolated from animal species and later to humans. Despite the fact that the antibiotic resistant data did not clearly indicate a direct transmission of resistant *E. coli* O157 strains through diet, the high proportion of isolates that were resistant to commonly used antibiotics among those from communal farms and human stool samples was a cause for concern. We recommend that proper hygiene practices should be enforced in the farms were animals are kept and during the slaughtering process. In all the rural areas sampled during the study, humans rely on ground water. This may have been an effective route for the transmission of these pathogens to humans since the microbial ecosystem of pigs, cattle and humans in those communities may have been interconnected.

Another objective of the study was to use a PCR technique to detect virulence genes in *E. coli* O157 isolated from the faeces samples of cattle and pigs and human stool samples in the North-West Province of South Africa. Although none of the *E. coli* O157 isolates obtained in the present study possessed the shiga toxin genes when compared to the positive control strain, the frequencies of the *eae* and *hlyA* genes were higher in isolates obtained from pig faeces than cattle and human stool samples. Similar observations had been reported (Frydendahl, 2002; Hornitzky et al., 2002), but our results contradict the finding that the *eae* gene exists in association with the shiga toxin genes (Pradel et al., 2000; Johnsen et al., 2001; Omisakin et al., 2003). The high prevalence of the *eae* gene in a large proportion of *E. coli* O157 isolated from non-diarrhoeal pigs indicated that pigs could serve as a potential source for the transmission of disease to humans. The *eae* gene codes for intimin that facilitates the intimate attachment of bacteria to host cell wall (Oswald et al., 2000) and has been identified as an important accessory virulence factor that correlates with disease (Gyles et al., 1998). However, the *eae* gene was detected in 1 of the 3 *E. coli* O157 isolated from human stool samples from diarrhoeal patients. Several reports have been documented that contradict the role of *eae* gene in the pathogenesis of human disease (Heuvelink et al., 1996; Chapman et al., 1997; Paton and Paton, 2002; Beutin et al., 2004). We recommend that more studies should be carried out to determine their exact contribution in the development of infections in humans.

The *hlyA* gene was absent in some of the *E. coli* O157 isolates that were phenotypically haemolytic and this was contrary to the notion that its presence confers such a property. However, its presence in a large proportion of the *E. coli* O157 isolates could have resulted from the fact that it can be easily transferred among bacterial species since it is plasmid encoded (Schmidt et al., 1999). In the present study, none of the isolates from human stool samples harboured both the *eae* and *hlyA* genes. Very few of the isolates from pigs and cattle possessed both the *eae* and *hlyA* genes despite a report that indicated that the *hlyA* gene is usually present in *eae* positive isolates (Tarr et al., 2002). However, the *hlyA* gene was more prevalent in *E. coli* O157 isolated from pigs than cattle and than humans. Our findings were similar to previously reported data (Frydendahl, 2002; Hornitzky et al., 2002) and this may suggest that pigs are easily colonised by *hlyA* positive *E. coli* strains.

Another goal of the study was to characterise antibiotic resistant *E. coli* O157 strains isolated from animal species and humans using PCR. This was motivated by the fact that a large proportion of the *E. coli* O157 isolated from both humans and animals were resistant to three or more antibiotics. A large proportion of the isolates were resistant to tetracycline (Table 2) and this highlighted the need to screen isolates for the presence of four different tetracycline resistant genes (*tetA* to *tetD*). Only a *tetB* gene was identified in *E. coli* O157 isolated from pig faeces. In a study carried out on *E. coli* isolated from humans and animals, the *tetB* gene was detected in 63% of the isolates (Bryan et al., 2004) while the *tetA* gene was most prevalent among isolates obtained in another study (Sengeløv et al., 2003). Resistance to tetracycline in the present study may have resulted from the presence of other *tet* genes that were not tested. Resistance may have also resulted from the presence of integrons that are easily transferred through conjugation (Zhao et al., 2001) but whose presence in the isolates were not investigated. However, no information has been documented on the distribution of integrons among *E. coli* O157 isolates from humans and animals in the sampled area. Further studies will be conducted to determine their contribution in the dissemination of antibiotic resistant genes among these pathogens. This would provide a clear picture of the health risks associated with the consumption of contaminated food products.

