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Induction of Shiga Toxins in *Escherichia coli* O157: H7 Isolated from Groundwater in the North West Province, South Africa Intended for Human Consumption Using Ampicillin and Tetracycline

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KEYWORDS *E. coli* O157:H7. Shiga-Toxin. Multiple Antibiotic Resistant (MAR). Ampicillin. Tetracycline. ELISA

ABSTRACT A total of 67 isolates from groundwater were used to determine their susceptibilities against 7 antibiotics and the Multiple Antibiotic Resistance (MAR) patterns were compiled. Most isolates were resistant to amocyxillin, ampicillin, chloramphenicol and penicillin G. MAR phenotype A-AP-K-NE-OT-C-PG was dominant among isolates from Rustenburg. However, in Carletonville and Delareyville the phenotypes A-AP-C-PG and A-AP-OT-PG were obtained at 87.5 percent and eighty percent, respectively. The isolates were screened for the presence of shiga toxin genes by PCR analysis and none were positive. Moreover, when the *E. coli* O157:H7 isolates were subjected to antibiotic treatment for the induction of shiga toxins using both ampicillin and tetracycline in broth cultures, no shiga toxins were detected with an ELISA assay after 24 hours of incubation. However, after 72 hours of treatment with these antibiotics shiga toxins were detected in a large proportion (89.6%) of *E. coli* O157:H7 isolates with ampicillin when compared to tetracycline in which only one of the isolates produced shiga toxins. Tetracycline and ampicillin are readily available over the counter and are most often used in animal medicine. The consumption of these antibiotics when suffering from *E. coli* O157:H7 infections may worsen the complications.

INTRODUCTION

In South Africa and the North West Province in particular, individuals who reside in most rural communities depend on untreated surface and groundwater for drinking and household activities (Momba et al. 2005; Ateba and Maribeng 2011, Ateba and Mbewe 2014, Ateba et al. 2014; Nkwe et al. 2015). Water from these sources is usually of poor quality resulting from chemical and microbial contamination (Momba et al. 2005, 2006) and this renders it unsafe for drinking. Some shiga toxin producing *E. coli* (STEC) and particularly the serotype O157:H7, are known to cause waterborne infections in humans worldwide (Licence et al. 2001; Zhu et al. 2005). Infections range from non-bloody to severe bloody diarrhea, hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) (Tozzi et al. 2003; Lynn et al. 2005; Cole et al. 2014; Eichhorn et al. 2015).

The treatment of infections caused by pathogenic microorganisms is usually achieved through the administration of antibiotics (Ball et al. 2004). However, there is some controversy involving the use of antibiotics in the treatment of *E. coli* O157:H7 infections in humans (Igarashi et al. 1999; Wong et al. 2000). Antibiotics have been reported to serve as inducers for bacteriophages in *E. coli* O157:H7 cells and the phages harbor the shiga toxin genes (Zhang et al. 2000). Therefore, in the presence of antibiotics, the shiga toxin genes are expressed, thus increasing the chances of the disease to progress to the more severe clinical forms (Wong et al. 2000; Zhang et al. 2000). On the contrary, it has also been demonstrated that infections such as HUS could be prevented in infected individuals when antibiotics are administered during the early stages of *E. coli* O157:H7 infections (Slutsker et al. 1997).

Faced with this confusion, it is suggested that the treatment of *E. coli* O157:H7 infections

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be based on supportive therapy, to replace fluids and electrolytes lost through diarrhea. However, a number of studies conducted worldwide have indicated the presence of multiple antibiotic resistant *E. coli* O157H7 strains from food products, water and humans (Magwira et al. 2005; Ateba and Bezuidenhout 2008; Ateba and Mbewe 2011; El-Shatoury et al. 2015; Iweriebor et al. 2015). It is therefore suggested that antibiotics may not have a significant effect on these antibiotic resistant isolates. The aim of this study is to investigate the occurrence of *E. coli* O157:H7 in water from boreholes and to determine antibiotic resistant profiles of the isolates. A further objective is to evaluate the ability of two antimicrobial agents (tetracycline and ampicillin) in inducing shiga toxin proteins in broth cultures of some selected isolates. This was designed to assess the health risks associated with the uncontrolled administration of antibiotics in diarrheal patients without determining the causative agents.

