

## ISOLATION OF *Escherichia coli*, *Staphylococcus aureus* AND *Listeria monocytogenes* FROM MILK PRODUCTS SOLD UNDER MARKET CONDITIONS AT AGRA REGION

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### ABSTRACT

*Escherichia coli*, *Staphylococcus aureus*, and *Listeria monocytogenes* were isolated from milk products i.e. curd and cottage cheese, sold at shops in the unorganized sector of Agra region. Of the 116 bacterial isolates from cottage cheese, 15 were confirmed as *E. coli*, 12 as *S. aureus*, and two as *L. monocytogenes*. Fifty-eight isolates were obtained from curd samples of which five were confirmed as *E. coli*, eleven as *L. monocytogenes*, and no *S. aureus* contamination was found in curd. The result indicates that strict preventive measures should be adopted to ensure contamination free milk products for the good health of all consumers.

Key words: milk products / curd / cottage cheese / microbiology / *Escherichia coli* / *Staphylococcus aureus* / *Listeria monocytogenes* / public health / India

### OSAMITEV BAKTERIJ *Escherichia coli*, *Staphylococcus aureus* IN *Listeria monocytogenes* IZ MLEČNIH PROIZVODOV, KI SO NA TRŽIŠČU V REGIJI AGRA (INDIJA)

### IZVLEČEK

Iz skute in svežega sira, ki ju prodajajo v neorganiziranih prodajalnah na področju Agra (Indija), smo osamili bakterije *Escherichia coli*, *Staphylococcus aureus* in *Listeria monocytogenes*. Od 116 bakterijskih izolatov iz svežega sira smo identificirali 15 izolatov *E. coli*, 12 *S. aureus* in dva *L. monocytogenes*. Med osemindesetimi izolati bakterij iz skute je bilo identificiranih pet izolatov *E. coli*, enajst *L. monocytogenes* in nobenega izolata *S. aureus*. Rezultati kažejo, da bi bilo za zagotavljanje zdravja potrošnikov nujno potrebno uvesti striktne ukrepe za preprečevanje okužbe mlečnih izdelkov.

Ključne besede: mlečni izdelki / skuta / sveži sir / mikrobiologija / *Escherichia coli* / *Staphylococcus aureus* / *Listeria monocytogenes* / zdravstveno varstvo / Indija

### INTRODUCTION

Milk is supposed to constitute a complex ecosystem for various microorganisms including bacteria. Milk products like cheese and curd are widely consumed and market for them has existed in many parts of the world for many generations. There is an increase demand by the consumer for high quality natural food, free from artificial preservatives, and contaminating microorganisms. Contamination of milk and milk products, with pathogenic bacteria is largely due to processing, handling, and unhygienic conditions. This paper describes the presence of

*Escherichia coli*, *Staphylococcus Aureus*, and *Listeria monocytogenes* in cottage cheese and curd available at shops comprising the unorganized sectors in Agra.

*E. coli* frequently contaminates food organism and it is a good indicator of fecal pollution (Diliello, 1982; Soomro *et al.*, 2002; Benkerroum *et al.*, 2004). 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 (Anon., 1975).

Of late *L. monocytogenes* has been recognized as a food born pathogen (Kaclikova, *et al.*, 2001) that can contaminate dairy products (Menendez *et al.*, 2001). Its virulent strain can cause a serious disease called listeriosis, particularly the risk populations including pregnant women, newborns, the very old, and people who are immune compromised (Fleming *et al.*, 1985; Bille, 1989).

Illness through *S. aureus* range from minor skin infection such as pimples, boils, cellulites, toxic shock syndrome, impetigo, and abscesses to life threatening disease such as pneumonia, meningitis, endocarditis, and septicemia. (Soomro *et al.*, 2003; Masud *et al.*, 1988).

## MATERIAL AND METHODS

### Standard strains

Standard strains of *E. coli* (MTCC-443), *L. monocytogenes* (MTCC-1143), and *S. aureus* (MTCC-3381) were procured from MTCC Chandigarh. All the isolates were confirmed through biochemical tests by comparing with the results of standard strains.

