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Prevalence and characterization of *Escherichia coli* O157:H7 isolates from meat and meat products sold in Amathole District, Eastern Cape Province of South Africa

Benard O. Abong'o [a,1], Maggy N.B. Momba [b,*]

[a] University of Fort Hare, Department of Biochemistry and Microbiology, School of Science and Technology, P/Bag X1314, Alice 5700, South Africa
[b] Department of Environmental, Water and Earth Sciences, Tshwane University of Technology - Arcadia Campus, 175 Nelson Mandela Drive, Arcadia, Pretoria 0002, South Africa

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ABSTRACT

Meat and meat products have been implicated in outbreaks of *Escherichia coli* O157:H7 in most parts of the world. In the Amathole District Municipality of the Eastern Cape Province of South Africa, a large number of households consume meat and meat products daily, although the microbiological quality of these types of food is questionable. The present study investigated the prevalence of *E. coli* O157:H7 isolated from selected meat and meat products (45 samples each of biltong, cold meat, mincemeat, and polony) sold in this area. Strains of *E. coli* O157:H7 were isolated by enrichment culture and confirmed by polymerase chain reaction (PCR). Also investigated were the antibiogram profiles of the *E. coli* O157:H7 isolates. Five (2.8%) out of 180 meat and meat products examined were positive for *E. coli* O157:H7 that carried the *fliC*$_{H7}$, *rfbE*$_{O157}$, and *eaeA* genes. Two of the *E. coli* O157:H7 isolates were resistant against all the eight antibiotics tested. To prevent *E. coli* O157:H7 infections, meat and meat products such as biltong, cold meat, mincemeat and polony should be properly handled, and packed in sterile polyvinyl wrappers.

1. Introduction

Escherichia coli O157:H7 is a human pathogen worldwide associated with meat and meat products, dairy products, vegetables, and water (Browning et al., 1990; Galane and Le Roux, 2001; Obi et al., 2004; Magwira et al., 2005). It is recognized as a bacterium causing hemorrhagic colitis (Olorunshola et al., 2000; Galane and Le Roux, 2001). Diarrheal diseases linked to *E. coli* O157:H7 infections are characterized by blood, cramping abdominal pain, fever, nausea, and vomiting (Calundungo et al., 1994; Germani et al., 1997; Yoh et al., 1996; Olorunshola et al., 2000; Koyange et al., 2004). African people living in countries such as Central African Republic, Democratic Republic of Congo, Angola, Kenya, Nigeria, Swaziland and Cameroon, Côte d'Ivoire, Malawi and others have suffered from diarrhea caused by *E. coli* O157:H7 (Germani et al., 1997, 1998; Paquet et al., 1993; Isaacson et al., 1993). The first case of *E. coli* O157:H7 infection in South Africa was in 1990 (Browning et al., 1990). Since then, several sporadic cases of bloody diarrhea and also an outbreak have been reported in South Africa (Effler et al., 2001, Galane and Le Roux, 2001). Research done on water distribution systems in the rural parts of South Africa has revealed worrying levels of water contaminated by *E. coli* (Müller et al., 2001, 2003), although *E. coli* O157:h7 was not isolated from the waters that were investigated.

Even though several studies have demonstrated that healthy ruminant animals such as sheep, goats, and deer harbor *E. coli* O157:H7 (Kudva et al., 1996; Pritchard et al., 2000; Fischer et al., 2001), the distribution of *E. coli* O157:H7 in cattle is ubiquitous (Meyer-Broseta et al., 2001; Grauke et al., 2002). In South Africa, approximately 56% of goat meat is sourced from the Eastern Cape. The province also produces 12 000 tons of beef, 8000 tons of mutton and 76 000 tons of pork every year (South African Government Information, 2000). Although the animals from which the meat and meat products are sourced have been reported to carry *E. coli* O157:H7, there have not been any reported cases of food-borne outbreaks of *E. coli* O157:H7 in the Eastern Cape Province; this could be related to lack of surveillance and poor reporting.

By the year 2002, a large proportion of the Eastern Cape population (68%) still lived below the South African national poverty line (UNDP, 2004) and approximately 11% and 38% of the population lived in informal and traditional structures, respectively. People continue with unsafe hygienic practices, such as unsafe human excreta disposal, unsafe solid and liquid waste disposal and

* Corresponding author. Tel.: +27 82 513 7395; fax: +27 012 382 6233.
E-mail addresses: melfelis@gmail.com (B.O. Abong'o), mombamnb@tut.ac.za (M.N.B. Momba).
[1] Tel.: +27 40 602 2427; fax: 086 625 6886.

