

PREVALENCE OF COAGULASE POSITIVE PATHOGENIC *Staphylococcus aureus* IN MILK AND MILK PRODUCTS COLLECTED FROM UNORGANIZED SECTOR OF AGRA

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Prevalence of coagulase positive pathogenic Staphylococcus aureus in milk and milk products collected from unorganized sector of Agra

The present study deals with the sampling, isolation and biochemical characterization of *Staphylococcus aureus* from raw milk of cow and buffalo, and milk products (khoa sweets and cottage cheese) collected from different unorganized sectors of Agra city, India. Samples of raw milk from cow, cottage cheese and khoa sweets indicated that *S.aureus* contamination was higher in east of Agra (Ram Bagh), whereas samples of raw milk from buffalo, collected from the west of Agra (Sikandra), showed higher *S.aureus* contamination. Least contamination was observed in samples of khoa sweets and cottage cheese collected from west of Agra (Sikandra) and in samples of cow and buffalo milk collected from North of Agra (Dayalbagh) and east of Agra (Ram Bagh), respectively. The incidence of high contamination in raw milk and milk products suggests that protective measures are inadequate and need to be enforced to avoid potential threat to public health.

Key words: milk / milk products / microbiology / *Staphylococcus aureus* / isolation / biochemical characterization

1 INTRODUCTION

Milk and milk products are home to complex microbial ecosystems; these are responsible for the broad variations in taste, aroma and texture of milk and milk products. Contamination of milk and milk products with pathogenic bacteria is mainly due to processing, handling and unhygienic environment. Milk and milk products like cottage cheese and khoa sweets are widely consumed since ancient times and its market demand is continuous throughout the world. The occurrence of pathogenic

Pogostnost okužb mleka in mlečnih proizvodov s patogenimi sevi bakterije Streptococcus aureus s koagulazno aktivnostjo na območju neorganizirane proizvodnje v okolici Agra (Indija)

Prispevek opisuje vzorčenje, osamitev in biokemijsko karakterizacijo bakterije *Staphylococcus aureus* iz surovega mleka krav in bivolov ter mlečnih proizvodov (khoa in skuta) na neorganiziranem proizvodnem območju mesta Agra v Indiji. Vzorci surovega kravjega mleka, skute in khoe kažejo, da je bila kontaminacija s *S. aureus* močnejša na vzhodu Agra (Ram Bagh), medtem ko so bili vzorci surovega mleka bivolov bolj kontaminirani na območju zahodnega dela Agra (Sikandra). Najnižjo raven kontaminacije smo opazovali v vzorcih khoe in skute z zahodnega dela Agra (Sikandra) in v vzorcih kravjega in bivoljega mleka s severnega (Dayalbagh) in vzhodnega dela Agra (Ram Bagh). Visoka stopnja kontaminacije mleka in mlečnih proizvodov kaže, da zaščitni ukrepi za preprečevanje kontaminacij niso ustrezni in jih je nujno treba izboljšati, da bi se izognili ogrožanju javnega zdravja.

Ključne besede: mleko / mlečni izdelki / mikrobiologija / *Staphylococcus aureus* / izolacija / biokemična karakterizacija

bacteria in these milk and milk products can cause severe health hazards to people. *S.aureus* is one of those bacteria that can cause minor skin infections (pimples, boils, cellulites, toxic shock syndrome, impetigo and abscesses) as well as life threatening diseases (pneumonia, meningitis, endocarditis and septicemia) (Soomro *et al.*, 2003).