Research Objectives

The present study aimed at investigating the occurrence of *E. coli* O157:H7 in water from boreholes that is intended for human consumption, determine antibiotic resistant profiles, and evaluate the ability of two antimicrobial agents (tetracycline and ampicillin) in inducing shiga toxin proteins in broth cultures of some selected isolates. This was designed to assess the health risks associated with the uncontrolled administration of antibiotics in diarrheal patients without determining the causative agents. Thus, the objectives of this study were:

1. To isolate and confirm the identities of *E. coli* O157:H7 isolates from groundwater samples.
2. To determine the percentage resistance of isolates to selected antimicrobial agents.
3. To compare the antibiotic resistant phenotypes of isolates.
4. To evaluate the ability of two antimicrobial agents (tetracycline and ampicillin) in inducing shiga toxin proteins in broth cultures of some selected isolates.

MATERIAL AND METHODS

Area of the Study

The research was conducted at the North West University at the Mafikeng Campus, North West Province. A total of 55 groundwater samples were collected from some rural communities in the North West Province, South Africa. Table 1 indicates the number of water samples collected from different sample sites.

Table 1: Areas where water samples were collected during the study

Sample source	*Sampling area*	*Number of samples*
Ground Water	Bekkersdal	2
	Khutsong	2
	Taung	2
	Zeerust	6
	Delaryville	2
	Deelpan	2
	Rustenburg	2
	Disaneng	8
	Tshidilamolomo	2
	Selosesha	2
	Makgobistad	2
	Logagane	2
	Mabule	2
	Dinareng	2
	Masamane	2
	Leporang	2
Tap Water	Stella	2
	Zeerust	3
	Rustenburg	2
	Deelpan	2
	Carltonville	2
Total		53

Sample Collection

A total of 55 water samples were collected from borehole taps and storage tanks in some rural communities in the North West Province of South Africa. Before collecting the samples, taps were allowed to run for about one minute and water samples were collected in 500 ml Durham Schott bottles. The water samples were clearly labeled and immediately transported on ice to the laboratory for analysis. Upon arrival in the laboratory, all the samples were analyzed for characters of *E. coli* within 6 hours.

Isolation of *E. coli* O157:H7

Water Samples

The membrane filtration method was used for the isolation of *E. coli* O157:H7 from water samples. Aliquots of 100 ml from each of the sample were filtered through 0.45µm Gridfilter-units

(Type HA) using a Gelman Little Gaint pressure/vacuum pump machine (model 13156 - Gelman Sciences, Michigan, USA). The filters were handled with sterile forceps and placed onto sorbitol-MacConkey agar (SMAC) for selective isolation of *E. coli* O157:H7. The plates were incubated at 37°C for 24 hours (Müller et al. 2001).

Purification of Presumptive *E. coli* Isolates

Eight presumptive *E. coli* O157:H7 colonies from each sample were sub-cultured onto sorbitol-MacConkey agar. The plates were incubated at 37°C for 24 hours (Meichtri et al. 2004). After incubation, colorless colonies were regarded as presumptive *E. coli* O157:H7 isolates. Pure cultures that were identified as sorbitol negative colonies and lactose positive were preserved for characterization and further biochemical identification using standard tests for *E. coli*.

Bacteria Identification

Presumptive *E. coli* isolates were identified based on the following criteria.

Cellular Morphology

Isolates were Gram stained using standard techniques (Cruikshank et al. 1975). *E. coli* species are Gram negative rods, thus all isolates that appeared pink were retained and subjected to primary and secondary biochemical identification tests.

Oxidase Test

The oxidase test was performed on all *E. coli* isolates using the TestOxidase™ reagent (PL.390) in accordance with the manufacturer's protocol (Mast Diagnostics, Neston, Wirral, U.K.).

Triple Sugar Iron (TSI) Agar Test

Triple sugar iron agar (Biolab, Merck, South Africa) was used to assay the *E. coli* content against three sugars (glucose, sucrose and lactose) that are present at concentrations 0.1 percent, 1.0 percent and 1.0 percent, respectively (Forbes and Weissfeld 1998). Results were interpreted based on standard protocols (Prescott 2002).

Simmons Citrate Agar Test

Simmons citrate agar was used to determine the ability of the isolates to utilize citrate and ammonium ions as the sole source of carbon and nitrogen, respectively (Simmons 1926).

All isolates that satisfied this preliminary identification criterion were subjected to confirmatory biochemical tests.

Confirmatory Biochemical Identification Tests for *E. coli* Isolates

Analytical Profile Index (API 20E) Test

API 20E was used for identification of *E. coli* according to the manufacturer's specifications (BioMérieux, Marcy l'Etoile, France). Indices were generated and the identities of the isolates were determined using the API web software.

