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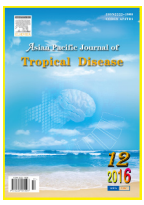

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Prevalence and antibiogram profiles of *Escherichia coli* O157:H7 isolates recovered from three selected dairy farms in the Eastern Cape Province, South Africa

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ABSTRACT

Objective: To investigate the occurrence and antibiotics susceptibility of *Escherichia coli* (*E. coli*) O157:H7 isolates from raw milk, cattle udder, milking machines and worker's hand swabs from three selected commercial dairy farms in the Amathole District Municipality, Eastern Cape Province, South Africa.

Methods: Raw milk samples were collected from bulk storage tanks and swab samples were collected from milking machines, cattle udders and worker's hands fortnightly over a six-month sampling regime between June and November 2014. A standard culture-based method was used for the enumeration and isolation of *E. coli* O157:H7, presumptive identification using sorbitol MacConkey agar (supplemented with cefixime (50 μg/L) and potassium tellurite (25 mg/L). A serological confirmation of the presumptive *E. coli* O157:H7 isolates was conducted using the O157 latex agglutination test kit.

Results: A total of 252 *E. coli* O157:H7 isolates were further subjected to PCR amplification of $rfbE_{O157}$ and $fliC_{H7}$ genes of which 27(11%) of the isolates were confirmed positive *E. coli* O157:H7. The percentage antibiotic resistance of the 27 *E. coli* O157:H7 isolates from the dairy farms revealed penicillin [23 (85%)], tetracycline [22 (81%)], erythromycin [19 (70%)], streptomycin [14 (52%)] and chloramphenicol [12 (45%)]. The highest resistances were penicillin [23 (85%)] and tetracycline [22 (81%)].

Conclusions: These findings revealed that the dairy farms are potential reservoirs of *E. coli* O157:H7 serotype, and harbor antibiotic-resistant determinants, a concern to public and environmental health.

1. Introduction

The emergence of *Escherichia coli* (*E. coli*) O157:H7 serotype dates back to 1982 when it was first discovered in an outbreak traced to contaminated Hamburgers[1]. Ever since its discovery to date *E. coli* O157:H7 remains as one of the most imperious foodborne pathogens, known to cause bloody diarrhoea, haemolytic uremic syndrome and hemorrhagic colitis in humans almost everywhere in the world[2].

Antimicrobial resistance has developed as an alarming health concern over time[3]. The Enterobacteriaceae, such as *E. coli* (with its variants) and some *Klebsiella* spp., produces different β-lactamase enzymes, some of which have activities against penicillin as well as second and third generations of cephalosporins. However, they have been reported to have improved their β-lactamases activity in recent years with the capability to hydrolyze the extended spectrum cephalosporin which led to the rapid evolution of extended spectrum β-lactamases with a capacity to confer resistance towards β-lactamase and non-penicillin antibiotics[4-6]. It is quite apparent that resistant bacteria evolves naturally when these bacterial strains self-replicate spontaneously or horizontally

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through genetic transfer mechanisms by microorganisms with resistant characteristics in conjunction with those that do not[7]. The multi-drug resistant isolates, particularly *E. coli*, have shown an alarming increase and wide resistance capability to broad-spectrum antimicrobials which are consequent causes of treatment failures, resulting to high mortality rates[6,8].

To facilitate and enhance the production among dairy farms, it has been conventional practices that most farmers tend to use antibiotics as growth promoters which may have a different interaction in the animal somehow enabling the spread and development of antibiotic resistance to some bacterial population[9]. Agricultural practices and the abnormal use of antimicrobials in veterinary medicine often promote the antimicrobial resistant bacteria and their positive selective pressure[3]. Inadequate clinical waste treatment may contribute in the prevalence and persistence of antimicrobial resistant bacteria and antibiotic residues in the environment, which becomes a major concern to global communities[10].

A number of studies suggest that the exploitative use of antimicrobial agents in humans and animals may support the increased resistance patterns by *E. coli* strains including O157:H7 to antimicrobials[11]. Antimicrobial resistance is reported as a massive setback towards effective prevention and treatment of the ever-increasing infections by bacteria, parasites, fungi and viruses, which is a global threat and a worrisome concern to the world of medicine[12]. The use of antibiotics for growth promotion and antimicrobial agents in dairy farms solely as treatment regime against *E. coli* is common in the Eastern Cape Province with the ever-increasing development of antimicrobial resistance by this bacterium[11]. To the best of our knowledge, there is scarcity of information on the prevalence and antibiogram characterization of *E. coli* O157:H7 in dairy farm surroundings in the Eastern Cape Province, South Africa. Hence, the present study elucidates the prevalence and antimicrobial susceptibility profiles of the confirmed *E. coli* O157:H7 isolates from three selected dairy farms in the Eastern Cape Province of South Africa.

2. Materials and methods

2.1. Description of the study location

Three selected commercial dairy farms under the Amathole District Municipality in the Eastern Cape Province, South Africa were used for this study and for confidentiality purpose were identified as farms A, B and C, respectively. Dairy farm A was surrounded by a number of villages and peri-urban settlements. It was located on the geographical coordinates 32°37'0" S and 27°07'0" E. This dairy coverws about 700 hectares of land with about 400 cows, a production capacity of 2 000 L of milk per day with 36 workers. Dairy farm B was located on the geographical coordinates of 32°49'0" S and 26°59'0" E and covered a terrain of about 280 hectares of land with 600 cows producing 2 000 L of milk per day with 16 permanent workers. Dairy farm C was situated along the geographical coordinates of 32°47'0" S, 26°50'0" E. About 800 cows were milked daily in the farm which produced an estimate of 10 000 L milk per day and had a total of 10 full-time workers. It supported both the local region and other regions abroad the Amathole District Municipality borders with its produce.

2.2. Sample collection

Samples were collected forth nightly over a period of six months (June–November, 2014). Samples included raw bovine milk samples from farm bulk storage tanks and were collected using pre-sterilized 50 mL centrifuge tubes (3 tubes for each farm), while sterile swabs sticks (Copan Group, Copan, Italia) were used to collect samples from milking machines, udder and hands of workers, and all samples were appropriately labelled. Samples were then transported on ice pack to the Applied and Environmental Microbiology Research Group Laboratory at the University of Fort Hare and analysed within few hours of collection.

2.3. Isolation and identification of E. coli O157:H7

Isolation of *E. coli* O157:H7 from raw milk samples was carried out following the protocol as described by Ateba and Mbewe[13] with some modifications. For raw milk samples, tenfold dilutions (10^{-1}–10^{-3}) of milk were made using sterile physiological buffer saline (PBS), where 1 mL of raw milk sample was transferred into 9 mL of sterile PBS (10^{-1} first dilution) and another 1 mL from the 10^{-1} dilution was transferred into another 9 mL of sterile PBS, and the process was repeated until 10^{-4} dilution was reached. One hundred microliter from each dilution was immediately spread-plated (in triplicates) on sorbitol MacConkey agar (Laboratorios Conda, Pronadisa, South Africa) plates supplemented with cefixime (50 μg/L) and potassium tellurite (25 mg/L) (Oxoid culture media supplements, UK) for the detection of *E. coli* O157:H7 and then incubated at 37 °C overnight. Colonies that appeared colourless or exhibited a beige colour on the agar were considered as presumptive *E. coli* O157:H7 positive isolates.

Swab samples from milking machines, cattle udders and the hands of workers collected across the three farms were inoculated into 10 mL of trypticasein soy broth (Laboratorios Conda, Pronadisa, South Africa) and incubated on a shaker at 37 °C overnight at 150 r/min. At the end of the incubation period,