### Collection of samples

Samples of cottage cheese and curd were collected during the period of I year from different regions of Agra city and examined for the presence of *E. coli*, *L. monocytogenes*, and *S. aureus*. Samples of each milk product were collected aseptically, transferred to sterile plastic bags and were directly transported to the laboratory under cold conditions. They were stored at 4 °C and analyzed within 24 hours.

### Microbiological analysis

A Portion (10 g or 10 ml) from the centre of each sample was extracted aseptically and homogenized with 90 ml sterile enrichment broth (lactose broth for *E. coli*, UVM-2 for *L. monocytogenes*, and peptone water for *S. aureus*) and incubated at 37 °C for 24 hours, for further biochemical analysis.

Table 1. Morphological and culture characteristics of isolated bacteria  
Preglednica 1. Morfološke značilnosti kultur izoliranih bakterij

Isolated bacteria	Gram staining	Culture characteristics on selective media
<i>E. coli</i>	Gram negative rods	Colonies showing metallic sheen
<i>S. aureus</i> .	Gram positive cocci (in clusters)	Jet black colonies surrounded by white halo
<i>L. monocytogenes</i>	Gram positive rods	Greenish-yellow glistening, iridescent and pointed colonies surrounded by diffuse black zone

### Media and growth conditions

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 37 °C for 24 hours. Morphologically typical colonies (at least 4 / plate) producing metallic sheen were taken into nutrient broth for further identification. *S. aureus* was isolated by using the technique given by Baird Parker (1962). Enriched samples were streaked on Baird Parker Agar (BPA) and the plate was incubated at 37 °C for 24–48 hours. Appearances of jet black colonies surrounded by white halo were considered to be presumptive *S. aureus*.

For the plating of *L. monocytogenes* Dominguez-Rodriguez Isolation Agar (DRIA) (Dominguez-Rodriguez *et al.*, 1984) was used. The inoculum from enriched broth was streaked on DRIA plate and incubated at 37 °C for 24–48 hours. The greenish-yellow glistening, iridescent and pointed colonies surrounded by diffuse black zone were suspected to be listeriae (Table 1).

### Physiological and biochemical examination

Four to five suspected colonies from each bacterial plate were picked, cultured and then identified by the various biochemical tests.

Biochemical tests were performed to confirm *E. coli* using Gram staining, Catalase test, Indole, Methyl red, Voges- Proskauer test, Nitrate reduction, Urease production, Simon citrate agar, and various sugar fermentation tests (Table 2).

Table 2. Biochemical characterization of *E. coli*  
Preglednica 2. Biokemijska karakterizacija *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	+

Confirmation of the genus, *Staphylococcus* was done by Gram staining and various biochemical tests including Catalase test, Oxidase test, Indole, Methyl red, Voges-Proskauer test, Nitrate reduction, acid from different sugars, and haemolysis on Sheep Blood Agar (S.B.A.) following the method of Cruickshank (1970), while the species, *S. aureus* was confirmed by Coagulase test as described by Monica (1991) (Table 3).

Table 3. Biochemical characterization of *S. aureus*  
 Preglednica 3. Biokemijska karakterizacija *S. aureus*

Biochemical test	Reaction	
Catalase	+	
Oxidase	-	
Indole Production	-	
Nitrate Reduction	+	
Methyl Red	+	
Voges- Proskauer	+	
Acid from sugar	(a) Glucose	+
	(b) Mannitol	+
	(c) Maltose	+
	(d) Lactose	+
	(e) Raffinose	-
	(f) Sucrose	+
Haemolysis	+	
Coagulase	+	

Morphologically typical colonies of *L. monocytogenes* were identified by Gram's staining. Catalase reaction, tumbling motility at 20–25 °C, Methyl red test, Voges-Proskauer test, Nitrate reduction, fermentation of sugars, and haemolysis on 5% Sheep Blood Agar were conducted (Table 4).

