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# Investigations on Methicillin-resistant *Staphylococcus aureus* (MRSA) isolation and identification from milk and environmental sources

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## Abstract
The objective of the current investigation was to determine the occurrence of Methicillin Resistant *Staphylococcus aureus* from Milk and Environmental sources during 2022-2023. 500 samples altogether were collected comprising raw milk, mastitic milk, pasteurised milk, animal nasal swabs, animal rectal swabs, teat skin swabs, animal farm environment, household environment, nosocomial infections and human clinical samples and were screened for the presence of *Staphylococcus aureus.* 233 samples turned out to be positive resulting in an overall occurrence of 46.6%. Of the 500 isolates, 37(74%), 38(76%), 09(18%), 33(66%), 12(24%), 28(56%), 24(48%), 23(46%), 14(28%), 17(34%) were isolated from raw milk, mastitic milk, pasteurised milk, animal nasal swabs, animal rectal swabs, teat skin swabs, animal farm environment, household environment, nosocomial infections and human clinical samples respectively. *S. aureus* isolates obtained were subjected to *in vitro* antibiotic sensitivity assay to ascertain the number of MRSA isolates resulting in 100% of the methicillin resistant *S. aureus* isolates. The above investigation revealed the presence of MRSA in our territory thus posing a serious threat to the public health safety.

**Keywords:** *Staphylococcus aureus*, occurrence, milk, public health, environment

## Introduction
*Staphylococcus aureus* recovered from milk and environment decipher an emergent hazard cornerstone policy in public health and food chain. Checkerboard contamination of raw and processed milk and dairy series with forbidden foodborne pathogens and their toxins and enzymes like biofilm producing and multidrug-resistant *S. aureus* represent risk policy in our food chain and so on proceeds to our lifestyle (FDA, 2020; CDCs, 2020) [15]. According to Chua *et al.* (2014) [5], *Staphylococcus aureus* is primarily pathogenic due to a combination of genetic factors mediating virulence, invasiveness, host immune system evasion, and antibiotic resistance. This bacterium has been linked to numerous diseases in both humans and animals. One of the most significant infectious mastitis pathogens in dairy cattle is *Staphylococcus aureus*. Controlling *S. aureus* is important for both public health and economic reasons in the dairy business because of its zoonotic potential (Kummel *et al.*, 2016) [23]. Milk and milk products, particularly those made from raw milk under unsanitary conditions, have the potential to spread foodborne pathogens such strains of S. aureus that are resistant to antibiotics (Kadariya *et al.*, 2014) [22]. When compared to methicillin-sensitive S. *aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA) related bacteremia is linked to higher mortality, morbidity, and healthcare expenses. (Cosgrove, 2003, 2005, and Shurland, 2007) [6, 7, 35].

Gram-positive, catalase-positive, and bacitracin-resistant *Staphylococcus aureus* (S. *aureus*) is a prevalent colonizer of the human body. According to Shaw *et al.* (1951) [34] and Tong *et al.* (2015) [40], these bacteria are frequently found on mucosal surfaces (such as the nares, the throat, and the rectum) and moist skin areas like the axilla, groin, and perineum. Over 50 species of the *Staphylococcus* genus have been described until now (Pyorala, and Taponen, 2009) [31]. Pathogens like *Staphylococcus aureus* are linked to significant hospital- and community-acquired illnesses. It is abundant in nature and has minimal dietary needs (Wu *et al.*, 2018) [24]. This foodborne virus, which is responsible for a wide range of symptoms and illnesses, is regarded as one of the top global causes of disease outbreaks linked to food intake.

