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Phenotypic and Genetic Characterization of Sorbitol-Fermenting *Escherichia coli* O157: H7 Isolated from Retail Beef and Mince Beef

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ABSTRACT *Escherichia coli* occur as normal flora in the gastrointestinal tract of humans and animals. However, shiga-toxin producing *E. coli* and most especially serotype O157:H7 are a major group of food-borne pathogens. The consumption of undercooked contaminated food products of animal origin particularly beef has identified as a potential source for the transmission of these pathogens to humans. The aim of this paper was to determine the level of *E. coli* O157:H7 contamination in beef and mince beef samples obtained from some supermarkets and evaluate the virulence gene profiles of the isolates. A total of 128 beef and mince beef samples were collected from selected supermarkets and butcheries in the North West Province of South Africa. Samples were analysed for heterotrophic bacterial counts on PCA and potential *E. coli* O157:H7 counts on SMAC. Preliminary (oxidase test, TSI agar test) and confirmatory biochemical (API 20E, O157 and H7 serological assays, rfb_{O157} and $fliC_{H7}$ PCR analysis) tests were used to identify *E. coli* O157:H7 isolates while the virulence profiles of the isolates were determine through amplification of the *stx*1, *stx*2, *eaeA* and *hlyA* virulence genes. Despite the fact that all the beef samples were contaminated with heterotrophic bacteria none of those from Ventersdorp and Rustenburg were contaminated with potential *E. coli* O157:H7. Beef samples from Swaartruggens had highest heterotrophic microbial count ($5.3x10^6$ CFU/mL) while those from Garankwa had the lowest level of contamination ($1.3x10^4$ CFU/mL). All (100 percent) the mince beef samples collected from the different sampling stations were contaminated with both heterotrophic and potential *E. coli* O157 bacteria. Mince beef samples from Potchefstroom, Rustenburg, Klerksdorp, Ventersdorp, Zeerust, Mafikeng, Swaartruggens, Vryburg, Bloemhof and Taung had very high levels ($1.8x10^3$ - $4.1x10^6$) of potential *E. coli* O157:H7 contamination. All the isolates from beef except for those isolated from Rustenburg and Ventersdorp as well as those from mince beef were Gram-negative rods. A large proportion (71.4 percent to 100 percent) of the isolates from both beef and mince beef were oxidase negative. Large proportions (92.9 percent to 100 percent) of isolates from beef as well as small proportions (14.3 percent to 42.9 percent) of those from mince beef fermented the sugars in the TSI medium. Small proportions (7.1 percent to 42.9 percent) of the isolates from both beef and mince beef produced gas and hydrogen sulphide from the TSI agar test. A total of 39 and 38 *E. coli* isolates were obtained from beef and mince beef samples respectively while a large proportion 27 (70.1 percent) of these isolates were positively identified as *E. coli* O157:H7 using a serological assay. Only 8 *E. coli* O157:H7 isolates were positively identified in the beef samples while large proportions (23) of the isolates from mince beef were positive for the $fliC_{H7}$ and rfb_{O157} gene fragments. Among the isolates from beef, the *stx*2 gene was present in all the 8 isolates while only 3 isolates possessed the *stx*1 gene. Similarly, the *stx*2 gene was frequently (13) detected among the isolates from mince beef when compared to the *stx*1 gene. Putative virulence genes *hlyA* and *eaeA* were detected in 12 and 6 isolates respectively from mince beef. Six isolates from beef possessed both the *hlyA* and *eaeA* gene fragments. The findings of this study revealed that beef and mince beef samples obtained from some supermarkets in the area may pose severe health risks to consumers if they are not properly cooked before consumption.

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INTRODUCTION

Escherichia coli occur as normal flora in the gastrointestinal tract of humans and animals (Harakeh et al. 2005; Ojo et al. 2010; Ateba and Mbewe 2011; Zheng et al. 2012; Díaz-Sánchez et al. 2013; Abraham et al. 2015; Tamang et al. 2015; Vijay et al. 2015; Liu et al. 2016). However, shiga-

