

Original Research Article

<https://doi.org/10.20546/ijcmas.2017.609.084>

## Prevalence of *Staphylococcus aureus* and Methicillin Resistant *Staphylococcus aureus* from Milk and Milk Products in and around Srinagar Area of Kashmir, India

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### ABSTRACT

The present study was focused on to determine the presence of *S. aureus* in milk and milk products and also to determine the resistance against methicillin. During this study it was found that there was high presence of *S. aureus* as well as MRSA in milk and milk products (curd, cheese and ice-cream). Out of 130 isolates only 60 (46.15%) samples were positive for *S. aureus* of which, 29 (22.30%) were phenotypically MRSA. *S. aureus* prevalence in milk was 15/25 (60%), 13/25 (52%) and 6/10 (60%) in cow, buffalo and goat respectively. Out of 34 *S. aureus* milk isolates, 15(44.1%) were MRSA positive. Prevalence of *S. aureus* in curd was 12/30 (40%), in cheese was 6/25 (24%) and in ice-cream was 8/15 (53.33%). The presence of *Staphylococcus aureus* strains in milk and milk products needs special attention towards public health hazards.

#### Keywords

Prevalence, Milk, *Staphylococcus aureus*, MRSA.

#### Article Info

##### Accepted:

14 July 2017

##### Available Online:

10 September 2017

### Introduction

Raw milk is an ideal growth medium for several microorganisms. Milk and its derivatives are considered vehicles of *Staphylococcus aureus* for infection in humans (Zecconi and Hahn, 2000). *S. aureus* is an important foodborne pathogen and causes a wide variety of diseases in humans and animals, ranging in severity from a mild skin infection to more severe diseases, such as pneumonia and septicemia (Lowy, 1998). In dairy cattle, *S. aureus* is frequently associated

with subclinical mastitis (Adesiyun *et al.*, 1998) and may contaminate milk and other dairy products (Capurro *et al.*, 1999).

*S. aureus* produces several staphylococcal virulence factors, including enterotoxins (SEA to SEE and SEG to SEQ), and other toxins, such as exfoliative toxin A and B, and toxic shock syndrome toxin (TSST-1) (Fagundes and Oliveira, 2004). Staphylococcal food poisoning (SFP) is

recognized as a cause of foodborne diseases (Jorgensen *et al.*, 2005).

Mammary glands infected by *S. aureus* are the main cause of milk contamination (Jayarao *et al.*, 2004). Contaminated milking equipment and the hands of the milkers are also common vehicles of transmission (Cullor, 1997). Although pasteurization kills *S. aureus* cells, thermostable SEs generally retain their biological activity. SFP risk related to pasteurized milk products may be minimized by adequate chilling of raw milk until heat treatment, followed by rigorous efforts to prevent recontamination (Jorgensen *et al.*, 2005). The aim of present study was to investigate the incidence of *S. aureus* and presence of enterotoxins in strains isolated in raw milk.

## Materials and Methods

The research work was carried out in the Division of Veterinary Microbiology, Faculty of Veterinary Sciences and Animal Husbandry, SKUAST, Jammu, Jammu and Kashmir. The period of study was from February 2017-April 2017(3 months). The milk samples were collected aseptically in pre-sterilized test tubes and other glassware and transported over ice within 2 hours of collection to the division for further processing and analysis.

Each sample was enriched in peptone water and incubated at 37<sup>0</sup>C for 24 hrs. Each inoculum was cultured on Baird Parker agar (selective medium for *Staphylococcus aureus*) and incubated at 37<sup>0</sup>C for 48 hrs. Then a single isolated colony was picked and streaked on mannitol salt agar and incubated at 37<sup>0</sup>C for 24-48 hrs and further the organisms were identified on the basis of their cultural, morphological, staining and various biochemical characteristics. All positive samples were subjected to coagulase test for

confirmation of *S. aureus* as described by Monica, 1991. Biochemical characterization of the presumptive *S. aureus* isolates was performed as described by Bennett and Lancette (2001). Thus, confirmed *S. aureus* isolates were inoculated and cultured on Oxacillin resistance screening agar (ORSA) for detection of Methicillin Resistant *S. aureus* (Pereira *et al.*, 2009).

## Processing of samples for *S. aureus* and MRSA

The isolation and identification of *S. aureus* isolates was carried by enrichment of samples in peptone water followed by incubation at 37<sup>0</sup>C for 24 hrs. Streaking of enriched inoculum on Baird-Parker (selective medium) and Mannitol Salt Agar followed by incubation at 37<sup>0</sup>C for 24-48 hrs. The presumptive pale-yellow colored friable colonies of *S. aureus* were inoculated in Brain Heart Infusion Broth and incubated at 37<sup>0</sup>C for 18-24hrs and stored in refrigerator for further processing. Biochemical identification of presumed *S. aureus* isolates. The confirmed *S. aureus* isolates were subjected to Disc diffusion antibiotic sensitivity test. The confirmed *S. aureus* isolates were inoculated and cultured on Oxacillin resistance screening agar (ORSA) for detection of Methicillin Resistance *S. aureus*. The presumptive blue colored colonies of MRSA on Oxacillin resistance screening agar were further inoculated in MRSA alert test kit for Methicillin Resistant *S. aureus* confirmation.