5. Conclusion

An evaluation of samples was made. Results indicated that the level of multiple antibiotic resistant (MAR) *E. coli* O157 was high in all samples examined. Although the study was not designed to demonstrate a direct link between the consumption of contaminated meat products and development of antimicrobial resistance in *E. coli* O157 isolated from humans, the high prevalence of MAR *E. coli* O157 in pigs suggest that these animals may pose a health risk in the sampled area. These isolates may have negative clinical implications and hence hamper the treatment of *E. coli* O157 infections. We suggest that there is a need to implement surveillance programmes that monitor the prevalence and/or antimicrobial resistance among zoonotic foodborne pathogens especially *E. coli* O157. This should contribute to reducing the occurrence of *E. coli* O157 infections in humans and ensure proper public health. Further work on conjugation experiments between the *E. coli* isolates obtained in this study and other pathogens should be conducted to evaluate the role these isolates play in the transfer of antibiotic resistant determinants. Such studies would be of more value if carried out in communal settings.

References

Ahmed, A.M., Kawamoto, H., Inouye, K., Hashiwata, Y., Sakaki, M., Seno, M., Shimamoto, T., 2005. Genomic analysis of a multidrug-resistant strain of enterohaemorrhagic *Escherichia coli* O157:H7 causing a family outbreak in Japan. Journal of Medical Microbiology 54, 867–872.

Anonymous, 2000. Waterborne Outbreaks of Gastroenteritis Associated with a Contaminated Municipal Water Supply. CDR, Walkerton, Ontario, Canada, pp. 20–26.

Armstrong, G.L., Hollingsworth, J., Morris Jr., J.R., 1996. Emerging foodborne pathogens: *Escherichia coli* O157:H7 as a model of entry of a new pathogen into the food supply of the developed world. Epidemiology Review 18, 29–51.

Ateba, C.N., Mbewe, M., Bezuidenhout, C.C., 2008. The Prevalence of *Escherichia coli* O157 strains in cattle, pigs and humans in the North-West Province, South Africa. South African Journal of Science 104, 7–8.

Bettelheim, K.A., Hornitzky, M.A., Djordjevic, S.P., Kuzevski, A., 2003. Antibiotic resistance among verocytotoxigenic *Escherichia coli* (VTEC) and non-VTEC isolated from domestic animals and humans. Journal of Medical Microbiology 52, 155–162.

Beutin, L., Geier, D., Zimmermann, S., Aleksic, S., Gillespie, H.A., Whittam, T.S., 1998. Epidemiological relatedness and clonal types of natural populations of *Escherichia coli* strains producing shiga-toxins in separate populations of cattle and sheep. Applied and Environmental Microbiology 63, 2175–2180.

Beutin, L., Krause, G., Zimmermann, S., Kaulfuss, S., Gleier, K., 2004. Characterisation of shiga toxin-producing *E. coli* strains isolated from human patients in Germany over a 3-year period. Journal of Clinical Microbiology 42, 1099–1108.

Browning, N.G., Botha, J.R., Sacho, et al., 1990. *Escherichia coli* O157:H7 haemorrhagic colitis. Report of the first South African case. South African Journal of Surgery 28, 28–29.

Bryan, A., Shapir, N., Sadowsky, J.M., 2004. Frequency and distribution of tetracycline resistant genes in genetically diverse, nonselected, and nonclinical *Escherichia coli* strains isolated from diverse human and animal sources. Applied and Environmental Microbiology 70, 2503–2507.

Brunder, W., Schmidt, H., Frosch, M., Karch, H., 1999. The large plasmids of shiga toxin-producing *Escherichia coli* (STEC) are highly variable genetic elements. Microbiology 145, 1005–1014.

Chapman, P.A., Siddons, C.A., Wright, D.J., Norman, P., Fox, J., Crick, E., 1993. Cattle as a possible source of verocytotoxin-producing *Escherichia coli* O157 in man. Epidemiology Infection 111, 439–447.

