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Review

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

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ABSTRACT

*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.



**Contents**

1. Introduction . . . 182
2. Materials and methods . . . 182
 2.1. Bacterial strains . . . 182
 2.2. Antibiotic susceptibility test . . . 182
 2.3. Haemolysis on blood agar . . . 182
 2.4. Extraction of genomic DNA and PCR . . . 182
 2.5. Sequence analysis . . . 183
 2.6. Statistical analysis . . . 183
3. Results . . . 183
 3.1. Antibiotic resistant data of *E. coli* O157 isolated from the different species at various sampling stations . . . 183
 3.2. MAR phenotypes of *E. coli* O157 isolated from pigs, cattle and humans . . . 185
 3.3. Cluster analysis of *E. coli* O157 for multiple antibiotic resistance (MAR) relationship on a dendogram . . . 185
 3.4. Detection of *E. coli* O157 virulence genes and tetracycline resistant genes . . . 185
4. Discussion . . . 185

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5. Conclusion . . . 186
Acknowledgements . . . 186
References. . . . 187

## 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