S.aureus 0.5–1.59µm in diameter are spherical Gram positive, non motile, non-spore forming facultative anaerobes which ferment most of the sugars except raffinose and salicin producing lactic acid during fermentation. They are catalase and coagulase positive and flour-

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ish between a pH of 7.4–7.6. They exist in air, dust water, sewage, meat and meat products, poultry and egg products, salad such as tuna, chicken, potato and macaroni, bakery products such as cream filled pastries, cream pies, chocolates éclairs, and sandwich fillings and in milk and milk products. Pathogenesis of *S.aureus* is due to repertoire toxins, exoenzyme adhesions and immune modulating protein that it produces. 20–30% of healthy people may carry this bacterium on their skin surface and nasal passage (MedicineNet.com). It causes a variety of superlative infections by producing leukocidin, a toxin that destroys the white blood cells and leads to the formation of pus and toxinosis in humans (Todar, 2005). The presence of *S.aureus* in food causes food poisoning by releasing enterotoxins into the food and it can also cause Toxic Shock Syndrome by release of super antigens into the blood stream (Todar, 2005).

Staphylococci possess protein receptors for many mammalian proteins such as fibronectin, fibrinogen and IgG, which facilitate Staphylococcal adhesion to host cell and tissue (Todar, 2005). It can grow at a temperature range from 15 to 45 °C and at NaCl concentration as high as 15 percent (Wenzel and Perl, 1995). About 50% strains of this organism are able to produce enterotoxin associated with food poisoning (Pyne and Wood, 1974). On heating at normal cooking temperature, the bacteria may be killed but the toxins remain (Prescott *et al.*, 2002). As little as 1.0 9g toxin in contaminated food produces symptoms of illness. This level of the toxin has been found at 105 cells /g of food; this toxin cannot be denatured after boiling (Ananthanarayana *et al.*, 2001). It can also grow at high salt which is generally 15% higher than normal. *S.aureus* also expresses certain virulence factors and due to these virulent determinants, it is tenacious, potentially destructive and shows increasing resistance to antimicrobial agents. The coagulase test is a standard criteria for the identification of *S.aureus* (Burriel, 1998).

Pathogenicity of *S.aureus* is due to the membrane active substances i.e. cytolytic toxins, consisting of four haemilysins and a leukocidin. This genus may have alpha, beta, gamma and delta haemolysin and the pathogenic members of species *aureus* display beta haemolysis (Prescott, 2002). The present study attempts to assess the prevalence and pathogenicity of *S.aureus* in milk (cow and buffalo) and milk products (khoa sweets and cottage cheese) collected from different unorganized sectors of Agra city, because unorganized sectors consist of all private enterprises whose activities are not regulated under legal provision. In Agra, there are many unorganized areas where milk and milk products are produced by traditional methods and these milk and milk products are usually consumed locally.

2 MATERIALS AND METHODS

Standard strains: Standard strain of *S.aureus* (MTCC-3103) was procured from MTCC Chandigarh. All the isolates were confirmed through biochemical tests by comparing with the results of standard strains. Collection of samples: Different samples of milk and milk products were collected from different regions of Agra city including Dayalbagh, Sikandra, Ram Bagh and Sultan Pura and examined for the presence of *S.aureus*. Raw milk samples were collected into sterile test tubes from individual cows and buffaloes while the milk products were collected aseptically, transferred to sterile plastic bags and directly transported to the laboratory under cold conditions and analyzed within 24 hrs.

Biochemical 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 peptone water and incubated at 37 °C for 24 hrs, for further biochemical analysis. *S.aureus* was isolated by using the Baird Parker's (1962) technique. Enriched samples were streaked on Baird Parker Agar (BPA) and the plate was incubated at 37 °C for 24–48 hrs. Appearance of jet black colonies surrounded by a white halo were considered to be presumptive *S.aureus* and were further analyzed by Gram's reaction and biochemical tests (Table 1).

Confirmation of the genus, *Staphylococcus* was done by Gram staining and biochemical tests-catalase test, oxidase test, indole, methyl red, Voges-Proskauer

Table 1: Biochemical characterization of *S.aureus* strains
Preglednica 1: Biokemijska karakterizacija sevov *S. aureus*

Reaction	<i>S.aureus</i>
Catalase	+
Oxidase	-
Indole Production	-
Nitrate Reduction	+
Methyl Red	+
Voges-Proskauer	+
Haemolysis	+
Coagulase	+
Acid from shugar	
Glucose	+
Mannitol	+
Maltose	+
Lactose	+
Raffinose	-
Sucrose	+

+ = Positive reaction / Pozitivna reakcija;
- = Negative reaction / Negativna reakcija

test, nitrate reduction, acid from different sugars while the species, *S.aureus* was confirmed by the coagulase test (Monica 1991). Pathogenicity of *S.aureus* was confirmed by beta haemolysis on Sheep Blood Agar (S.B.A.) following the method of Cruickshank (1970).