Chapman, P.A., Siddons, C.A., Gerdan Malo, A.T., Harkin, M.A., 1997. A 1-year study of *Escherichia coli* O157 in cattle, sheep, pigs and poultry. Epidemiology Infection 119, 245–250.

Cruikshank, R., Duguid, J.P., Marmoin, B.P., Swain, R.H., 1975. Medical Microbiology, 12th ed. Longman, New York, pp. 3–4. chap 2.

Dundas, S., Todd, W.T., Stewart, A.I., Murdoch, P.S., Chaudhuri, A.K., Hutchinson, S.J., 2001. The central Scotland *Escherichia coli* O157:H7 outbreak: risk factors for the haemolytic uraemic syndrome and death among hospitalized patients. Clinical and Infectious Disease 33, 923–931.

Dunn, J.R., Keen, J.E., Moreland, D., Thompson, R.A., 2004. Prevalence of *Escherichia coli* O157:H7 in white-tailed deer from Louisiana. Journal of Wildlife Disease 40, 361–365.

Doyle, J.J., Doyle, J.L., 1990. Isolation of plant DNA from fresh tissues. Focus 12, 13–15.

Frydendahl, K., 2002. Prevalence of serogroups and virulence genes in *E. coli* associated with postweaning diarrhoea and oedema disease in pigs and a comparison of diagnostic approaches. Veterinary Microbiology 85, 169–182.

Galland, J.C., Hyatt, D.R., Crupper, S.S., Acheson, D.W., 2001. Prevalence, antibiotic susceptibility and diversity of *Escherichia coli* O157:H7 isolates from a longitudinal study of beef cattle feedlots. Applied and Environmental Microbiology 67, 1619–1627.

Gioffré, A., Meichtri, L., Miliwebsky, E., Baschkier, A., Chillemi, G., Romano, M.I., Estani, S.S., Cataldi, A., Rodríguez, R., Rivas, M., 2002. Detection of shiga toxin producing *Escherichia coli* by PCR in cattle in Argentina. Evaluation of two procedures. Veterinary Microbiology 87, 301–313.

Gyles, C., Johnson, R., Gao, A., Ziebell, K., Pierard, D., Aleksic, S., Boerlin, P., 1998. Association of enterohemorrhagic *Escherichia coli*, haemolysin with serotypes of shiga-like toxin producing *E. coli* of human and bovine origins. Applied and Environmental Microbiology 64, 4134–4141.

Heuvelink, A.E., Wernars, K., de Boer, E., 1996. Occurrence of *Escherichia coli* O157 and other verocytotoxin-producing *E. coli* in retail raw meats in the Netherlands. Food Protection 59, 1267–1272.

Hornitzky, M.A., Vanselow, B.A., Walker, K., Bettelheim, K.A., Corney, B., Gill, P., Bailey, G., Djordjevic, S.P., 2002. Virulence properties and serotypes of shiga toxin producing *Escherichia coli* from healthy Australian cattle. Applied and Environmental Microbiology 68, 6439–6445.

Igarashi, T., Inatomi, J., Wake, A., Takamizawa, M., Katayama, H., Iwata, T., 1999. Failure of pre-diarrheal antibiotics to prevent haemolytic uraemic syndrome in serologically proven *Escherichia coli* O157:H7 gastrointestinal infection. Journal of Pediatrics 135, 768–769.

Isaäcson, M., Canter, P.H., Effler, P., Arntzen, L., Bomans, P., Heenan, R., 1993. Haemorrhagic colitis epidemic in Africa. Lancet 341, 961.

Johnsen, G., Yngvild, W., Heir, E., Berget, O.I., Herikstad, H., 2001. *Escherichia coli* O157:H7 in faeces from cattle, sheep and pigs in the southwest part of Norway during 1998 and 1999. International Journal of Food Microbiology 65, 193–200.

Kirby, W.M.M., Bauer, A.W., Sherris, J.C., Turck, M., 1966. Antibiotic susceptibility testing by single disc method. American Journal Clinical Pathology 45, 4.

Meng, J., Zhao, S., Doyle, M.P., Joseph, S.W., 1998. Antibiotic resistance of *Escherichia coli* O157:H7 from animals, food and humans. Journal of Food Protection 61, 1511–1514.