toxin producing *E. coli* and most especially serotype O157:H7 are a major group of food-borne pathogens (Harakeh et al. 2005, Rangel et al. 2005; Seto et al. 2007; Amani et al. 2015; Kargar and Homayoon 2015). Despite the fact that *E. coli* O157:H7 may be present in the gastrointestinal tract of healthy domestic animals such as cattle, sheep and goats without any clinical symptoms (Müller et al. 2002; Cobbold et al. 2000) the pathogen has been associated with a variety of diseases in humans ranging from non-bloody to severe bloody diarrhoea, haemorrhagic colitis, haemolytic uraemic syndrome and thrombotic thrombocytopenic purpura which may be very lethal in immuno-compromised patients (Banatvala et al. 2001; Momba et al. 2008). These infections are usually the cause of renal failure in infected subjects (Weir 2000). Virulent *E. coli* O157:H7 strains harbour a variety of virulence gene determinants including Stx_1, Stx_2 and variants of Stx_2, *hlyA* and *eaeA* gene (Paton and Paton 1998; Law 2000; Schouten et al. 2005). These toxins invade the cells of the colon, epithelia and the endothelium of the human GIT tract resulting in disease (Paton and Paton 1998). *E. coli* O157:H7 infections usually result from the consumption of undercooked contaminated food products of animals origin and meat particularly beef has identified as a potential source for the transmission of these pathogens to humans (Meichtri et al. 2004; Stampi et al. 2004; Ojo et al. 2010; Ateba and Mbewe 2011; Gordillo et al. 2014; Ahmed and Shimamoto 2015; Dong et al. 2015; Hessain et al. 2015; Xu et al. 2016). In most developing countries, proper hygiene practices are not always strictly implemented during the handling and processing (Harakeh et al. 2005). Against this background meat, the equipment used for cutting meat and the environment in which the meat is stored may become contaminated with pathogenic bacteria strains including *E. coli* O157:H7 during slaughter (Elder et al. 2000; Blanco et al. 2003; Conedera et al. 2004; Magwira et al. 2005). Outbreaks of *Escherichia coli* O157:H7 infections through consumption of contaminated foods including undercooked meat products have brought a great safety concern (Gordillo et al. 2014; Ahmed and Shimamoto 2015; Dong et al. 2015; Hessain et al. 2015; Xu et al. 2016). The objectives of this study was to determine the level of *E. coli* O157:H7 on beef and mince beef and to evaluate the virulence gene profiles of the isolates.

MATERIAL AND METHODS

Sampling and Isolation of Bacteria

A total of 128 beef and mince beef samples were collected from selected supermarkets and butcheries in the North West Province of South Africa (Table 1). Fresh beef and minced beef portions were aseptically collected and properly packed in sterile Whirlpak bags. The samples were properly labeled and transported on ice to the laboratory for analysis. One gram from each sample was washed in 5 ml of 2 percent (w/v) peptone water. Ten-fold serial dilutions were prepared and an aliquot of 100 µL from each dilution was spread-plated on Sorbitol MacConkey agar (SMAC) agar and Plate Count agar (PCA) medium. The plates were incubated aerobically at 37°C for 24 hours. Colonies on PCA and SMAC agar plates were counted and results were re-

Table 1: Areas, sources and types of samples collected

Areas of collection	*Source samples*	*Nature of samples*	*Number of samples collected*
Zeerust	Bovine	Meat	4
	Bovine	Mince meat	4
Lichtenburg	Bovine	Meat	4
	Bovine	Mince meat	4
Koster	Bovine	Meat	4
	Bovine	Mince meat	4
Mafikeng	Bovine	Meat	4
	Bovine	Mince meat	4
Swaartruggens	Bovine	Meat	4
	Bovine	Mince meat	4
Delareyville	Bovine	Meat	4
	Bovine	Mince meat	4
Swarzrenerke	Bovine	Meat	4
	Bovine	Mince meat	4
Rustenburg	Bovine	Meat	4
	Bovine	Mince meat	4
Vryburg	Bovine	Meat	4
	Bovine	Mince meat	4
Taung	Bovine	Meat	4
	Bovine	Mince meat	4
Garankwa	Bovine	Meat	4
	Bovine	Mince meat	4
Brits	Bovine	Meat	4
	Bovine	Mince meat	4
Potchefstroom	Bovine	Meat	4
	Bovine	Mince meat	4
Ventersdorp	Bovine	Meat	4
	Bovine	Mince meat	4
Bloemhof	Bovine	Meat	4
	Bovine	Mince meat	4
Klerksdorp	Bovine	Meat	4
	Bovine	Mince meat	4

corded. Isolates on SMAC were later sub-cultured on SMAC agar and plates were incubated aerobically at 37°C for 24 hours. The isolates on SMAC were stored at room temperature and used for bacteria identification.