## Characterization of *Staphylococcus* isolates

### Gram's staining

All the presumed cultures of *Staphylococcus* spp. were subjected to Gram's staining and observed under a light microscope for Gram's reaction, size, shape and cell arrangements. The Gram-stained smears from typical

colonies that showed Gram-positive cocci occurring in bunched grape like irregular clusters were taken as presumptive *Staphylococcus* species (Figure 1).

### **Tube coagulase test**

3 ml sterile distilled water was added aseptically to rehydrate 0.1gm of rabbit coagulase plasma. 0.5 ml of the rehydrated content was added to 0.05ml overnight broth culture of *S.aureus*. The tubes were incubated at 37<sup>0</sup>C for 4-6 hours during which these were observed for clot formation which was taken as positive reaction for *S. aureus*.

### **Biochemical characterization**

All the isolates were further subjected to series of biochemical tests which included Indole test, Methyl red test, Voges-Proskauer test and Citrate utilization test.

### **Indole test**

This test was performed to demonstrate the ability to decompose the amino-acid tryptophan to indole. The test was performed by inoculating the test culture in 2 percent tryptone water, incubated at 37<sup>0</sup>C for 48 to 96 hours. Kovac's reagent 0.5 ml was added to the culture medium and shaken gently. Appearance of Red colour indicated positive reaction.

### **Methyl red test**

The test was performed by inoculating glucose phosphate broth with test organism and incubated at 37<sup>0</sup>C

### **Voges- Proskauer test**

The bacteria that ferment carbohydrates with the production of acetyl methyl carbinol or its reduction product 2, 3, butylene glycol can be

tested by a colorimetric reaction between diacetyl (which is formed during the test by oxidation of acetyl methyl carbinol or 2, 3, butylene glycol) and a guanidine group. 5 ml of glucose phosphate peptone water (MR-VP Broth) Hi-Media Pvt. Ltd. was inoculated with isolated organism and incubated at 37<sup>0</sup>C for 48 hours. Then 1 ml of 40 percent potassium hydroxide and 3 ml of 5 percent  $\alpha$ -naphthol in absolute ethyl alcohol were added. Change in colour to red indicated a positive reaction.

### **Citrate utilization test**

Simmons's citrate medium was used to detect ability of the organism to utilize citrate as the sole source of carbon and energy for growth and an ammonium salt as the sole source of nitrogen. The test was carried out by inoculating Simmon's citrate media with test organism and incubating at 37<sup>0</sup>C up to 48-96 hrs. Change in green solid slant to blue indicated a positive reaction for *Staphylococcus*.

### **Catalase test**

On a single bacterial colony grown over nutrient agar plate, a loopful of 3 percent H<sub>2</sub>O<sub>2</sub> was added. Prompt effervescence indicated catalase production confirming the test.

### **Oxidase test**

In order to carry out this test oxidase discs were used. A loopful of colonies was rubbed over the moistened discs. No change in colour indicated negative reaction for *Staphylococcus* spp.

### **MRSA detection**

The confirmed *Staphylococcus aureus* isolates were further inoculated on to Oxacillin Resistance Screening Agar. The medium

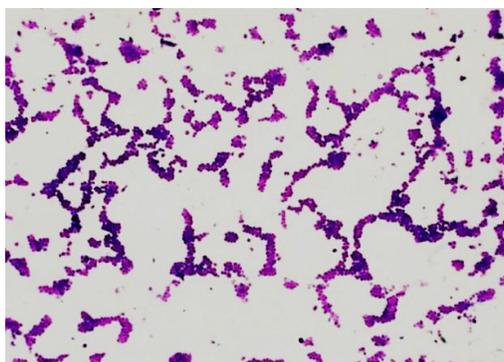
incorporates an optimized salt level, lithium chloride and polymyxin B to suppress competing flora; aniline blue as an indicator dye for mannitol fermentation and oxacillin at a level of 2 mg/l. The colonies that were blue in color were taken as presumptive MRSA (Figure 2).

### **Results and Discussion**

Prevalence of MRSA in raw milk and milk products *vis-a-vis* the emerging threat of *S. aureus* and MRSA. Thus, more prevalence studies are required to generate data about occurrence *S. aureus* and MRSA in raw milk and milk products. Hence, a study of problem of *S. aureus* including MRSA with respect to its presence in raw milk and milk products was carried. One hundred ten (130) samples from milk and milk products were analysed.

These included 60 samples each from raw milk (cow-25, buffalo-25 and goat-10), 30 samples from curd, 25 samples from cheese and 15 samples from ice-cream. The samples were processed to reveal the prevalence of *S. aureus* and methicillin resistance using various techniques. Sixty (60) isolates of *S. aureus* were obtained and identified on the basis of cultural, morphological and biochemical characters. The isolates thus obtained were further processed to detect the prevalence of Methicillin resistant *S. aureus* (MRSA). The overall occurrence of *S. aureus* in milk and milk products was 46.15% with 60%, 60% and 52 % in raw milk of cow, goat and buffalo respectively and 53.3%, 40% and 24% in the processed milk and milk products like ice-cream, curd and cheese respectively. The presence of MRSA in cow milk was 8/15 (32 %).