Miranda, C.D., Kehrenberg, C., Ulep, C., Schwarz, S., Roberts, M.C., 2003. Diversity of tetracycline resistant genes from bacteria isolated from Chilean salmon farms. Antimicrobial Agents and Chemotherapy 47, 883–888.

Mi-Yeong, J., Ji-Hyun, K., Jae-Hyang, L., Mi-Young, K., Hong-Bum, K., Yong-Ho, P., Do-Young, Y., Joon-Seok, C., Seong-Kug, E., Lee, J.H., 2004. Prevalence and characteristics of *Escherichia coli* O157 from major animals in Korea. International Journal of Food Microbiology 95, 41–49.

Muhldorfer, I., Hacker, J., Keusch, G.T., Acheson, D.W., Tschape, H., Kane, A.V., Ritter, A., Olschlager, T., Donohue-Rolfe, 1996. Regulation of shiga-like toxin II operon in *Escherichia coli*. Infection Immunity 64, 495–502.

Müller, E.E., Ehlers, M.M., Grabow, W.O.K., 2001. The occurrence of *E. coli* O157:H7 in Southern African water sources intended for direct and indirect human consumption. Water Research 35, 3085–3088.

Muyzer, G., Teske, A., Wirsen, C.O., Jannasch, H.W., 1995. Phylogenetic relationships of *Thiomicrospira* species and their identification in deep-sea hydrothermal vent samples by denaturing gradient electrophoresis of 16S rDNA fragments. Archives in Microbiology 164, 165–172.

National Committee for Clinical Laboratory Standards, 1999. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. National Committee for Clinical Laboratory Standards, Wayne, Pennslyvania, Approved Standard M 31-A19 (11).

Olsen, S.J., Miller, G., Breuer, T., Kennedy, M., Higgins, C., Walford, J., McKee, G., Fox, K., Bibb, W., Mead, P., 2002. A waterborne outbreak of *Escherichia coli* O157 infections and haemolytic uraemic syndrome: implications for rural water systems. Emerging Infectious Diseases 8, 370–375.

Omisakin, F., Macrae, M., Ogden, I.D., Strachan, N.J., 2003. Concentration and prevalence of *Escherichia coli* O157 in cattle faeces at slaughter. Applied and Environmental Microbiology 69, 2444–2447.

Oswald, E., Schmidt, H., Morabito, S., Karch, H., Marches, O., Capioli, A., 2000. Typing of intimin genes in human and animal enterohaemorrhagic and enteropathogenic *Escherichia coli*: characterisation of a new intimin variant. Infection Immunity 68, 64–71.

Paquet, C., Perea, W., Grimont, F., Collin, M., Guillod, M., 1993. Aetiology of haemorrhagic colitis epidemic in Africa. Lancet 342, 175.

Paton, C.J., Paton, A.W., 1998. Pathogenesis and diagnosis of shiga toxin-producing *Escherichia coli* infections. Clinical Microbiology Reviews 11, 450–479.

Paton, A.W., Paton, C.J., 2002. Direct detection and characterisation of shiga toxigenic *Escherichia coli* by multiplex PCR for stx_1, stx_2, eae, ehxA and saa. Journal of Clinical Microbiology 40, 271–274.

Pradel, N., Livrelli, V., DeChamps, C., Palcoux, J.B., Reynaud, A., Scheutz, F., Sirot, J., Joly, B., Forestier, C., 2000. Prevalence and characterisation of shiga toxin-producing *Escherichia coli* isolates from cattle, food and children during a one-year prospective study in France. Journal of Clinical Microbiology 38, 1023–1031.

Pritchard, G.C., Willshaw, G.A., Bailey, J.R., Carson, T., Cheasty, T., 2000. Verocytotoxin-producing *Escherichia coli* O157 on a farm open to the public: outbreak investigation and longitudinal bacteriological study. Veterinary Records 147, 259–264.

Riley, L.W., Remis, R.S., Helgerson, S.D., McGee, H.B., Wells, J.G., Davis, B.R., Hebert, R.J., Olcott, E.S., Johnson, L.M., Hargrett, N.T., Blake, P.A., Cohen, M.L., 1983. Haemorrhagic colitis associated with a rare *Escherichia coli* serotype. New England Journal of Medicine 308, 681–685.