Serotyping

Representative *E. coli* isolates from each sample were further analyzed for characteristics of *E. coli* O157:H7 using the slide agglutination test with *E. coli* O157 and H7 specific antisera obtained from Mast Diagnostics (U.K). Agglutination that was strong and clearly visible within one minute was recorded as positive results.

Molecular Characterization of *E. coli* O157:H7 Isolates

Extraction of Genomic DNA

Genomic DNA was extracted from all presumptive *E. coli* O157:H7 isolates using a modified cell boiling method (Tunung et al. 2007). Fresh cultures were prepared by spread plating the isolates onto nutrient agar plates to revive the cells. Plates were incubated at 37°C for 24 hours. After incubation, 500 µl of sterile water was placed in a 1.5 ml microfuge tube and pure cultures of the isolates were transferred into the tubes. The tubes were vortexed vigorously to prepare a homogenous suspension. The cell suspension was incubated at 100°C in a heating block (Biorad, Digital dry bath) for 15 minutes and this was followed by centrifugation for two minutes at 13,500 rpm. After centrifugation, the tube was placed on ice for 5 minutes and the supernatant was transferred to a new tube. An aliquot of 5 µl of this supernatant was used for PCR analysis.

Multiplex PCR for the Detection of Two E. coli Housekeeping Genes

A multiplex PCR analysis was performed on all positively identified *E. coli* isolates to detect the presence of two housekeeping genes - the *mdh* (Tarr et al. 2002) and *lacZ* (Ram and Shanker 2005).

Identification of Suspected E. coli O157:H7 Isolates by PCR

The identities of the suspected *E. coli* O157:H7 isolates were confirmed through amplification of the *rfb*O_{157} gene fragments (Morin et al. 2004) and the *fliC*$_{H7}$ gene fragments (Reischl et al. 2002), respectively. Amplifications were performed using a Peltier Thermal Cycler (model-PTC-220 DYAD™ DNA ENGINE). Standard 25 ml PCR reactions that constituted 1 mg/ml of the template DNA, 50 pmol of each oligonucleotide primer set, 1X PCR master mix, 1U *Taq* DNA polymerase and RNase free distilled water were performed. All the PCR reagents used were Fermentas, USA products and supplied by Inqaba Biotec Ltd, South Africa. All PCR products were stored at 4^{o}C until electrophoresis.

PCR for the Detection of Shiga Toxin Genes in E. coli O157:H7 Isolates

Specific PCR analysis was performed on all positively identified *E. coli* O157:H7 isolates to detect the presence of the shiga toxin genes (Pass et al. 2000).

Electrophoresis of PCR Products

The PCR products were resolved by electrophoresis on a 2 percent (w/v) agarose gel. A Gene Genius Bio Imaging System (Syngene, Synoptics, UK) was used to capture the image using GeneSnap (version 6.00.22) software. GeneTools (version 3.07.01) software (Syngene, Synoptics, UK) was used to analyze the images in order to determine the relative sizes of the amplicons.

Antibiotic Susceptibility Tests

The antibiotic susceptibility test was performed on *E. coli* O157:H7 isolates to determine their antibiotic resistance profiles using the Kirby-Bauer disk diffusion method (Bauer et al. 1966). The antibiotic inhibition zone diameters were measured and the results obtained were used to classify organisms as being resistant, intermediate and susceptible to a particular antibiotic based on standard reference values (NCCLS 2003). The antibiotics used were those to which isolates from animals and humans have been found to be resistant against in the area (Ateba and Bezuidenhout 2008; Moneoang and Bezuidenhout 2009).

Multiple Antibiotic Resistance (MAR) Phenotypes

MAR phenotypes were generated for isolates that were resistant to three and more antibiotics (Rota et al. 1996). Phenotypes were generated using the abbreviations that appeared on the antibiotic discs.

ELISA Assay for the Detection of Shiga Toxins in Antibiotic Induced Broth Cultures of Confirmed E. coli O157:H7 Isolates

E. coli O157:H7 isolates that were positively identified by PCR analysis to possess the shiga toxin genes were incubated for 24, 48 and 72 hours, respectively in Luria Bertani broth while shaking at 170 rpm. Prior to shaking, either ampicillin or tetracycline was added to the broth at concentrations of 10 µg/ml or 30 µg/ml, respectively. After incubation, the entire broth sample was centrifuged for 15 minutes at 3000 rpm. Aliquots of 100 µl from the clear supernatants were used in performing the ELISA test. The ELISA test was performed using a RIDASCREEN® Verotoxin (C2201). The test is an *in vitro* diagnostic assay for the quantitative determination of shiga like toxins 1 and 2 of *E. coli* strains in stool and enrichment cultures.