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unsafe drinking water (Phaswana-Mafuya and Shukla, 2005). There are also cultural practices that encourage communal eating for instance during funerals, weddings, political rallies and circumcision ceremonies. The aforementioned unsafe practices are possible breeding avenues for pathogenic microorganisms such as *E. coli* O157:H7. When these bacteria contaminate meat and meat products, they may finally infect humans. Infections commonly associated with consumption of meat and meat products contaminated with *E. coli* O157:H7 can easily spread throughout the entire community, leaving behind a trail of destruction of lives mostly to people with reduced immune systems such as HIV/AIDS patients.

One of the major problems that accompany *E. coli* O157:H7 infection is the danger of treating such patients with antibiotics (Wong et al., 2000; Okoli et al., 2005). Wong et al. (2005) warn that treating *E. coli* O157:H7 infections may result in the release of shigatoxins into the blood stream of the infected individuals. It is believed that the release of such toxins affects the kidneys resulting in a condition described as hemolytic uremic syndrome (Wong et al., 2000). This therefore presents a great challenge in the treatment approach to be adopted in the case of *E. coli* O157:H7 infections. In the present study, the prevalence of *E. coli* O157:H7 in selected meat and meat products often consumed in the Province has been determined as well as the molecular characteristics and antibiogram profiles of the *E. coli* O157:H7 isolates.

2. Materials and methods

2.1. Study areas and sample collection

One hundred and eighty samples of meat and meat products (45 samples of biltong, cold meat, mincemeat, and polony) were used in the study. The meat and meat products were purchased from butcheries, shops, supermarkets, and open air markets serving the communities of Alice, Fort Beaufort, and Mdantsane from March 2005 to August 2006. Biltong is a South African product typically made from raw fillets of meat cut into strips following the grain of the muscle and dried. It is similar to beef jerky in that both are spiced dried meats but they differ significantly in typical ingredients, taste, and production process. The word *biltong* is from the Dutch word "*bil*" meaning rump and "*tong*" meaning strip or tongue (Stephanie, 2006). The samples were placed in sterile polyvinyl bags, put in a cooler box with ice blocks, and transported to the laboratory where the microbiological examination started within 24 h after collection of the samples.

2.2. Culture-based enrichment, recovery, and identification of E. coli *O157:H7*

2.2.1. Enrichment and recovery of E. coli *O157:H7*

For enrichment, 10 g of each meat and meat product samples were added to 90 ml of modified EC (mEC) broth containing novobiocin (n) (Merck, SA, 20 μg ml^{-1}) (Cagney et al., 2004). The samples were incubated for 8 h at 37 °C on a rotary shaker with centrifugation at 100 rpm (Gallenkamp, Loughborough, UK). One milliliter of each enriched sample culture was then added to an Eppendorf tube (Eppendorf, SA) containing 20 μl of magnetic beads coated with antibodies to *E. coli* O157 (Dynal, Oslo Norway). Immunomagnetic separation (IMS) was carried out as described by the manufacturer (Dynal Product Brochure, 2006). Finally, the beads were re-suspended in 1 ml of sterile 1% (w/v) phosphate-buffered water (Merck, SA) and the suspension was spread in duplicate (50 μl each) onto sorbitol–MacConkey agar supplemented with cefixime (0.05 mg l^{-1}) and potassium tellurite (2.5 mg l^{-1}) (CT-SMAC) (Merck, SA) (Cagney et al., 2004). Five typical sorbitol-non-fermenting colonies were selected, sub-cultured onto Nutrient Agar (NA) (Merck, SA), and subjected to a confirmatory test as described below.

Prior to the conventional indole–methyl red–Voges-Proskauer citrate (IMViC) tests, Gram-negative colonies were tested for oxidase activity (Müller et al., 2003; Feng and Monday, 2000). Then biochemical characterization using an API 20E kit (bioMerieux) was performed following the manufacturer's instructions. The strips were read and final identification was secured using API LAB PLUS computer software (bioMérieux) (Momba et al., 2006).

2.3. Molecular characterization of E. coli *O157:H7 using the polymerase chain reaction (PCR)*

2.3.1. Bacterial DNA extraction

DNA was extracted from sorbitol non-fermenting colonies identified as *E. coli* and from a positive control strain for *E. coli* O157:H7 (ATCC 43895) following the method of Torres et al. (2003). A loop-full of overnight culture of presumptive *E. coli* O157:H7 colonies was suspended in 200 μl of sterile Milli-Q PCR grade water (Merck, SA) and the cells were lysed using a Dri-Block DB.2A (Merck, SA) for 15 min at 100 °C. The cell debris was removed by centrifugation at 13 400 rpm for 2 min using a MiniSpin microcentrifuge (Merck, SA). The lysate supernatant was placed on ice for 5 min.