According to Scallan *et al.* (2011) [33] and Kadariya *et al.* (2014) [22], *S. aureus* is responsible for over 241,000 instances of food poisoning each year in the US. Methicillin-resistant *S. aureus* (MRSA) is clinically the most significant pathogen because of its frequent ability to resist antibiotics which makes S. *aureus* infections particularly difficult. MRSA infections had greater rates of hospitalization, mortality, and morbidity as compared to infections brought on by methicillin-sensitive S. aureus (MSSA) (Ippolito *et al.*, 2010) [21]. According to Stefani *et al.* (2012) [37], methicillin resistance rates among clinical isolates vary substantially by nation, ranging from single-digit percentages in Scandinavian nations to over 50%, for instance, in the U.S. and China. The skin and subcutaneous tissues are the most frequently affected by MRSA-related infections, which are followed by invasive illnesses like osteomyelitis, meningitis, pneumonia, lung abscess and empyema. MRSA-induced infectious endocarditis is connected to intravenous drug misuse and has higher morbidity and fatality rates than endocarditis produced by any other bacterium.

Due to its abundant availability of important nutrients and high water content, milk offers a favourable environment for a variety of microorganisms. According to Viljoen (2001) [43], bacteria, yeast, and mold are frequently found as contaminants in milk. Some microbes can contribute to the spoiling and degradation of milk and dairy products (Eneroth *et al.*, 2000) [12]. As a result, bacteria in milk have a substantial impact on its quality and can be harmful to both the dairy industry and the general public's health (Li *et al.*, 2018) [24]. The primary paths for raw milk contamination are direct contact with contaminated materials in the dairy environment, such as dirt, excrement, feed, water, air, milking equipment, sick animals, as well as secretions from infected animals' udders (Mcauley *et al.*, 2014) [26]. The microbial ecology of dairy farms is quite complex, and there are many challenging situations there. As a result, the dairy environment is home to a variety of harmful bacteria (Oliver *et al.*, 2005). Pathogens are often rendered inactive during the pasteurization process. However, the post-processing of raw milk in dairy processing factories does not completely eradicate pathogen contamination of the milk or dairy products. According to Mcauley *et al.* (2014) [26], such contamination could make customers sick from eating. Considering the extreme importance of *S. aureus* as a potential public health threat, the current investigation was performed to assess the occurrence of *S. aureus* from milk and environment sources.

## Materials and Methods

### *S. aureus* sampling and isolation

A total of 500 samples were collectively taken from milk and environmental sources (50 samples from each source) including raw milk, mastitic milk, pasteurised milk, animal nasal swabs, animal rectal swabs, teat skin swabs, animal farm environment, household environment, nosocomial infections and human clinical samples (pus, burn and wound samples). Raw and mastitic milk samples, animal nasal swabs, animal rectal swabs, teat skin swabs and animal farm environment samples were collected from local farmers of Srinagar and Ganderbal and Mountain Livestock Research Institute, Mansbal, Ganderbal. Whereas the pasteurised milk samples were collected from different shops in different areas of Srinagar and Ganderbal between the years 2022-2023. Samples from Animal farm environment included milkers' hands, water samples, cow shed area and paddock area. The samples were subsequently brought to the Division of Veterinary Public Health's laboratory in a refrigeration box filled with ice.

According to Yambise *et al.*, 2020 [44], the samples were treated right away for the isolation and identification of *S. aureus.* According to Palilu and Budiarso (2017) [30], each collected sample was cultured in 90 mL of BHI (Brain Heart Infusion) broth after being taken (10 ml) and incubated for 16-18 hours. The incubated sample (1 ml) was then diluted into a solution with a concentration ranging from 10-1 to 10-6 using 9 ml of 0.1% peptone water, and it was then homogenized using a vortex. The surface of the BPA (Baird Parker Agar) medium was inoculated with a diluted sample (0.1 ml) from concentrations of 10-4, 10-5, and 10-6, and then incubated for 48 hours at 37 °C. In BPA (Baird Parker Agar) medium, suspected Staphylococcus colonies were expressed as dark grey to shiny black coloured colonies. These putative colonies were purified by taking the individual colonies and utilizing the streak plate technique to inoculate them in BPA (Baird Parker Agar) medium to obtain the single colony. After streaking into MSA (Mannitol Salt Agar) media, this single colony of probable *Staphylococcus* was cultured for 24 hours. The single isolate was obtained by separating the colony expressed in yellow colour into a different BPA (Baird Parker Agar) medium. Finally, this isolate was cultured on Nutrient Agar and obtained as a possible *S. aureus* isolate on subjecting to Gram's staining the characteristic Gram-positive organisms were isolated with the characteristic cocci arranged in bunches (Plate 3). The isolates were further subjected to drug sensitivity testing against methicillin to determine the resistance pattern of all the isolates.