Rota, C., Yanguela, J., Blanco, D., Carraminana, J.J., Arino, A., Herrera, A., 1996. High prevalence of multiple resistance to antibiotics in 144 *Listeria* isolates from Spanish dairy and meat products. Journal of Food Protection 59, 938–943.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning, A Laboratory Manual, Second edition. Cold spring harbour laboratory press.

Schmidt, H., Geitz, C., Phillips, I.T., Mathias, F., Karch, H., 1999. Non-O157 pathogenic shiga toxin producing *Escherichia coli*: phenotypic and genetic profiling of virulence traits and evidence for clonality. Infectious Disease 179, 115–123.

Schroeder, C.M., Zhao, C., DebRoy, C., Torcolini, J., Zhao, S., White, D.G., Wagner, D.D., McDermott, P.F., Walker, R.D., Meng, J., 2002. Antimicrobial resistance of *Escherichia coli* O157:H7 isolated from humans, cattle, swine and food. Applied Environmental Microbiology 68, 576–581.

Sengeløv, G., Agersø, Y., Halling-Sørensen, B., Baloda, S.B., Anderson, J.S., Jensen, L.B., 2003. Bacterial antibiotic resistance levels in Danish farmland as a result of treatment with pig manure slurry. Environment International 28 (7), 587–595.

Smith, S.T., Aboaba, O.O., Odeigha, P., Shodipo, K., Adeyeye, J.A., Ibrahim, A., Adebiyi, T., Onibokum, H., Odunukwe, N.N., 2003. Plasmid profile of *Escherichia coli* O157:H7 from apparently healthy animals. African Journal of Biotechnology 2 (9), 322–324.

Tarr, C.L., Large, T.M., Moeller, C.L., Lacher, D.W., Tarr, P.I., Acheson, D.W., Whittam, T.S., 2002. Molecular characterization of a serotype 0121:H19 clone, a distinct shiga toxin-producing clone of pathogenic *Escherichia coli*. Infection Immunity 70, 6853–6859.

Vali, L., Wisely, K.A., Pearce, M.C., Turner, E.J., Knight, H.I., Smith, A.W., Sawada, T., 2004. High-level genotypic variation and antibiotic sensitivity among *Escherichia coli* O157 strains isolated from two Scottish beef cattle farms. Applied and Environmental Microbiology 70, 5947–5954.

Vidovic, S., Korber, D.R., 2006. Prevalence of *Escherichia coli* O157 in Saskatchewan Cattle: characterisation of isolates by using random amplified polymorphic DNA PCR, antibiotic resistance profiles, and pathogenicity determinants. Applied and Environmental Microbiology 72, 4347–4355.

WHO, 1997. Consultations and workshops. Prevention and control of enterohaemorrhagic *Escherichia coli* infections. Report of a WHO Consultation, Geneva, Switzerland. 8 April–1 May.

Wilkerson, C., Samadpour, M., van Kirk, N., Roberts, M.C., 2004. Antibiotic resistance and distribution of tetracycline resistance genes in *Escherichia coli* O157:H7 isolates from humans and bovines. Antimicrobial Agents and Chemotherapy 48, 1066–1067.

Wong, C.S., Jelacic, S., Habeeb, R.L., Watkins, S.L., Tarr, P.I., 2000. The risk of haemolytic-uraemic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 infections. New England Journal of Medicine 342, 1930–1936.

Yoh, M., Honda, T., 1997. The stimulating effect of fosfomycin, an antibiotic in common use in Japan, on the production/release of verotoxin-1 from enterohaemorrhagic *Escherichia coli* O157:H7 in vitro. Epidemiology Infection 119, 101–103.

Zhao, S., White, D.G., Ge, B., Ayers, S., Friedman, S., English, L., Wagner, D., Gaines, S., Meng, J., 2001. Identification and characterisation of integron-mediated antibiotic resistance among shiga toxin-producing *Escherichia coli* isolates. Applied Environmental Microbiology 67, 1558–1564.