2.3.2. Amplification of $fliC_{H7}$, $rfbE_{O157}$ and eaeA genes

Three sets of oligonucleotide primer mixtures were prepared according to the method previously used by Wang et al. (2002). A total volume of 10 μl of the extracted DNA was used in each PCR reaction. The PCR assays for $fliC_{H7}$, $rfbE_{O157}$ and *eaeA* (A/E) genes were carried out in a 50 μl reaction volume containing 10× SuperTherm GOLD buffer, 1.5 mM $MgCl_2$, each of the four deoxynucleoside triphosphates (dNTPs) (Southern Cross Biotechnology, SA) at a concentration of 0.25 mM, 100 pmol each of $fliC_{H7}$, $rfbE_{O157}$ and *eaeA* specific primers, 5 U of *Taq* DNA polymerase (Southern Cross Biotechnology). The reaction was carried out in the Eppendorf model AG 22331 Thermocycler (Merck, SA). The PCR conditions adopted in this study were similar to those previously used by Wang et al. (2002). *Escherichia coli* O157:H7 (ATCC 43895 strain) and sterile Milli-Q PCR grade water (Merck, SA) were included in each PCR assay as positive and negative controls respectively.

2.3.3. DNA electrophoresis

The amplicons (10 μl aliquots) were resolved in a 2% (w/v) agarose gel (Merck, SA) in 1× TAE buffer (40 mM Tris–HCl, 20 mM Na-acetate, 1 mM EDTA, pH 8.5) and stained with 0.5 μg ml^{-1} ethidium bromide (EtBr) (Merck, SA) (Wang et al., 2002; Cagney et al., 2004) before being visualized and photographed under the BioDoc-It System (UVP Upland, CA 91786, USA). A 100-bp DNA ladder (Promega, USA) was included on each gel as a molecular size standard. The electrophoresis was carried out at 76 V for 1 h.

2.3.4. Anti-microbial susceptibility test

The anti-microbial susceptibility test was determined using the Bauer and Kirby disk diffusion technique on Mueller–Hinton Agar (Merck, SA) (Jorgensen et al., 1999) using the antibiotics listed in Table 1. To achieve the antibiotic susceptibility of *E. coli* O157:H7 the measurement for the zones of inhibition was based on the breakpoints of the zone diameters for individual antibiotic agents. The results were interpreted according to the guidelines of the National Committee for Clinical Laboratory Standards for anti-microbial susceptibility testing (National Committee for Clinical Laboratory Standards, 1999). *Escherichia coli* (ATCC 43895) strain was included as a positive control.

Table 1
Antimicrobial susceptibility of five *E. coli* O157:H7 isolated from meat and meat products.

Antibiotic agent	Antibiotic disc content (μg)	No. of isolates[a]		
		R	I	S
Gentamycin	10	4	0	1
Ampicillin	10	2	2	1
Chloramphenicol	30	0	3	2
Nalidixic acid	30	1	4	0
Amikacin	30	0	0	5
Ceftriaxone	30	0	4	1
Erythromycin	15	5	0	0
Tetracycline	30	1	1	3

[a] R, resistant; I, intermediate; S, susceptible.

3. Results

3.1. Prevalence of E. coli O157:H7 in meat and meat products

The prevalence of *E. coli* O157:H7 is based on the number of meat and meat products that were positive for *E. coli* O157:H7, which carried the three target genes ($fliC_{H7}$, $rfbE_{O157}$, and *eaeA*) detected in the isolates by PCR analysis. The results show that five out of the 180 meat and meat product samples that were tested were positive for *E. coli* O157:H7 as verified by PCR for $rfbE_{O157}$ and $fliC_{H7}$. All isolates were positive for *eaeA*. These five meat and meat products were made up of two polony samples (one from Fort Beaufort and another from Mdantsane), one mincemeat and a cold meat sample (from Mdantsane), and a biltong sample from Alice.

3.2. Drug susceptibility testing

The results of the antibiotic susceptibility testing are summarized in Table 1.