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 analysed 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 bacteria using a modification of the hot (65°C) CTAB (cetyltrimethylammonium bromide)–PVP (Polyvinylpyrrolidone) DNA extraction procedure (Dolye and Dolye 1990).

The quality and purity of DNA was checked using the spectrophotometric method as described earlier by Sambrook et al. (1989).

Polymerase Chain Reaction (PCR) for the Amplification of *rfb*$E_{O157:H7}$, 16S rRNA and *fliC*$_{H7}$ Gene Fragments

The PCR protocols were used to amplify bacterial *rfbE*$_{O157:H7}$, 16SrRNA and *fliC*$_{H7}$ gene fragments as described earlier by Paton and Paton (1998), Muyzer et al. (1995) and Gannon et al. (1997), respectively. Oligonucleotide primers sequences that were used in the study are shown in Table 2. The PCR reactions were performed to amplify *rfb*$E_{O157:H7}$ gene by using a DYAD DNA engine model (Model - PTC- 220 DYAD™ DNA Engine Thermocycler with the following conditions: initial denaturation 95°C for 5 minutes, followed by 34 cycles at 95°C for 30 seconds, 65°C for 30 seconds, 72°C for 30 seconds cycles, and finally elongation at 72°C for 3 minutes. For the amplification of 16S rRNA gene amplification following thermal conditions were used: initial denaturation at 94°C for 5 minutes followed by 31 cycles consisting of denaturation at 94°C for 1 minute, annealing at 67°C for 40 seconds and extension at 72°C for 45 seconds, followed by final extension at 72°C for 2 minutes. The *fliC*$_{H7}$ gene segment was amplified by using the fol-

Table 2: Oligonucleotide primers sequences that were used in the study

Primer	*Sequence (5' – 3')*	*Specificity*	*Amplicon size BP*
O157F[a]	CGGACATCCATGTGATATGG	$rfbE_{O157:H7}$	259
O157R[a]	TTGCCTATGTACAGCTAATCC		
GM5F[b]	CCGTCAATTCCTTTGAGTTT	16S bacterial ribosomal genes	550
907R[b]	CCGTCAATTCCTTTGAGTTT		
*fliCh*7 F[c]	GCGGCTGTCGAGTTCTATCGAGC	H7 gene	625
*fliCh*7 R[c]	CAACGGTGACTTTATCGCCATTCC		

[a]Paton and Paton (1998), [b]Muyzer (et al.) 1995, [c]Gannon (et al.) 1997

lowing PCR conditions: initial denaturation at 96°C for 3 minutes, followed by 26 cycles at 96°C for 10 seconds, 51°C for 7 seconds and 62°C for 15 seconds; followed by final extension at 62°C for 4 minutes. The amplified PCR amplicons products were stored at 4°C until used.

Agarose Gel Electrophoresis

PCR products were separated by electrophoresis on a 1 percent (w/v) agarose gel using a horizontal Pharmacia biotech equipment system (model Hoefer HE 99X) for 2 hours at 80V using 1 X TAE buffer. A 100 bp DNA molecular weight marker was included in each run and was used to compare the sizes of the amplified fragments. Gels were stained in ethidium bromide (0.001µg/ml) and visualized under UV light (Sambrook et al. 1989). A Gene Genius Bio Imaging System (Syngene, Synoptics.UK) was used to capture images using the gene snap (version). The images were analyzed using the gene tools (version) software (Synegene, Synoptics, UK).

RESULTS

Detection of Potential *E. coli* Isolates in the Samples

A total of 128 samples of raw beef and mince were examined for the presence of *E. coli* O157:H7 and results revealed that large proportion (60%) of the samples was contaminated with *E. coli* O157:H7 based on morphological characteristics. This included 87.5 percent of beef samples analysed in the study. Beef samples from Garankwa were the most contaminated with 31.8 percent potential *E. coli* O157:H7 contamination when compared to those from Swaartruggens that had the lowest contamination levels (Table 3). On the contrary, none of the samples from Ventersdorp and Rustenburg were contaminated with potential *E. coli* O157:H7 strains based on colonial morphologies.