### Statistical analysis

The study's final results were analyzed using the Graph Pad Prism 5.0 program, and the Chi-square test was employed for the analytical evaluation.

## Results and Discussion

### Occurrence of *Staphylococcus aureus* from milk and environmental sources

A total of 500 samples were collected from milk and environmental sources in Srinagar and Ganderbal districts of J&K, India in which 233 samples turned out to be positive for *S. aureus* resulting in an overall occurrence of 46.60%. The samples included raw milk, mastitic milk, pasteurised milk, animal nasal swabs, animal rectal swabs, teat skin swabs, animal farm environment, household environment, nosocomial infections and human clinical samples. 37(74%), 38(76%), 09(18%), 33(66%), 11(22%), 27(54%), 24(48%), 23(46%), 14(28%) and 17(34%) isolates were recovered from the above samples respectively. The highest prevalence of *S. aureus* was recorded in mastitic milk (76%) whereas the contamination was comparatively least in pasteurized milk (24%). The highest percentage of S. aureus was found in swabs taken from milkers' hands (45.83%), where 11 isolates were found. These samples were followed by samples from the paddock and water, where 7 (29.16%) and 6 (25%) isolates were found, respectively.

The findings of the current research are in line with the conclusions of Taj *et al.*, (2012) [39], Abebe *et al.*, (2016) [1] Dweba *et al.*, (2019) [10] and El Faramaway *et al.*, (2019) [11] who reported an occurrence of 54.78%, 51.26%, 53.8% and 46.5% respectively. Islam *et al.*, 2016 reported a higher

prevalence of 75% of *Staphylococcus aureus* samples which indicates a potential threat of *Staphylococcus aureus* in the environment and milk samples. The increased presence of *S. aureus* in raw milk (74%) may pose a risk to the dairy industry and the dairy workers globally. It may subsequently act as a source of contamination of various dairy products, particularly unpasteurized milk. *S. aureus* is one of the primary causes of udder infection in dairy cows. It also has a key role in the emergence of intramammary infections (IMI) and the emergence of clinical and subclinical mastitis associated with an increase in somatic cell number (SCC).The highest prevalence of *S. aureus* in Mastitic milk (76%) may be due to Hygienic and management factors (including breeds, size of the farm, absence of teat disinfection before and after milking, lack of diagnostic facilities for the detection of subclinical and chronic mastitis, absence of dry cow therapy, and procedure followed during hand milking) in the studied dairy farms. Lastly, risk factors for MRSA infection and colonization include the overuse of antibiotics, extended hospital stays, particularly in ICUs, intravascular catheterization, and immune-compromised states (Enright, 2003; Hidron *et al.*, 2005) [13, 18].

## Conclusion

The present study has revealed that the public in Srinagar city of Kashmir valley are exposed to the potential hazard of the MRSA as their presence has been demonstrated in milk and the environment. Hence there is a need for several interventions to reduce the MRSA burden. This research also highlights the need of implementing strict hygiene and sanitation standards in all the household environments of Srinagar city followed by proper cleaning and disinfection. There is a need to strictly monitor the animal Farm environment to ensure proper sanitation among animal workers and milkers. The animals should be regularly monitored for any disease conditions. The farm premises should be kept clean and regular disinfection and sanitization should be followed. Many academics have focused on the financial damages that mastitis causes to the dairy sector, which justifies the continuous interest in this field.

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## Ethics approval

Following approval from the Institutional Review Bureau, GMC Srinagar, this study was carried out in the faculty of F.V.Sc & A.H., SKUAST K.

## Competing interests

There are no competing interests, according to the authors.

## Authors' contributions

The first author completed this work. The study's analyses were overseen by the authors, who also provided direction for the research.

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## Disclaimer

The author's views and opinions are those of the article, not necessarily the official viewpoint or policy of any organization to which the authors are affiliated.

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