4. Discussion

The study showed that five (2.8%) of the 180 samples of meat and meat products from Alice, Fort Beaufort, and Mdantsane were contaminated with *E. coli* O157:H7. These *E. coli* were found to be positive for the three target genes $fliC_{H7}$, $rfbE_{O157}$ and *eaeA* genes that are characteristics of *E. coli* O157:H7. A previous study carried out in Pretoria/South Africa revealed much higher levels (74.5%) of *E. coli* in meat and meat products (Vorster et al., 1994), however, the authors did not investigate for *E. coli* O157:H7. Nevertheless, the results of the current study confirm the most recent findings of similar research conducted in one African State (Magwira et al., 2005). In Botswana, Magwira et al. (2005) isolated *E. coli* O157:H7 from 5.22% of chunk meat, 3.76% of mincemeat, and 2.26% of sausages. In France, Vernozy-Rozand et al. (2002) also isolated the bacterium from 0.12% of industrial mincemeat. In Switzerland *E. coli* O157:H7 was isolated from 2.3% of mincemeat investigated (Chinen et al., 2001). The findings of the current study report a very low prevalence (0.03%) of *E. coli* O157:H7 in meat and meat products consumed within the Amathole District. These findings corroborate those of Vernozy-Rozand et al. (2002) since both fall within a similar percentage range. However, it must be noted that the presence of these pathogens in foods meant for human consumption is of great concern owing to the very low infectivity dose (10–100 cells) of *E. coli* O157:H7 (Hawker et al., 2001).

The most probable reason for the high prevalence of *E. coli* O157:H7 in mincemeat may be due to the spread of these bacteria during the mincing process. It is also suspected that mixing of meat from several cows, when only one carcass is fecally contaminated with *E. coli* O157 may result in contamination of the whole batch of minced meat. Further cross-contamination of the minced meat may be acquired from the mincing blades. *E. coli* O157:H7 may also be harbored in raw beef that is used in the manufacture of other meat products (Flores and Stewart, 2004).

Recent studies have revealed a trend towards increased antibiotic resistance of *E. coli* O157:H7 (Amornrut et al., 2000; Magwira et al., 2005). For instance, in 2005, about 35% of *E. coli* O157:H7 strains isolated from meat and meat products in Gaborone, Botswana, were resistant to cephalothin, sulfatriad, colistin sulfate and tetracycline (Magwira et al., 2005).

In this study, *E. coli* O157:H7 isolates were resistant to 5 out of 8 antibiotics namely: gentamicin, ampicillin, nalidixic acid, erythromycin, and tetracycline. There was double and multiple resistances (R) of four isolates. However, some isolates were either intermediately susceptible (I) and/or fully susceptible (S) to some antibiotics (Table 1).

Antibiotic resistance may occur either spontaneously by selective pressure or because of antibiotic miss-use by humans or overuse by farmers on their beef cattle (Schroeder et al., 2002). Although antibiotic resistance is common, antibiotics are still indicated in the management of life threatening diseases like diarrhea. However, the use of antibiotics in the management of *E. coli* O157:H7 infections in humans is still controversial due to the possible development of hemolytic uremic syndrome (HUS) (Wong et al., 2000).

Noteworthy is the fact that even though cold meat and polony are always packed in polyvinyl wrappers, the samples that were used in this study were purchased from butcheries while unpacked and sliced in small quantities hence this could have exposed them to contamination by *E. coli* O157:H7. In addition, the biltong samples that were used in this study were displayed hanging from hooks attached to the ceiling of butchery buildings and this may be a factor for cross-contamination of such meat products by flies and cockroaches feeding on the meat.

Mincemeat could have been contaminated with *E. coli* O157:H7 during the mincing process. Meat needs to be washed and cooked thoroughly to prevent human infection as proper cooking at very high temperatures actually destroys the cells of *E. coli* O157:H7. Meat products need to be handled while observing great hygiene and safety. During the study, improper handling of meat and meat products in the shops and butcheries was observed. Such uncontrolled handling of meat and meat products might lead to their cross-contamination with *E. coli* O157:H7.

5. Conclusion and recommendation

The results of the present study indicated a low prevalence of *E. coli* O157:H7 in meat and meat products sold in Amathole District Municipality. Only 5 (2.8%) out of 180 meat and meat products examined were positive for *E. coli* O157:H7 that carried the $fliC_{H7}$, $rfbE_{O157}$, and *eaeA* genes. The low prevalence of *E. coli* O157:H7 in meat and meat products should not be underestimated as this bacterium has been implicated in disease outbreaks worldwide. Frequent surveillance for *E. coli* O157:H7 in meat and meat products sold in the Amathole District and South Africa as a whole is therefore recommended in order to ascertain the exact prevalence level of the bacterium. The study also revealed that 2 of the *E. coli* O157:H7 isolates were resistant against all the eight antibiotics tested. Regardless of the effects of some antibiotics against the *E. coli* O157:H7 isolates, treating of patients infected with bacteria is still a subject of discussion in the scientific fraternity and is therefore beyond the scope of the current study.

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