Table 3: Total heterotrophic bacterial counts and potential *E. coli* isolates obtained from beef samples

Areas of collection	*Colony forming units on plate count agar*	*Colony forming units on Sorbitol Macconkey Agar*
Brits	$9.9x\ 10^5$	$8.5\ x\ 10^2$
Mafikeng	$1.1x10^5$	$4.7\ x10^2$
Garankwa	$1.3x10^4$	$0.4\ x10^4$
Koster	$1.2x10^5$	$0.3\ x10^3$
Potchefstroom	$0.9x10^6$	$0.1\ x10^4$
Klerksdorp	$1.8x10^5$	$3.3\ x10^3$
Ventersdorp	$2.9x10^4$	0
Zeerust	$1.5x10^4$	$0.2\ x\ 10^3$
Lichtenburg	$0.9x10^6$	6.5 x10
Swaartruggens	$5.3x10^6$	$3.7\ x10^2$
Vryburg	$1.4x10^6$	$6.9\ x10^3$
Delaryville	$2.8x10^6$	$6.3\ x10^3$
Swazrenecke	$5.3x10^5$	$1.5\ x10^3$
Bloemhof	$1.4x10^4$	$0.6\ x10^3$
Taung	$1.3x10^5$	$6.4\ x10^3$
Rustenburg	$1.7x10^6$	0

Determination of the Level of Contamination with Heterotrophic Bacteria and Potential *E. coli* isolates in Samples

The level of microbial contamination with potential *E. coli* O157:H7 isolates with respect to heterotrophic bacteria was determined for both beef and mince beef samples and results are shown in Tables 3 and 4 respectively. Despite the fact that all the beef samples were contaminated with heterotrophic bacteria none of those from Ventersdorp and Rustenburg were contaminated with potential *E. coli* O157:H7 (Table 3). Beef samples from Swaartruggens had highest heterotrophic microbial count ($5.3x10^6$ CFU/ml) while those from Garankwa had the lowest level of contamination ($1.3x10^4$ CFU/ml). In

addition, samples from Vryburg, Delareyville and Taung had very high levels of contamination with potential *E. coli* O157 contaminants (Table 3). All (100%) the mince beef samples collected from the different sampling stations were contaminated with both heterotrophic and potential *E. coli* O157 bacteria (Table 4). Samples from Potchefstroom, Rustenburg, Klerksdorp, Ventersdorp, Zeerust, Mafikeng, Swaartruggens, Vryburg, Bloemhof and Taung had very high levels ($1.8x10^3$ - $4.1x10^6$) of potential *E. coli* O157:H7 contamination. This was a cause for concern and it is suggested that these samples may facilitate the transmission of *E. coli* O157:H7 strains to humans especially if the food products are consumed undercooked.

Table 4: Total heterotrophic bacterial counts and potential *E. coli* isolates obtained from mince beef samples

Area of collection	*No. of colony farming units in plate count Agar*	*No. of colony farming units in Sarbitol Macconkey Agar*
Garankwa	$2.03x10^3$	$2x10^2$
Brits	$2.52x10^5$	$3.68x10^2$
Koster	$8.3x10^5$	$1.2\ x10^3$
Potchefstroom	$9.1x10^6$	$4.1x10^6$
Klerksdorp	$3.4x10^4$	$8.5x10^3$
Ventersdorp	$1.38x10^5$	$8.5x10^3$
Zeerust	$2.2x10^6$	$2.5x10^4$
Lichtenburg	$1\ x10^4$	$5.5x10^2$
Mafikeng	$1.9x10^5$	$1.8x10^3$
Swaartruggens	$19.5x10^4$	$5.5x10^4$
Vryburg	$9\ x10^4$	$6.5x10^3$
Delaryville	$1.22x10^5$	$2.3x10^3$
Swazrenecke	$6.37x10^5$	$8.7x10^2$
Bloemhof	$6.1x10^4$	$9.5x10^4$
Taung	$1.3x10^6$	$8.5x10^4$
Rustenburg	$1.4x10^6$	$1.2x10^4$

Preliminary Identification of *E. coli* Isolates from Beef and Mince Beef Samples