In performing the test 100 µl of the positive control, negative control and the test samples were pipetted into the wells of a 96 well microtitre plate. The samples were incubated at room temperature (20-25^{0}C) for 60 minutes. The samples were emptied and the plate inverted on absorbent paper in order to remove the residual moisture. The plate was washed five times using 300 µl of the wash buffer. After the wash, 100 µl of the enzyme conjugate was added to the wells and incubated at room temperature for 30 minutes. The plate was washed five times using 300 µl wash buffer and 100 µl of the substrate added to each well. The plate was then incubated at room temperature for 15 minutes in the dark. After the incubation, the reactions were stopped by add-

ing 50 µl of the stop reagent to each well. The extinction was measured photometrically using a micro-plate reader (LT-4000MS from Labtech International Ltd, Sussex, UK) at a wavelength of 450 nm. In validating the results obtained, the test was considered correctly carried out if the extinction value of the positive control was greater than 0.8 at 450 nm and that of the negative control less than 0.2 nm at 450 nm. In calculating the cut-off, the following formula was used:

Culture Supernatant: Cut-off = Extinction for the negative control + 0.1

Therefore, 0.2 was considered the threshold above which all tests were seen to be positive for the shiga toxin genes and results were recorded as positive when their extinction was greater than the calculated cut-off value.

RESULTS

PCR for the Identification of *E. coli* O157:H7 Isolates Using rfb_{O157} and $Flic_{H7}$ Primers

A total of 57 and 31 *E. coli* isolates that possessed the *mdh* and *lacZ* genes were subjected to specific PCR analysis for identification as *E. coli* O157:H7. This was achieved through amplification of the $rfbO_{157}$ and $fliC_{H7}$ gene fragments specific for *E. coli* O157:H7 isolates. Only 15 (40.1%) of the isolates were positively identified and results are shown in Table 2, and the number of *E. coli* O157:H7 isolates were higher in samples obtained from Zeerust (47.8%) and Taung (30.4%).

PCR for the Detection of stx Genes of *E. coli* O157: H7

Results obtained revealed that none of the isolates from the different sample stations were positive for shiga toxin genes. These isolates may still have the potential to cause water and foodborne infections in humans if they possess other *E. coli* O157:H7 accessory virulence gene determinants that were not screened.

Percentage Antibiotic Resistant Data of *E. coli* Isolated from the Groundwater

All the *E. coli* O157H7 isolates from water samples obtained from Bekkersdal, Carltonville, and Taung were resistant to amocyxillin, ampicillin, chloramphenicol and penicillin G (Table 3).

Table 2: Proportion of isolates that were positive for preliminary and confirmatory identification tests

Sample source	*Gram staining (-ve rod)*	*Oxidase (+ve)*	*Lactose (+ve)*	*Glucose (+ve)*	*H_2S (-ve)*	*Gas (+ve)*	*SCT (-ve)*	*Serotyping*	*API 20E*	*No of isolates tested by PCR*	*No of isolates positive for the targeted genes*				
											mdh	*lacZ*	*rfb_{O157}*	*$fliC_{H7}$*	*stx*
Carltonville	8	6	5	5	6	6	8	8	4	8	8	0	3	3	0
Khutsong	16	10	11	10	15	10	11	0	10	0	0	0	0	0	0
Bekkersdal	16	9	10	11	10	9	10	2	14	2	2	2	0	0	0
Taung	20	8	7	8	4	5	5	10	4	10	10	10	7	7	0
Vryburg	16	7	11	11	11	11	7	0	5	0	0	0	0	0	0
Delaryville	16	15	10	11	10	10	7	5	12	5	0	0	0	0	0
Zeerust	100	80	55	55	65	66	40	25	75	25	22	14	11	11	0
Deelpan	24	18	15	15	14	3	0	0	0	0	0	0	0	0	0
Tshidilamolomo	8	7	5	5	6	5	2	0	0	0	0	0	0	0	0
Rustenburg	100	12	8	10	75	70	33	5	32	5	3	0	0	0	0
Dingateng	8	5	6	7	4	3	5	0	1	0	0	0	0	0	0
Disaneng	8	7	7	6	4	5	5	0	2	0	0	0	0	0	0
Logagane	8	4	6	6	6	7	6	3	1	3	0	0	0	0	0
Leporang	8	4	4	6	3	6	6	2	1	2	1	1	2	2	0
Makgobistad	8	6	4	4	5	4	7	0	1	0	0	0	0	0	0
Mabule	8	7	4	4	4	5	4	2	3	2	1	1	0	0	0
Masamane	8	5	5	4	4	4	4	1	0	1	0	0	0	0	0
Selosesha	8	4	6	5	7	4	3	1	0	1	3	0	0	0	0
Stella	12	4	4	5	6	6	5	3	1	3	7	3	0	0	0
Total No.	**400**	**218**	**188**	**259**	**239**	**168**	**270**	**67**	**166**	**67**	**57**	**31**	**23**	**23**	**0**