Table 4. Biochemical characterization of *L. monocytogenes*  
 Preglednica 4. Biokemijska karakterizacija *L. monocytogenes*

Biochemical test	Reaction	
Catalase	+	
Oxidase	-	
Indole Production	-	
Nitrate Reduction	-	
Methyl Red	+	
Voges- Proskauer	+	
Haemolysis	+	
Acid from sugar	(a) Rambose	+
	(b) $\alpha$ methyl d mannoside	+
	(c) Xylose	-

## RESULTS

The present research findings pertain to the isolation of *E. coli*, *L. monocytogenes*, and *S. aureus* from milk products. Table 5 depicts the sampling data which consists of various numbers of samples analyzed and confirmed as *E. coli*, *L. monocytogenes*, and *S. aureus*. Out of 60 isolates 21 isolates were confirmed as *E. coli* (six from curd and 15 from cottage cheese); out of 63 isolates, 12 isolates were confirmed as *S. aureus* (from cottage cheese), out of 51 isolates, 13 isolates were confirmed as *L. monocytogenes* (11 from curd and two from cottage cheese) on the basis of morphological and biochemical characterization (Table 2, 3, and 4).

According to these results a higher contamination with *E. coli* and *S. aureus* was found in cottage cheese as compared to curd. No curd samples yielded *S. aureus* though incidence of *L. monocytogenes* was higher in curd rather than cottage cheese (Table 5).

Table 5. *E. coli*, *L. monocytogenes*, and *S. aureus* detected in the test sample  
Preglednica 5. Število v vzorcih odkritih *E. coli*, *L. monocytogenes* in *S. aureus*

Organism	Number of sampling		Number of samples in which colonies appear		Number of isolates taken		Number of positive samples	
	curd	Cottage cheese	curd	Cottage cheese	curd	Cottage cheese	curd	Cottage cheese
<i>E. coli</i>	13	13	8	7	32	28	6	15
<i>S. aureus</i>	13	13	1	13	4	59	-	12
<i>L. monocytogenes</i>	13	13	5	8	22	29	11	2

## DISCUSSION

*E. coli*, *S. aureus*, and *L. monocytogenes* occur frequently in milk products, such as curd and cottage cheese (Mary *et al.*, 1992; Oranusi *et al.*, 2007). These milk products are mostly prepared and consumed in the unorganized sector in Agra region. The method of their manufacturing, handling and sale is entirely based on the traditional systems. Such systems could provide a favorable environment for bacterial contamination. The unclean hands of workers, poor quality of milk, unhygienic conditions of the manufacturing unit and water supplied for washing the utensils could be the source for accelerating the bacterial contamination of milk products beside the post manufacturing contamination (Tariq Masud *et al.*, 1988; Johnson, 1961; Elmahmood *et al.*, 2007).

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 (Hahn, 1996), yet the pathogenic strains if present could be harmful to consumers. The risk is magnified when the same samples of curd and cottage cheese are contaminated with *L. monocytogenes*, because *L. monocytogenes* can survive at temperatures of refrigeration where these milk products are normally stored (EI-Kest *et al.*, 1991).

*S. aureus* on the other hand releases a toxic chemical, enterotoxin. As little as 1.0 µg of the toxin in contaminated food produces symptoms of illness. This level of the toxin has been found at 10<sup>5</sup> cells /g of food (Ananthanarayna *et al.*, 2001).

Milk is one of the most important nutrients and protein dense food, because it is an excellent source of nine essential nutrients and casein, a major milk protein. Dairy products like curd and

cottage cheese made from milk and their consumption plays a significant role in the supply of important nutrients and protein required for good health. These milk products are very essential in the Indian diet so their contamination can cause varied health hazards. This can be of serious concern to the local consumers and also to the innumerable tourists flocking Agra from the different parts of India as well as the world.

The results of the present study indicate that strict preventive measures should be adopted to ensure contamination free milk products for the good health of all consumers. For this, consciousness and care is required from the point of generation to the point of consumption of these widely consumed milk products.

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