A total of 448 presumptive *E. coli* O157:H7 isolates that were different based on colonial morphologies were obtained from samples collected from the different locations and these isolates were subjected to preliminary identification tests that are specific for *E. coli* (Gram staining, oxidase test, TSI agar test and Simmons citrate agar test). The makeup included 224 isolates from beef and mince beef samples. Results obtained for isolates obtained from beef and mince beef samples are shown in Tables 5 and 6 respectively. All the isolates from beef were Gram-positive rods except for those isolated from Rustenburg and Ventersdorp. However, all the isolates from mince beef were Gram-negative rods. A large proportion (71.4% to 100%) of the isolates from both beef and mince beef were oxidase negative. Despite the fact that a large proportion (85.7% to 92.9%) of the isolates from beef in Vryburg and Lichtenburg did not ferment the sugars from the TSI medium, large proportions (92.9% to 100%) of those from the other stations fermented these sugars. However, none of the isolates from Rustenburg and Ventersdorp as well as only a small proportion (7.1% to 5 (35.7%) produced gas from the TSI medium. In addition, only 33 of the 224 isolates from beef

Table 5: Proportion of isolates from beef samples that were positive for preliminary biochemical tests

Areas of collection	*Gram staining (Negative rod)*	*Oxidase test (Negative)*	*Simmons Citrate agar test (Negative)*	*Colour change or acid production*	*Gas production*	*Hydrogen sulphide production*
Brits	14 (100%)	13 (92.9%)	6 (42.9%)	1 (7.1%)	1 (7.1%)	1 (7.1%)
Garankwa	14 (100%)	14 (100%)	1 (7.1%)	2 (14.3%)	3 (21.4%)	0
Rustenburg	0	0	0	0	0	7 (50%)
Vryburg	14 (100%)	13 (92.9%)	12 (85.7%)	1 (7.1%)	7 (50%)	0
Koster	14 (100%)	13 (92.9%)	3 (21.4)	3 (21.4%)	4 (28.6%)	4 (28.6%)
Bloemhof	14 (100%)	12 (85.7%)	9 (64.3%)	3 (21.4%)	6 (42.9%)	2 (14.3%)
Taung	14 (100%)	4 (28.6%)	4 (28.6%)	1 (7.1%)	0	1 (7.1%)
Swaartruggens	14 (100%)	11 (78.6%)	0	1 (7.1%)	1 (7.1%)	1 (7.1%)
Klerksdorp	14 (100%)	12 (85.7%)	3 (21.4%)	3 (21.4%)	1 (7.1%)	0
Ventersdorp	0	0	0	0	0	4 (28.6%)
Zeerust	14 (100%)	6 (42.9%)	16.1	3 (21.4%)	2 (14.3%)	2 (14.3%)
Delareyville	14 (100%)	11 (78.6%)	6 (42.9%)	1 (7.1%)	0	8 (57.1%)
Swaartruggens	14 (100%)	10 (71.4%)	0	3 (21.4%)	2 (14.3%)	0
Lichtenburg	14 (100%)	12 (85.7%)	13 (92.9%)	3 (21.4%)	1 (7.1%)	0
Potchefstroom	14 (100%)	13 (92.9%)	3 (21.4%)	5 (35.7%)	4 (28.6%)	0
Mafikeng	14 (100%)	7 (50%)	3 (21.4%)	1 (7.1%)	7 (50%)	3 (21.4%)

produced hydrogen sulphide gas (Table 5). Only a small proportion (14.3% to 42.9%) of the isolates from mince beef as well as none of those from Ventersdorp produced acid from breakdown of carbohydrates in the TSI medium. Similarly, none of the isolates from Taung, Ventersdorp, Delareyville and Rustenburg including a small proportion (7.1% to 42.9%) of those from mince beef in the other sample stations produced gas (Table 6). Interestingly, only 30 of the 224 isolates from mince beef produced hydrogen sulphide gas which is generally not a biochemical characteristic of *E. coli* strains.

Serological and API 20E Tests for Isolates from Beef and Mince Beef Samples

None of the beef samples from Rustenburg, Koster, Taung, Swaartruggens, Ventersdorp and Zeerust were positive for *E. coli* contamination using the API 20E assay. Despite this, 39 *E. coli* isolates were obtained from beef in the study. Similarly, 38 *E. coli* isolates were also obtained from mince beef samples. Among these isolates only small proportion 11 (28.2%) of the isolates from beef were positively identified as *E. coli* O157:H7. On the contrary, a large proportion 27 (70.1%) of the *E. coli* isolates were positive for *E. coli* O157 and H7 serological assays (Table 7). Interestingly *E. coli* O157:H7 was not detected in both beef and mince beef samples from Taung.