3 RESULTS

Samples were collected in different regions of Agra city. Table 2 depicts the sampling data, which showed the percentage of prevalence of pathogenic *S.aureus* isolated from the various samples of cow milk, buffalo milk, khoa sweets and cottage cheese collected from different unorganized sectors of Agra city. A total of 64 cow milk samples, 52 buffalo milk samples, 71 cottage cheese samples and 69 khoa sweet samples were analyzed for the isolation of coagulase positive pathogenic *S.aureus*, collected from different sites of Agra city including Ram Bagh (East), Sikandra (West), Dayalbagh (North) and Sultan Pura (South) (Fig. 1).

Cow milk samples: Out of 64 cow milk samples, 42 isolates were confirmed as coagulase positive *S.aureus* and from these 25 were confirmed as pathogenic *S.aureus*.

Buffalo milk samples: Out of 52 buffalo milk samples, 33 isolates were confirmed as coagulase positive *S.aureus* of which 15 were confirmed as pathogenic *S.aureus*.

Cottage cheese samples: Out of 71 cottage cheese samples, 43 isolates were confirmed as coagulase positive *S.aureus* of which 9 were confirmed as pathogenic *S.aureus*.

Khoa sweets samples: Out of 64 khoa sweets samples, 47 isolates were confirmed as coagulase positive *S.aureus* and from these 13 were confirmed as pathogenic *S.aureus*.

Data analysis showed that *S.aureus* contamination was higher in samples of cow milk, cottage cheese and khoa sweets collected from the eastern part (Ram Bagh) of Agra city. Buffalo milk samples collected from the in western part (Sikandra) of Agra city showed a higher contamination. Khoa and cottage cheese samples from



Figure 1: Map of Agra showing different sites of sampling.
Slika 1: Zemljevid z mesti vzorčenja.

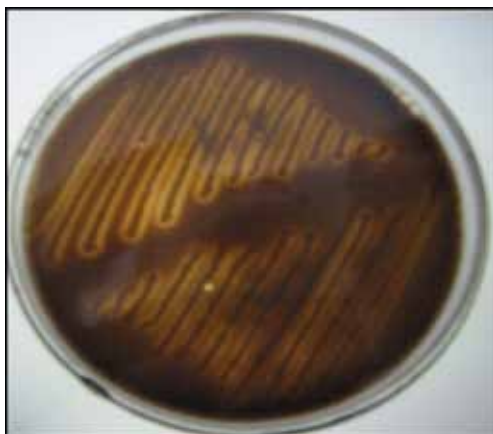


Figure 2: Plate showing beta haemolysis on 5% sheep blood agar.
Slika 2: Test hemolizne aktivnosti na 5 % krvnem agarju iz ovčje krvi

Sikandra showed the least contamination. Raw milk samples both from cow and buffalo procured from Dayalbagh and Ram Bagh were least contaminated. (Fig. 3, Table 2)

4 DISCUSSION

Milk is normally sterile in the udder of the cow and buffalo provided they do not suffer from mastitis (udder infection). If they have mastitis, a large number of generally Gram positive bacteria such as *Streptococcus* and *Staphylococcus* sps. may be present in milk when it leaves the udder (Holm and Jespersen, 2003). Further, milk may be often contaminated on its way to the bulk tank as rarely the hygienic procedure is followed by the local dairy. Khoa is made from the thickening of the milk at high temperature, which expectedly kills all the bacteria incriminated during handling, storage and transportation.