SCT=Simmons Citrate agar test result; API = analytical profile index; +ve=positive; -ve=negative

Table 3: Percentage antibiotic resistance data for *E. coli* O157:H7 isolates

Sampling site	*No. of isolates*	*A*	*AP*	*K*	*NE*	*OT*	*C*	*PG*
Carltonville	8	100	100	0	0	12.5	100	100
Khutsong	0	0	0	0	0	0	0	0
Bekkersdal	2	100	100	50	0	50	100	100
Taung	10	100	100	30	0	100	60	100
Vryburg	0	0	0	0	0	0	0	0
Delaryville	5	100	100	20	0	100	0	100
Zeerust	25	100	100	40	8	80	36	100
Deelpan	0	0	0	0	0	0	0	0
Tshidilamolomo	0	0	0	0	0	0	0	0
Rustenburg	5	100	100	80	60	100	100	100
Dingateng	0	0	0	0	0	0	0	0
Disaneng	0	0	0	0	0	0	0	0
Logagane	3	100	100	0	0	100	66.7	100
Leporang	2	100	100	0	0	100	100	100
Makgobistad	0	0	0	0	0	0	0	0
Mabule	2	100	100	100	100	100	100	100
Masamane	1	0	100	0	0	100	100	100
Selosesha	1	100	100	100	100	100	100	100
Stella	3	100	100	66.7	0	100	66.7	100

Moreover, all the isolates from Delaryville, Logagane, Leporang, Mabule, Selosesha and Stella were resistant of amocyxillin, ampicillin, oxytetracycline and penicillin G. On the contrary, none of the isolates from Khutsong, Vryburg, Deelpan, Tshidilamolomo, Dingateng, Disaneng and Makgobistad were resistant to any of the antimicrobial agents tested. A large proportion (66.7% to 100%) of the isolates from Mabule, Selosesha, Stella and Rustenburg were resistant to kanamycin. However, none of the isolates from Carltonville, Khutsong, Vryburg, Deelpan, Tshidilamolomo, Dingateng, Disaneng, Logagane, Leporang Makgobistad and Masemane were resistant to kanamycin and neomycin. Despite this, 8.3 percent to seventeen percent of the isolates from the aforementioned villages including Stella and Taung were resistant to neomycin. In general, ampicillin, amoxicillin, tetracycline and penicillin G are the drugs to which most of the isolates from the different sources were resistant (Table 3). However, these isolates were highly susceptible to neomycin.

Multiple Antibiotic Resistant Phenotypes of E. coli Isolates from Groundwater

All the isolates from Mabule and Selososha were resistant to all the antibiotics tested. Thus, the multiple antibiotic resistant phenotypes (MAR) for these isolates were A-AP-K-NE-OT-C-PG. Moreover, a large proportion (60%) of the isolates from Rustenburg possessed this phenotype (Table 4). The predominant MAR phenotypes for isolates from Carltonville and Delaryville were A-AP-C-PG and A-AP-OT-PG

Table 4: Predominant multiple antibiotic resistant (MAR) phenotypes for *E. coli* O157:H7 isolates from groundwater

Sampling site	*Phenotypes*	*No. observed*	*Percentage (%)*
Carltonville	A-AP-OT-C-PG	1	12.5
	A-AP-C-PG	7	87.5
Bekkersdal	A-AP-OT-C-PG	1	50
	A-AP-K-C-PG	1	50
Taung	A-AP-K-OT-C-PG	3	30
	A-AP-OT-PG	4	40
	A-AP-OT-C-PG	3	30
Delaryville	A-AP-OT-PG	4	80
	A-AP-K-OT-PG	1	20
Zeerust	A-AP-K-NE-OT-C-PG	2	8
	A-AP-K-OT-C-PG	7	28
	A-AP-K-OT-PG	1	4
	A-AP-OT-PG	10	40
	A-AP-PG	5	20
Rustenburg	A-AP-K-NE-OT-C-PG	3	60
	A-AP-K-OT-C-PG	1	20
	A-AP-OT-C-PG	1	20
Logagane	A-AP-OT-C-PG	2	66.7
	A-AP-OT-PG	1	33
Leporang	A-AP-OT-C-PG	2	100
Mabule	A-AP-K-NE-OT-C-PG	2	100
Selososha	A-AP-K-NE-OT-C-PG	1	100
Masamane	AP-OT-C-PG	1	100
Stella	A-AP-K-OT-C-PG	2	66.7
	AP-OT-PG	1	33