Genotypic Characterization and Virulence Genes Detection in *E. coli* O157:H7

In order to avoid bias all the 448 isolates were subjected to a universal 16S rRNA PCR assay as well as *E. coli* O157:H7 specific PCR designed to amplify the $fliC_{H7}$ and rfb_{O157} gene fragments. A further task was to assess the virulence profiles of the isolates through amplification of the *stx*1 and *stx*2 as well as putative virulence factors enterohemolysin (*hlyA*) and intimin (*eaeA*). Detailed results are shown in Tables 8 and 9 for beef and mince beef isolates respectively. Despite the fact that only 8 *E. coli* O157:H7 isolates were positively identified in the beef samples a large proportion (23) of the isolates from mince beef was positive for the $fliC_{H7}$ and rfb_{O157} gene fragments. Among the isolates from beef, the *stx*2 gene was present in all the 8 isolates while only 3 isolates possessed the *stx*1 gene. Similarly, the *stx*2 gene was frequently (13) detected among the isolates from mince beef when compared to the *stx*1 gene. Putative virulence genes *hlyA* and *eaeA* were detected in 12 and 6 respectively of the mince beef isolates. Among the isolates from beef 6 isolates possessed both the *hlyA* and *eaeA* gene fragments. It was also identified that beef samples from Mafikeng and mince beef samples from Brits had the highest level of contamination with *E. coli* O157:H7 (Tables 8 and 9). Despite the fact that *E. coli* O157:H7 was not detected in both beef samples from Koster, Bloemhof, Taung, Swaartruggens, Zeerust,

Table 6: Proportion of isolates from mince beef samples that were positive for preliminary biochemical tests

Areas of collection	*Gram staining (Negative rod)*	*Oxidase test (Negative)*	*Simmons Citrate agar test (Negative)*	*Colour change or acid production*	*Gas production*	*Hydrogen Sulphide production*
Brits	14 (100%)	8 (57.1%)	3 (21.4%)	2 (14.3%)	3 (21.4%)	0
Garankwa	14 (100%)	13 (92.9%)	1 (7.1%)	2 (14.3%)	2 (14.3%)	0
Mafikeng	14 (100%)	2 (14.3%)	0	2 (14.3%)	1 (7.1%)	6 (42.9%)
Vryburg	14 (100%)	10 (71.4%)	3 (21.4%)	5 (35.7%)	5 (35.7%)	0
Koster	14 (100%)	6 (42.9%)	5 (35.7%)	2 (14.3%)	3 (21.4%)	0
Bloemhof	14 (100%)	5 (35.7%)	3 (21.4%)	6 (42.9%)	6 (42.9%)	0
Taung	14 (100%)	6 (42.9%)	3 (21.4%)	2 (14.3%)	0	1 (7.1%)
Swaartruggens	14 (100%)	8 (57.1%)	3 (21.4%)	2 (14.3%)	1 (7.1%)	1 (7.1%)
Klerksdorp	14 (100%)	10 (71.4%)	13 (92.9%)	2 (14.3%)	1 (7.1%)	0
Zeerust	14 (100%)	4 (28.6%)	3 (21.4%)	2 (14.3%)	2 (14.3%)	2 (14.3%)
Delaryville	14 (100%)	5 (35.7%)	0	2 (14.3%)	0	9 (64.3%)
Swarzrenerke	14 (100%)	13 (92.9%)	3 (21.4%)	2 (14.3%)	2 (14.3%)	0
Lichtenburg	14 (100%)	13 (92.9%)	2 (14.3%)	2 (14.3%)	1 (7.1%)	0
Potchefstroom	14 (100%)	13 (92.9%)	12 (85.7%)	5 (35.7%)	4 (28.6%)	0
Rustenburg	14 (100%)	14 (100%)	4 (28.6%)	2 (14.3%)	0	7 (50%)
Ventersdorp	14 (100%)	12 (85.7%)	4 (28.6%)	0	0	4 (28.6%)