Sweets made from khoa may be kept for days or weeks and may then be contaminated with the bacteria. On the other hand, cottage cheese in industries is manufactured by automated process, so the chances of contamination are fewer. In villages and unorganized sectors cheese is prepared by traditional methods that increases the probability of contamination during the processing and may cause the transfer of undesirable microorganisms in the end products. Proper hygiene and safe food practices are essential to prevent the presence of organisms such as *Listeria monocytogenes* and *S.aureus* in the milk products.

Negligence of hygienic condition such as improper cleaning of bulk tank, dirty udders, milking equipments, cooking temperature, milk handling technique and improper storage will increase the proportion of Gram-positive and Gram-negative bacteria in the bulk tank milk (Vasavada, 1988; Bonfoh *et al.*, 2003).

These parameters play an important role in determining the characteristics of milk and milk products, which are one of the major sources of protein in a vegetarian's diet. If these foods are contaminated with *S.aureus*, they mean a serious health problem. Raw milk is used to collect cream and this cream is further used to produce various value added products, however, this cream may serve as a major vehicle for transmission of pathogens. The analyzed raw milk samples both from buffalo and cow were contaminated with pathogenic *S.aureus*, therefore, the presence of pathogenic bacteria in milk becomes a major concern to public health, its consumption, or use in the production of dairy products such as sweets, cheese, butter, cream and ice-cream, without pasteurization or subjection to a high temperature for sufficient time may lead to various health problems among masses. As Agra is the city where innumerable tourists flock from different parts of India as well as the world, therefore it can be a serious problem to the local consumers and the

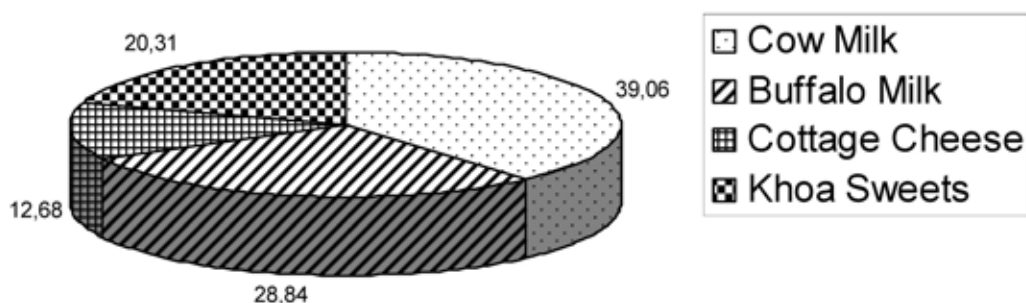


Figure 3: Percentage of prevalence of *S.aureus* in milk and milk products.
Slika 3: Pogostnost pojavljanja *S. aureus* v mleku in mlečnih izdelkih.

Table 2: Percentage of incidence of *S.aureus* in different areas of Agra city**Preglednica 2:** Pogostnost pojavljanja *S. aureus* na različnih območjih mesta Agra

Source	Area	Total No. of isolates analyzed	No. of isolates confirmed as <i>S.aureus</i>	No. of isolates confirmed to be pathogenic <i>S.aureus</i>	Percentage of isolates confirmed to be pathogenic
Cow Milk	Dayalbagh	20	10	2	10
	Sikandra	8	7	4	50
	Sultan Pura	24	21	12	50
	Ram Bagh	12	11	7	58
Buffalo Milk	Dayalbagh	20	12	5	25
	Sikandra	4	4	4	100
	Sultan Pura	16	8	4	25
	Ram Bagh	12	9	2	16
Cottage cheese	Dayalbagh	21	9	2	9.5
	Sikandra	20	12	0	0
	Sultan Pura	19	13	4	21
	Ram Bagh	11	9	3	27
Khoa sweets	Dayalbagh	13	8	2	15.3
	Sikandra	8	3	0	0
	Sultan Pura	20	14	2	10
	Ram Bagh	28	22	9	32

tourists when the food is incriminated with the pathogenic *S.aureus*.

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