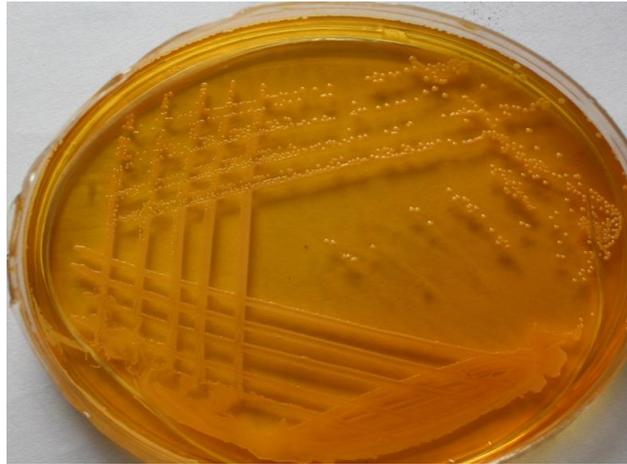
**Fig.1** Gram's staining showing characteristic violet colored grapes bunch like appearance



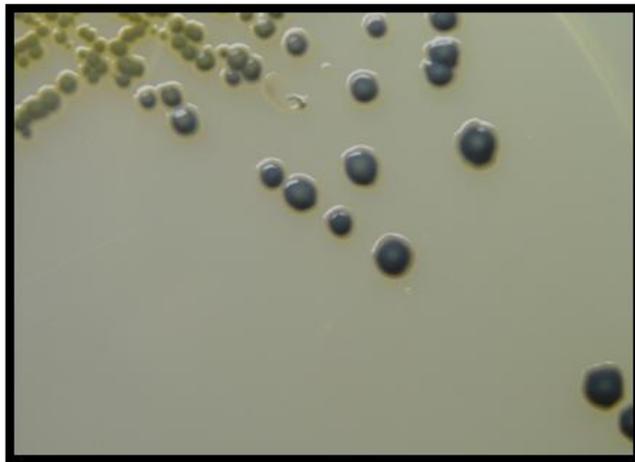
**Fig.2** Characteristic deep blue colonies of Methicillin Resistant *S. aureus* (MRSA) on Oxacillin resistance screening agar media



**Fig.3** Characteristic yellow colored, friable colonies of *S. aureus* on Mannitol salt agar media



**Fig.4** Jet black colonies of *S. aureus* surrounded by a light halo on Baird-Parker agar media



**Fig.5** Characteristic effervescence shown by presumptive *S. aureus* colonies on catalase test



The buffalo and goat milk however, recorded much less per cent of MRSA. The buffalo milk showed 6/13 (24%) and goat milk 1/6 (10%) MRSA. The presence of MRSA in curd samples and cheese samples was recorded as 7/12 and 4/6 respectively (16%) whereas 3/8(20%) in ice-cream samples.

The bacteriological quality of the samples was assessed by standard methods. Isolation and identification of *S. aureus* was carried out using specific biochemical media. All the isolates fermented mannitol, showed growth on Baird-Parker agar media (Figures 3 and 4). The isolates were catalase positive (Figure 5), oxidase negative, indole positive, MR-VP positive and coagulase positive following the methods as described by Monica (1991), the findings corresponded well with the findings of Singh and Prakash (2008). Coagulase production has generally been regarded as a specific property of pathogenic *Staphylococci*; the organisms may sometimes lose coagulase production ability due to various reasons (Baird-Parker, 1965). The work of health inspection in municipalities is crucial to raise consumer awareness on the consumption of good-quality milk through educational programs and the inspection of establishments that insist on selling raw milk. Dairy inspection and the adoption of good fabrication practices can also contribute to a reduction in contamination of pasteurized milk.

The counts of *Staphylococcus aureus* in milk analyzed indicate the product is improper for human consumption, and the population of Srinagar is vulnerable to toxic infections. The presence of *Staphylococcus aureus* in raw and pasteurized milk underlines the importance of preventive measures to ensure the quality of the milk from milking through to the processing in the dairy industry, in order to avoid the occurrence of pathogenic microorganisms and consequently to prevent

risks of transport of toxic infections by the consumption of contaminated milk.

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**How to cite this article:**

Ridwana, G.A. Badroo, Ajaz Ahmad, U. Rasool and Fayaz, S. 2017. Prevalence of *Staphylococcus aureus* and Methicillin Resistant *Staphylococcus aureus* from Milk and Milk Products in and around Srinagar Area of Kashmir, India. *Int.J.Curr.Microbiol.App.Sci.* 6(9): 690-696. doi: <https://doi.org/10.20546/ijcmas.2017.609.084>