and were obtained at 87.5 percent and eighty percent, respectively. Moreover, the MAR phenotypes A-AP-PG, A-AP-K-OT-C-PG and A-AP-OT-PG were obtained at percentages of twenty percent, twenty-eight percent and forty percent, respectively among *E. coli* O157:H7 isolated from Zeerust. Similarly, the MAR phenotypes for isolates from Taung included A-AP-K-OT-C-PG and A-AP-OT-C-PG, which occurred at thirty percent each and A-AP-OT-PG was identified among forty percent of the isolates. These results indicate that in the present study, MAR *E. coli* O157:H7 were isolated from groundwater. It is therefore suggested that these MAR isolates may have severe health implications in individuals who consume water from these sources.

ELISA Assay for the Detection of Shiga Toxins

A total of 52 *E. coli* O157:H7 isolates were selected randomly for the ELISA assay. These isolates were subjected to an ELISA test to determine the potential of two antimicrobial agents (ampicillin and tetracycline) in Luria Bertani broth cultures to induce shiga-like toxins. Figure 1 shows the results of the ELISA test for the isolates after they had been grown in the LB broth for 24, 48 and 72 hours in the presence of ampicillin or tetracycline. As shown in Figure 1, none of the isolates were positive for the shiga toxins after 24 hours induction with both ampicillin and tetracycline. This is mainly due to the fact that the extinction (O.D) values for the isolates and the negative control were less than 0.2 at 450 nm. However, it is evident that the extinction values for the positive controls were 2.054 for tetracycline and ampicillin, respectively. Furthermore, at 48 hours all the isolates were negative for shiga-like toxins. At 72 hours of induction with tetracycline, only one of the isolates [Z10-2(4)] and the positive control were positive for the shiga toxins when compared to the negative control. On the contrary, when these isolates were induced with ampicillin, all except for three of the isolates (KV1, KV2 and KV6) and the negative control were positive for the shiga toxins (Fig. 1). Interestingly, all these three isolates were obtained from the same sampling site (Carltonville). From these results, it was identified that the duration of exposure of *E. coli* O157:H7 isolated may significantly affect the release of shiga toxins in broth cultures. Moreover, tetracycline other than ampicillin could serve as a better inducer for shiga toxins in these pathogens. These results also indicate that the uncontrolled usage of these drugs during diarrheal cases without consulting might pose severe health risk on humans.

DISCUSSION

The primary objective of the study was to isolate and identify *E. coli* O157:H7 from groundwater obtained from some rural communities around the North West Province, South Africa. In the present study, *E. coli* O157:H7 was successfully isolated and identified in some of the water samples. The number of isolates was higher in water samples obtained from villages in Zeerust and Taung compared to the others. The exact reason for the distribution pattern observed for this pathogen cannot be described at the moment. However, during the collection of samples, it was identified that the boreholes in these villages were constructed very close to pit toilets. This may favor the transmission and cross contamination with pathogenic microorganisms. *E. coli* O157:H7 has a very low infectious dose and therefore, the results obtained in the present study may have a significant public health implication.

Water from boreholes and surface water bodies tested are not treated and individuals in these rural communities, who do not have access to treated water supplies, use the water. This therefore exposes them to a number of *E. coli* O157:H7 infections (Olsen et al. 2002; Petridis et al. 2005; Schets et al. 2005). This is even aggravated by the fact that most residents in these communities do not have any knowledge about the health implications of these pathogens. Considering the burden of diseases caused by *E. coli* O157:H7 in humans in countries with more advanced public and healthcare systems (Allison et al. 2000; Vogt and Dippold 2005; CDC 2012), there is a need to reduce contact with these pathogens especially in rural communities within South Africa, where proper hygiene is not practiced. Despite the fact that water and foodborne outbreaks of *E. coli* O157:H7 infections have not been reported to date in South Africa, the pathogen has been isolated from animals and humans in the area (Ateba et al. 2008; Ateba and Mbewe 2011). This therefore indicates that there is a possibility of these isolates contaminating either surface or groundwater through improper deposition of feces from both humans