Table 7: Proportion of beef and mince beef isolates that were positive for confirmatory biochemical tests

Areas of collection	*API 20E*	*O157 serotyping*	*H7 serotyping*	*API 20E*	*O157 serotyping*	*H7 serotyping*
	Beef isolates			*Mince beef isolates*		
Brits	3 (21.4%)	1 (7.1%)	1 (7.1%)	3 (21.4%)	2 (14.3%)	2 (14.3%)
Garankwa	2 (14.3%)	1 (7.1%)	1 (7.1%)	2 (14.3%)	2 (14.3%)	2 (14.3%)
Rustenburg	0	0	0	1 (7.1%)	0	0
Vryburg	1 (7.1%)	1 (7.1%)	1 (7.1%)	5 (35.7%)	3 (21.4%)	2 (14.3%)
Koster	0	0	0	4 (28.6%)	4 (28.6%)	4 (28.6%)
Bloemhof	3 (21.4%)	1 (7.1%)	1 (7.1%)	6 (42.9%)	6 (42.9%)	6 (42.9%)
Taung	0	0	0	0	0	0
Swaartruggens	0	0	0	1 (7.1%)	1 (7.1%)	1 (7.1%)
Klerksdorp	1 (7.1%)	1 (7.1%)	1 (7.1%)	2 (14.3%)	1 (7.1%)	1 (7.1%)
Ventersdorp	0	0	0	0	0	0
Zeerust	0	0	0	2 (14.3%)	1 (7.1%)	1 (7.1%)
Delareyville	4 (28.6%)	0	0	1 (7.1%)	0	0
Swarzrenerke	6 (42.9%)	1 (7.1%)	1 (7.1%)	2 (14.3%)	1 (7.1%)	1 (7.1%)
Lichtenburg	9 (64.3%)	1 (7.1%)	1 (7.1%)	3 (21.4%)	1 (7.1%)	1 (7.1%)
Potchefstroom	6 (42.9%)	1 (7.1%)	1 (7.1%)	5 (35.7%)	4 (28.6%)	4 (28.6%)
Mafikeng	4 (28.6%)	3 (21.4%)	3 (21.4%)	1 (7.1%)	1 (7.1%)	1 (7.1%)
Total	**39**	**11**	**11**	**38**	**27**	**27**

Table 8: Proportion of *E. coli* O157:H7 isolates and *E. coli* O157:H7 specific virulent determinants in isolates from beef samples

Sample areas	*No of isolates screened*	*No positive for the rfb_{O157} gene fragment*	*No positive for the $fliC_{H7}$ gene fragment*	*No of isolates positive for the stx1 gene fragment*	*No of isolates positive for the stx2*	*No of isolates positive for theeae*	*No of isolates positive for the hylA*
Brits	14	1	1	1	1	1	1
Garankwa	14	1	1	0	1	1	1
Mafikeng	14	2	2	1	2	2	2
Vryburg	14	1	1	1	1	0	0
Koster	14	0	0	0	0	0	0
Bloemhof	14	0	0	0	0	0	0
Taung	14	0	0	0	0	0	0
Swaartruggens	14	0	0	0	0	0	0
Klerksdorp	14	1	1	0	1	0	0
Zeerust	14	0	0	0	0	0	0
Delareyville	14	0	0	0	0	0	0
Swarzrenerke	14	0	0	0	0	0	0
Lichtenburg	14	1	1	0	1	1	1
Potchefstroom	14	1	1	0	1	1	1
Rustenburg	14	0	0	0	0	0	0
Ventersdorp	14	0	0	0	0	0	0
Total	**224**	**8**	**8**	**3**	**8**	**6**	**6**

Delareyville, Swartzrenerke, Rustenburg and Ventersdorp as well as mince beef samples from Taung, Zeerust, Delareyville, Rustenburg and Ventersdorp but the detection of the pathogen in some of the samples was a cause for concern. The findings of this paper revealed that beef and mince beef samples obtained from some supermarkets in the area may pose severe health risks on consumers if not properly cooked before consumption.

