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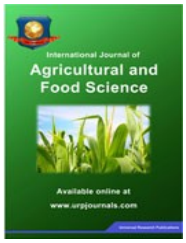

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Original Article

Detection of *Escherichia coli* in unpasteurized raw Milk

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Abstract

The present study was undertaken to estimate the incidence of opportunistic pathogen, *Escherichia coli* (*E. coli*), in raw cow's milk from different localities of Sfax governorate in Tunisia (El Amra, Esaadi, Nakta and Menzel cheker). Forty raw milk samples were randomly collected and inoculated on the relevant bacteriological media. Confirmation was performed using a series of biochemical tests. The results revealed that 32.5% of the samples were *E. coli* positive. The highest numbers of milk samples contaminated with *E. coli* were obtained from El Amra and Esaadi. The highest mean value of coliform was found in milk from El Amra farms with 3.34 ± 0.31 $\log_{10}$ cfu/ml, while the lowest mean count of 2.61 ± 0.40 log10 cfu/ml was detected in milk obtained from Nakta farms.

Key words: unpasteurized raw milk; pathogenic bacteria; coliform bacteria; *E. coli.*

1. Introduction

Raw or processed milk is a well-known good medium that supports the growth of several microbes with resultant spoilage of the product or infections/ intoxications in consumers [1, 2]. Microbes may gain entry into raw milk directly from dairy cows, from the farm environment particularly the water source [3] and utensils used for the storage of milk on farm or during transportation.

Markets and consumers for raw milk and their products have existed in many parts of the world. Raw unpasteurized milk is consumed directly by a large number of people in rural areas and indirectly by a much larger segment of the population via consumption of several types of cheeses. Among the main reasons that people may believe that the raw milk and their products have advantages or value over the pasteurized one. Being a highly nutritious medium, therefore many bacteria including spoilage and pathogenic bacteria can grow and propagate in it. The presence of food-borne pathogens in unpasteurized raw milk either directly or indirectly increases the risk of ingestion and transmission of food-borne pathogens and ingestion of potentially harmful toxins. Many microorganisms can get access to milk and products, among these are *E. coli*. Coliforms and *E. coli* are often used as marker organisms. Recovery and counting of *E. coli* is used as reliable indicator of fecal contamination and indicates a possible presence of enteropathogenic and/or toxigenic microorganisms which constitute a public health hazard. *E. coli* is one of the main inhabitants of the intestinal tract of most mammalian species, including humans. Most *E. coli* are harmless, but some are known to be pathogenic bacteria, causing severe intestinal and extra intestinal diseases in man [4]. The output of dairy and dairy products from Tunisia is increasing day by day in their internal market. Considering its economic potential, extensive and intensive exploitation of cow milk can both contribute to the nutrient requirements of the Tunisian public and increase the income of farmers. In view of the growing public awareness about food safety and quality, knowledge of the microbial and chemical composition of milk is of great significance for further development of its hygienic processing into high quality consumer products. The objective of this study was to investigate the occurrence of the opportunistic pathogen *E. coli* in cow's milk from different localities of Sfax governorate, Tunisia.

2. Materials and methods

All the samples were collected in sterilized container at random from different farms of localities of Sfax governorate, and were brought in ice box to the Food Analysis Laboratory, National Engineering School of Sfax, for the isolation of *E. coli*.

2.1 Microbiological methods

2.1.1 Preparation of serial dilutions:

Ten ml from each sample of unpasteurized raw milk were transferred to 90 ml sterile peptone water (0.1%) and thoroughly mixed to give 1:10 dilution 'first dilution'; serial dilutions were prepared by transferring one ml from first dilution to 9ml peptone water and so on as described by

Harrigan and McCance (1976).

2.1.2 Coliform bacteria, *E. coli*:

The coliform test was done according to Harrigan [5] by plating one ml sample onto Mac Conkey agar media. The plates were incubated at 37ºC for 48 h and the counts were presented as colony forming unites per gram (cfu/ml). Plates showing positive coliform were subjected to the confirmatory test using Brilliant green bile lactose broth in test tubes with inverted Durham tubes and incubated at 44ºC for 48 h. Each positive tube was subcultured into E.C. broth medium and then incubated at 44.5ºC for 24 h. Tubes showing gas productions were considered *E. coli* positive.

All the samples positive for *E. coli* contamination were confirmed using Gram's staining, cultural and biochemical examinations. For the isolation and identification of *E. coli*, the enriched sample was cultured on selective medium Levine Eosin Methylene Blue (EMB) Agar and incubated at 37ºC for 24 h [6]. Morphologically typical colonies (at least 4/plate) producing metallic sheen were taken into nutrient broth for further identification. Biochemical tests were performed to confirm *E. coli* using Gram staining, Catalase test, Indole, Methyl red, Voges- Proskauer test, Nitrate reduction, Urease production, Simmon's citrate agar, and various sugar fermentation tests (Table 1).

