# PATHOGENIC *ESCHERICHIA COLI* STRAINS IN RAW MILK OBTAINED FROM TWO FARMS OF THE EASTERN CAPE PROVINCE, SOUTH AFRICA

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## ABSTRACT

Production of maximum quantities of high-quality milk is an important goal of every dairy operation. High-quality milk must contain a low number of somatic cells and a low bacteria count, and must be free of human pathogens and antibiotic residues. In this study 400 milk samples were collected from two different commercial farms in the Eastern Cape, South Africa in which 200 samples were collected from each farm. The *E. coli* isolates were screened for markers of *E.coli* uidA, EHEC (flicH7) and EAEC (eagg) using PCR assays. Middledrift dairy had the highest amount of *E.coli* 48 out of 200 (24%) isolated from the milk samples and Fort Hare farm with the lowest *E.coli* 33 out of 200 (16.5%) present in the milk samples. This means that hygiene is not practiced in these farms and since a lot of people still drink raw milk, especially in rural areas, this emphasises the need for educational efforts on health risks associated with consumption of raw unpasteurized milk.

**Key words**: milk, *Escherichia coli*, Enterohaemorrhagic *E. coli (*EHEC*)*, Enteroaggregative *E.coli* (EAEC)

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## INTRODUCTION

Raw milk is a well-known good medium that supports the growth and multiplication of many kinds of microorganisms due to its complex biochemical composition and high water activity (Oliver *et al*., 2005). Many microorganisms can get access to raw milk and, among these are *Escherichia coli. E.coli* is an environmental pathogen found in the immediate surroundings of the cow such as the soil, grass, manure and the bedding of housed cows. It is therefore easy for the organism to be found in the udder of cow thereby gaining entrance to the milk. Its status as a true pathogen is of public health significance. It produces toxins that destroy cell membrane and can directly damage milk-producing tissues which can lead to bovine mastitis (Jones *et al*., 1998). It therefore became the objective of this study to determine the prevalence of *E. coli* in unpasteurized recovered from Middledrift and Fort Hare dairy, to characterize the identified isolates into different pathotypes and to determine the antibiogram of the isolates.

## MATERIAL AND METHODS

Four hundred samples were collected, 200 samples from each of the two commercial farms, Middledrift dairy and Fort Hare dairy Trust. For the isolation and identification of *E.coli*, the milk samples were cultured on selective medium Violet Red Bile-MUG Agar (MERCK,SA) and incubated at 37 ℃ for 24 hours . The identified *E.coli* isolates were then subjected to antibiotic susceptibility testing by the disc diffusion method (Bauer *et al*., 1966). Ten antibiotics were used (erythromycin, gentamicin, neomycin, streptomycin, chloramphenicol, thrimethroprim, kanamycin, amoxicillin and penicillin G). DNA was extracted from positive *E.coli* isolates following the boiling method in which isolates were suspended in eppendorf tubes containing 250 µl of sterilized distilled and then placed them on a heat block at 94-95°C for 15 minutes and then centrifuged at 15000rpm for 15 minutes at 4°C. The supernatant was used as a template DNA (Maugeri *et al*., 2004). DNA was used in PCR with oligonucleotide primers targeting a specific gene(uidA, eagg and flicH7). The PCR products (10 µl aliquots) were resolved in 1.8 % agarose gel containing 5 µl Ethidium bromide in 1X TBE buffer before being visualized and photographed under the Alliance System. The electrophoresis was carried out at 100 V for 1 hour.

## RESULT AND DISCUSSION

*Table 1-Antibiotic susceptibility testing of E.coli isolates from Fort Hare farm*

| | *E.coli* (n=33) | | |
|---|---|---|---|
| Antibiontics | S | I | R |
| Streptomycin (10 µg) | 33 (100%) | 0 (0%) | 0 (0%) |
| Neomycin (30 µg) | 33 (100%) | 0 (0%) | 0 (0%) |
| Gentamicin (10 µg ) | 33 (100%) | 0 (0%) | 0 (0%) |
| Penicillin G (10 µg) | 0 (0%) | 0 (0%) | 33 (100%) |
| Thrimethropim (5 µg) | 25 (76%) | 0 (0%) | 8 (24%) |
| Tetracycline (30 µg) | 8 (24%) | 8 (24%) | 17 (51%) |
| Amoxycillin (20 µg) | 0 (0%) | 8(24%) | 25 (76%) |
| Kanamycin (30 µg) | 33 (100%) | 0 (0%) | 0 (0%) |
| Erythromycin (15 µg) | 0 (0%) | 0 (0%) | 33 (100%) |
| Chloramphenicol (30µg) | 33 (100%) | 0 (0%) | 0 (0%) |