DISCUSSION

Shiga-toxigenic *E. coli* particularly those belonging to the serotype O157:H7 have received remarkable attention recently even in countries

Table 9: Proportion of *E. coli* O157:H7 isolates and *E. coli* O157:H7 specific virulent determinants in isolates from mince beef samples

Sample areas	*No of isolates screened*	*No positive for the* rfb_{O157} *gene fragment*	*No positive for the* $fliC_{H7}$ *gene fragment*	*No of isolates positive for the stx1 gene fragment*	*No of isolates positive for the stx2*	*No of isolates positive for theeae*	*No of isolates positive for the hylA*
Brits	14	2	2	1	2	2	2
Garankwa	14	2	2	0	1	2	2
Mafikeng	14	1	1	1	1	0	1
Vryburg	14	3	3	3	3	0	3
Koster	14	4	4	1	3	0	2
Bloemhof	14	5	5	0	0	0	0
Taung	14	0	0	0	0	0	0
Swaartruggens	14	1	1	0	0	0	0
Klerksdorp	14	1	1	0	1	0	0
Zeerust	14	0	0	0	0	0	0
Delareyville	14	0	0	0	0	0	0
Swarzrenerke	14	1	1	0	0	0	0
Lichtenburg	14	1	1	0	1	1	1
Potchefstroom	14	2	2	1	1	1	1
Rustenburg	14	0	0	0	0	0	0
Ventersdorp	14	0	0	0	0	0	0
Total	**224**	**23**	**23**	**7**	**13**	**6**	**12**

with advance more public health and health care facilities and this mainly due to their potential to cause a number of food-borne diseases in humans (Belongia et al. 1991; Meichtri et al. 2004; Harakeh et al. 2005; Amani et al. 2015; Kargar and Homayoon 2015). Sporadic cases of infections and outbreaks of *E. coli* O157:H7 infections usually result from the consumption of undercooked contaminated beef (Williams et al. 2000; Gordillo et al. 2014; Ahmed and Shimamoto 2015; Dong et al. 2015; Hessain et al. 2015; Xu et al. 2016). The primary aim of this paper was to determine the level of microbial contamination in beef and mice beef samples obtained from some supermarkets in the major cities in the North West Province. Based on colonial appearance large proportion (60%) of the samples was contaminated with potential heterotrophic bacteria as well as potential *E. coli* O157:H7.

A further objective of the paper was to identify *E. coli* O157:H7 using preliminary and confirmatory biochemical tests. Despite the fact that only a small proportion (8 and 11) of the isolates from beef and mince beef respectively were positively identified as *E. coli* O157:H7 the detection of the pathogen in some of the meat samples was a cause for concern. Similar observations have been reported (Ateba and Mbewe 2011). It has been reported that food products of animals origin particularly beef has identified as a potential source for the transmission of these pathogens to humans (Meichtri et al. 2004; Ateba et al. 2008; Ojo et al. 2010; Ateba and Mbewe 2011; Evans et al. 2011; Ateba et al. 2013; Gordillo et al. 2014; Ahmed and Shimamoto 2015; Dong et al. 2015; Hessain et al. 2015; Xu et al. 2016). In most developing countries, proper hygiene practices are not always strictly implemented during the handling and processing (Harakeh et al. 2005). The equipment used for cutting meat and the environment in which the meat is stored as well as the hygiene status of employees in the meat processing plant and proper farm management techniques have been reported to contribute significantly to the level of *E. coli* O157:H7 contamination (Elder et al. 2000; Blanco et al. 2003; Conedera et al. 2004; Loneragan et al. 2005; Magwira et al. 2005; Dong et al. 2015; Hsu et al. 2015). In addition, strategies that limit person-to-person transmission of *E. coli* O157:H7 may greatly reduce both sporadic and outbreak cases of infections in huamns (Seto et al. 2007). It is therefore important to constantly determine the occurance of this pathogen in food products so as to reduce human infections. In the present paper *E. coli* O157:H7 isolates possesed virulence gene *stx*1, *stx*2, *eaeA* and *hlyA*. The presence of these genes in *E. coli* O157:H7 are re-

sponsible for their pathogenicity (Oswald et al. 2000; Brusa et al. 2012; Lorente et al. 2014; Claudia et al. 2015).

CONCLUSION

In conclusion, *E. coli* O157:H7 was successfully isolated in the study and the isolates possessed virulence gene determinants. Given that this pathogen can be introduced into the food chain if proper hygiene practices are not strictly implemented during the handling and processing of food. Against this background there is need to develop and implement control measures that will limit the transmission of this pathogen to humans.

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