Table 1: Biochemical characterization of *E. coli*

Biochemical test	Reaction
Lactose fermentation	+
Catalase	+
Simmon's Citrate	-
Indole Production	+
Nitrate Reduction	+
Methyl Red	+
Voges- Proskauer	-
Urease	-
Acid from Sugar	
(a) Glucose	+
(b) Mannitol	+
(c) Lactose	+
(d) Salicin	+
(e) Sucrose	+

2.2 Statistical analysis:

All analytical determinations were performed at least in triplicate. Values of different tests were expressed as the mean ± standard deviation (x ± SD). SPSS packet program for Windows was used for the statistical analysis. Significant differences between mean ($P< 0.05$) were determined by using a one-way ANOVA (Duncan's test).

3. Results and Discussion

The results of the present study are summarized in the Table 2 and 3. According to Table 2, the highest number of *E. coli* contaminated samples was recorded in El Amra and Esaadi milk samples (5 out of 10 samples), followed by raw milk samples obtained from Menzel cheker (2 out of 10 samples). The least number of contaminated samples was detected in raw milk samples obtained from Nakta (1 out of 10 samples). From Table 3 it is shown that all tested samples were found to be contaminated with Coliform bacteria and with *E. coli*. The least contamination with Coliforms and specifically *E. coli* was detected in samples collected from Nakta farms, 2.61 ± 0.40 and 2.08 ± 0.11 $\log_{10}$ cfu/ml for Coliforms and *E. coli,* respectively. The highest contamination with *E. coli* was shown in samples of Fresh cow milk collected from El Amra and Esaadi farms, 2.69 ± 0.12 and 2.64 ± 0.08 $\log_{10}$ cfu/ml, respectively. Coliform bacteria can be carried into milk duct of the cow during milking by suction of the milking machine and then flushed out during subsequent milking without causing clinical symptoms of infection. Previous studies provided evidence that *Escherichia coli* are frequently occurring organism in milk. The methods of production, transportation, handling and sale of milk are entirely unhygienic. The results of the present study showed that 13 out of 50 milk samples (32.5%) were contaminated with *E. coli*. High incidence of *E. coli* was found in different types of milk by many researchers [7, 8, 9]. Contamination of milk and milk products, with pathogenic bacteria is largely due to processing, handling, and unhygienic conditions. *E. coli* is one of the bacteria that exist in the normal microflora of the intestinal tract of humans and warm blooded animals. *E. coli* is, furthermore, a known causative agent of diarrhea and other food-borne related illnesses through the ingestion of contaminated foodstuffs.

Table 2: Summarizes the overall percentages of *E. coli* contamination in milk samples

Sfax locality	Total number of analyzed samples	Number of *E. coli* positive samples	Percentage (%) of *E. coli* positive
El Amra	10	5	50
Esaadi	10	5	50
Nakta	10	1	10
Menzel cheker	10	2	30
Total	40	13	

Table 3: Mean counts of coliform, *E. coli* in raw cow milk sample collected from different localities of Sfax governorate.

Sfax locality	Coliform ($\log_{10}$ cfu/ml)	*E .coli* ($\log_{10}$ cfu/ml)
El Amra (n=10)	3.34 ± 0.31^{a}	2.69 ± 0.12^{a}
Esaadi (n=10)	3.23 ± 0.51^{a}	2.64 ± 0.08^{a}
Nakta (n=10)	2.61 ± 0.40^{b}	2.08 ± 0.11^{b}
Menzel cheker (n=10)	3.04 ± 0.25^{c}	2.23 ± 0.02^{c}

Different letters in the same column indicate significant difference ($P<0.05$).

Pathogenic members of the coliform group as well as the Enterobacteriacae family are represented by genera such as *Salmonella* and *Shigella* and are found in the intestines of humans and animals [10]. Most strains of *E. coli* are nonpathogenic [11]. However some strains differ from Commensal in that they express virulence factors [12]. *E. coli* frequently contaminates food and it is a good indicator of fecal pollution [13]. Presence of *E. coli* in milk products indicates the presence of enteropathogenic microorganisms, which constitute a public health hazard. Enteropathogenic *E. coli* can cause severe diarrhoea and vomiting in infants, and young children. The incidence of the species of *E. coli* itself in milk and milk products, as a possible cause of food born disease, is not significant if *E. coli* is normally a ubiquitous organism [14], yet the pathogenic strains if present could be harmful to consumers.

4. Conclusions

The results obtained in this study concluded that raw Cow's milk available to consumers in Sfax governate 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, pasteurization of milk before distribution to consumers and eradication of diseased animals from the herd are highly recommended. In this respect pasteurization and immediate cooling to 5ºC of milk could be more effective.

The magnitude of the problem of bacterial contamination deserves more elaborative studies from the point of production of milk and milk products to the point of consumption and at all intermediary levels. To identify potential milk safety hazards and to ensure production of safe and high quality product, food safety management programmed, the Hazard Analysis Critical Control Point (HACCP), should be implemented and highly considered.

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