*S= susceptible, I= intermediate, R= resistant*

Globally, higher percentage of *E. coli* was reported by many authors including Egypt where the presence of coliform bacteria in raw milk was shown (Aly and Galal, 2002), India the raw milk and products were heavily contaminated by *E. coli* (Soomro *et al*., 2002), South Africa where a higher percentage of *E. coli* in raw milk was detected (Lues *et al*., 2003) a figure higher than in our study. Multiple resistances of the pathogenic *E.coli* isolates were found in the tested antimicrobial agents. Penicillin G, amoxycillin and erythromycin showed the highest resistance our results (see in *Table 1* and *2*) are similar to what Okoh and Osode (2008) have reported. Prevalence of pathogenic E.coli in raw milk indicated by the presence of the target gene marker are presented in *Table 3*. *uidA* gene which encodes the beta glucuronidase enzyme was amplified to identify *E.coli* strains.

*Table 2- Antibiotic susceptibility testing of E.coli isolates from Middledrift farm*

| | *E.coli* (n=48) | | |
|---|---|---|---|
| Antibiotics | S | I | R |
| Streptomycin (10 µg) | 41 (87.5%) | 6 (12.5%) | 0 (0%) |
| Neomycin (30 µg) | 48 (100%) | 0 (0%) | 0 (0%) |
| Gentamicin (10 µg ) | 48 (100%) | 0 (0%) | 0 (0%) |
| Penicillin G (10 µg) | 0 (0%) | 0 (0%) | 48 (100%) |
| Thrimethropim (5 µg) | 32 (66.7%) | 0 (0%) | 18 (37.5%) |
| Tetracycline (30 µg) | 48 (100%) | 0 (0%) | 0 (0%) |
| Amoxycillin (20 µg) | 18 (37.5%) | 18(37.5%) | 12 (25%) |
| Kanamycin (30 µg) | 48 (100%) | 0 (0%) | 0 (0%) |
| Erythromycin (15 µg) | 0 (0%) | 6 (12.5%) | 41 (87.5%) |
| Chloramphenicol(30 µg) | 36 (75%) | 0 (0%) | 12 (25%) |

*S= susceptible, I= intermediate, R= resistant*

The PCR assays successfully amplified the target gene *fliCH7* which is characteristic of the Enterohaemorrhagic *Escherichia coli* O157:H7 has been amplified from 37 isolates. An outbreak of *E. coli* O157:H7, which affected 16 people and caused 5 HUS cases among children, was linked to a yogurt made on a farm from pasteurized milk. According to my level of knowledge this gene has also been isolated from water by Okoh and Osode (2008). Enteroaggregative *Escherichia coli* characterized by *eagg* gene was amplified. EAEC is an emerging diarrheagenic pathogen associated with diarrheal illnesses among patients in developed and developing countries. and environmental samples ( Falcao *et al*., 2004).

*Table 3- Prevalence of pathogenic E.coli in raw milk indicated by the presence of the target gene marker*

| Location | uidA | flicH7 | eagg |
|---|---|---|---|
| Middledrift | + (48) | + (24) | + (20) |
| Fort Hare | + (33) | + (13) | + (8) |

## CONCLUSIONS

The results obtained in this study concluded that raw cow's milk available to consumers in the Eastern Cape, South Africa was contaminated with the opportunistic pathogen *E.coli*. High and strict preventive measures like regular washing and sterilization of dairy equipment, utensils,

milker's hands, and animal udders, and eradication of diseased animals from the herd are highly recommended. satisfactory. Given the growing number of reports of multidrug resistant to pathogenic *E.coli* isolates, it is evident that further research is still needed in this area.

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