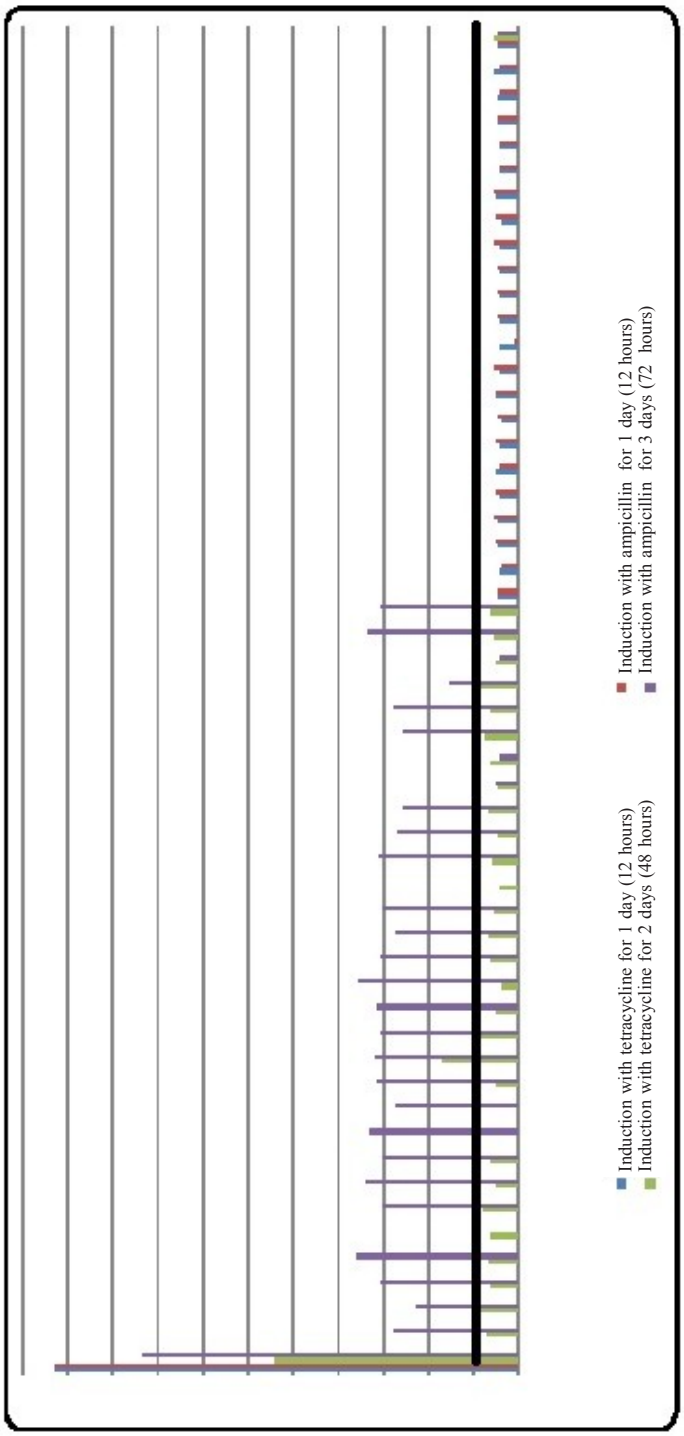

Fig. 1. ELISA resits for shiga-toxins of *E. coli*O157:H7 isolates induced with ampicillin and tetracycline after 1 day (24 hours) of incubation. Z= Zeerust; TAU=Taung

and animals. Contamination of these water bodies could also result through leaching from rainfall runoffs.

Another objective of the study was to determine the presence of shiga toxins genes that also account for pathogenicity in *E. coli* O157:H7 isolates using specific PCR analysis. In the present study, none of the isolates were positive for the *stx* genes and similar observations have been reported earlier (Hornitzky et al. 2002; Ateba and Bezuidenhout 2008). Despite the fact that the *stx* genes have been reported to be highly associated with other *E. coli* O157:H7 virulence genes (Pradel et al. 2000; Johnsen et al. 2001), some isolates from diseased humans did not possess any of the shiga toxin genes (Ateba and Bezuidenhout 2008). Unfortunately, the *E. coli* O157:H7 obtained in the present study were not screened for the presence of other accessory virulence genes.

A further study was to determine the antibiotic resistance profiles of *E. coli* O157:H7 isolates obtained from water samples. Similar to a previous finding (Ateba and Bezuidenhout 2008), in the present study, multiple antibiotic resistance (MAR), defined as resistance to three or more different classes of antibiotics, was observed in all (100%) of the *E. coli* O157:H7 isolated. A large proportion of the isolates were resistant to amocyxillin, ampicillin, chloramphenicol and penicillin G thus the MAR phenotype A-AP-C-PG was present in most phenotypes. In a previous study, only a small proportion (0% to 37.8) of *E. coli* O157:H7 isolated were resistant to chloramphenicol and ampicillin (Ateba and Bezuidenhout 2008). However, these isolates were obtained from animal and human stool samples. On the contrary, only a small proportion (23.8% to 25.4%) of *E. coli* O157:H7 isolates were resistant to tetracycline and ampicillin, respectively (Ateba and Bezuidenhout 2008). More than one third of the isolates screened in this study were resistant to tetracycline. Despite the fact that some isolates obtained in the present study were highly resistant to kanamycin, a large proportion of these isolates were susceptible to this drug. These findings are similar to those obtained in previous studies (Ateba and Bezuidenhout 2008). However, results obtained from a study conducted in Gaborone, Botswana, indicated that *E. coli* O157:H7 strains isolated from meat and meat products were highly resistant to cephalothin, sulfatriad, colistin sulfate and tetracycline (Magwira et al. 2005). It is therefore suggested that the antibiotic resistant profiles of isolates may depend on level of exposure and varies between regions. These resistant determinants may be transferred easily to other microbes with which they share common habitats (Lin and Biyela 2005). Moreover, these isolates may find their way into the environment, water bodies and grazing animals (Kidd et al. 2002; Dancer 2004). It is therefore suggested that the widespread and inappropriate use of antibiotics veterinary and human medicine, animal husbandry, aquaculture, agriculture and food technology may contribute in the development and spread of bacterial resistance to antimicrobial agents (Barbosa and Levy 2000; Mincey and Parkulo 2001). The data obtained from this study could be useful in controlling the development of bacterial antibiotic resistance within populations.

Another objective of the study was to determine the potential of two antimicrobial agents (ampicillin and tetracycline) in inducing the release of shiga toxins in broth cultures. The antimicrobial agent ampicillin was more effective in the release of shiga toxins in *E. coli* O157:H7 broth cultures compared to tetracycline. Moreover, the ability of tetracycline to induce these shiga toxin proteins in broth cultures depended largely on the duration of exposure of *E. coli* O157:H7 cells. To the best of the researchers' knowledge, in the present study, they report for the first time that *Escherichia coli* O157:H7 from environmental sources that do not possess shiga toxin genes by PCR analysis produce cytotoxins in broth cultures of ampicillin and tetracycline. Physicians may put patients suffering from diarrheal infections, and since the administration of antimicrobial agents for shiga toxigenic *E. coli* infection is under discussion (Wong et al. 2000; Zhang et al. 2000), the findings of this study indicate that these isolates may have severe public health implications on humans and therefore presents a huge challenge towards therapy.

A number of studies have assessed the ability of some antimicrobial agents such as mitomycin and norfloxacin to release cytotoxins in shiga toxigenic *E. coli* isolates (Aertsen et al. 2005; Wegrzyn and Wegrzyn 2005). Unfortunately, these drugs are either rarely or not used at all for the treatment of human infections. However, in another study, the antibiotic fosfomycin was able to produce a marked increase of shiga tox-

ins after 5 hours of incubation in broth cultures of enterohaemorrhagic *E. coli* O157:H7 (Yoh et al. 1997). In the present study, similar observations were obtained with ampicillin and tetracycline. It is important to mention that the ability to induce these potent toxins was significantly higher with ampicillin when compared to tetracycline. Therefore ampicillin rather than tetracycline was considered a more potential inducer of shiga toxin in broth cultures of *E. coli* O157:H7 isolates obtained in the study area. In South Africa, tetracycline and ampicillin are easily accessible over the counter and this therefore amplifies the need to perform proper laboratory identification test to determine the causative bacteria strain in diarrheal cases before antibiotic treatment is administered. The controversy surrounding the use of antibiotics for the treatment of shiga toxin producing *E. coli* infections coupled with the present findings indicate that further studies are necessary to actually evaluate the exact effects of these antibiotics and the shiga toxins produced gut epithelial cells. Results obtained may indicate whether the administration of these antimicrobial agents actually worsen an EHEC infections in humans.

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