Characterisation and Antimicrobial Resistance Profiling of *Staphylococcus aureus* from Raw Milk and Milk products sold in Puducherry, India

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**Abstract**

A study was carried out with aim to isolate *Staphylococcus aureus* from raw milk and milk products (Ice cream and Pedha) and also to determine antimicrobial profiling pattern of *S. aureus* isolates from raw milk and milk products sold in Puducherry. A total of 60 raw milk and milk product samples (ice cream and Pedha) were collected from different places in and around Puducherry city such as milk collection centre of Co-operative milk dairies, individual household, milk vendors and sweet shops. The samples were collected under aseptic precautions and were enriched in Peptone Water (PW) followed by direct plating on selective media. The isolates were identified as per the standard microbiological procedure. Antimicrobial resistance profiling pattern of isolates was done by using common antimicrobial agents by disk diffusion method. The result revealed that out of total 60 samples of milk (40) and milk products i.e. ice cream (10) and Pedha (10) resulted in the isolation of 21 isolates (35.00 %) of *S. aureus*. In the present study *S. aureus* isolates were found variably resistant to the antibiotics tested. The isolates showed highest 100% sensitivity towards cephalothin, co-trimoxazole, cephalexin, tetracycline and erythromycin followed 90.00 %, 80.00 %, 70.00 %, 60.00 % by clindamycin, ciprofloxacin, oxacillin and streptomycin respectively. The pattern clearly indicated that the overall high percent of *S. aureus* isolates were 100.00 % resistant to Penicillin-G, metronidazole, methicillin, cephalixin, ampicillin and oxytetracycline followed by intermediate sensitivity with, ciprofloxacin (20.00%) and carbenicillin (10.00 %). Results clearly indicated a possibility of potential public health threat of *S. aureus* resulting from contamination of milk and milk products with pathogenic bacteria is mainly due to unhygienic processing, handling and unhygienic environment.

**Key words**: antimicrobial profiling pattern, raw milk, milk products, Staphylococcal food poisoning

**Introduction**

Food-borne diseases (FBD) are defined by the World Health Organization as diseases of infectious or toxic nature caused by, or thought to be caused by the consumption of food or water. The pathogenesis of bacteria causing food-borne poisoning depends on their capacity to produce toxins after ingestion (in the digestive tract) or intoxication (ingestion of preformed toxins in foodstuff). Among the bacteria predominantly involved in these diseases, *Staphylococcus aureus* is a leading cause of gastroenteritis resulting from the consumption of contaminated food. Staphylococcal food poisoning is due to the absorption of Staphylococcal enterotoxins preformed in the food.

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Loir et al. (2003) and Chapaval et al. (2010) found that staphylococcal enterotoxins are produced in milk when milk is stored at temperatures of 37°C to 42°C or exposed to variations in temperature. When milk is heated at normal cooking temperatures, the bacteria can be killed, but the toxins remain active (Presscott et al., 2002). Staphylococcal Illness caused by S. aureus ranges from minor skin infections such as pimples, boils, cellulitis, toxic shock syndrome, impetigo, and abscesses to life-threatening diseases such as pneumonia, meningitis, endocarditis, and sepsis (Soomro et al., 2003). Especially in India, the rate of infection is still higher due to warm and humid climates (Bhatia and Zahoor, 2007). For many years, S. aureus was the only staphylococcal species known to produce enterotoxins (Haluk et al., 2010).

Recently, this organism has acquired resistance to different antibiotics and developed resistance to antimicrobial agents. Staphylococci are frequently associated with hospital and community-acquired infections (Chowdhry et al., 2013). Strains of S. aureus resistant to β-lactam antibiotics are known as methicillin-resistant S. aureus (MRSA). It is also called multidrug-resistant (MDR) S. aureus. The emergence of Multi-drug resistant bacteria is a major problem for treatment of diseases using antibiotics. In recent years it has been reported that the clinical administration of antibiotics against the pathogenic bacteria is gradually prohibited due to emergence of MDR bacterial strains including S. aureus. (Kumar et al., 2010; Akindele et al., 2010; Hoerlle and Brandelli, 2010, Efuntayo et al., 2011). Thus, the objective of this study was to investigate the occurrence of S. aureus in milk and milk products (Ice cream and Pedha) and determine antimicrobial profiling pattern of S. aureus isolates.

Materials and Methods
Sample Collection

From February 2015 to April 2015, a total of 60 samples, which includes raw milk (40 samples), ice cream (10 samples) and Pedha (10 samples) were collected from different places in and around Puducherry city. The majority of samples were from collection centres of Cooperative milk dairies, individual household, milk vendors and sweet shops. The samples were collected in sterilized milk collecting tubes and polyethylene bags and transported in an icebox to department laboratory for further processing and microbiological analysis. All the agar, chemical reagents and antibiotic disk used in this study were procured from HiMedia Pvt. Ltd Mumbai.

Isolation and Identification of Staphylococcus aureus

Isolation of S. aureus was attempted according to Deepa et al. (2015), with slight modification. The 10 ml or 10 g of sample was homogenized with 90 ml sterile enrichment peptone water and enriched for 24 hrs at 37°C. The selective medium used for isolation of S. aureus was Baird Parker Agar (BPA). A loopful of inoculum from enrichment were streaked on Baird Parker agar and incubated for 28 to 48 hours at 37°C.
Characteristic appearance of jet black colonies surrounded by a white halo were considered to be presumptive *S. aureus*. The colonies were then gram's stained and pure cultures was streaked on Nutrient agar and incubated for 24 hours at 37°C and were further characterized by biochemical tests like Catalase test, Coagulase test, DNase test, Oxidase test and D-mannitol fermentation.

**Antimicrobial Profiling Pattern**

The isolates were subjected for some common antimicrobial agents was done by disk diffusion method using commercial disks. The antimicrobial agents tested were the following: ampicillin (10µg), cephalothin (30µg), co-trimoxazole (25µg), cephalexin (30 µg), ciprofloxacin (5µg), gentamicin (10µg), methicillin (5µg), oxy-tetracycline (30µg), oxacillin (1µg), penicillin-G (10units), streptomycin (10µg), tetracycline(30µg), metronidazole (5µg), carbenicillin (100µg) and cepoxitin (30 µg) respectively.

**Results**

The results indicated that out of total 60 samples of milk (40) and milk products i.e. ice cream (10) and Pedha (10) resulted in the isolation of 21 isolates (35.00 %) of *S. aureus*. Of the 40 milk samples, 10 ice cream and 10 Pedha samples, 10 (25.00%), 7 (70.00%) and 4 (40.00 %) respectively were found to be positive for *S. aureus* according to standard microbiological procedure. In the present study, *S. aureus* isolates were found variably resistant to the antibiotics tested. The *S. aureus* isolates were found variably resistant to the antibiotics tested. The isolates showed highest 100% sensitivity towards cephalothin, co-trimoxazole, cephalexin, tetracycline and erythromycin followed 90.00 %, 80.00 %, 70.00 %, 60.00 % by clindamycin, ciprofloxacin, oxacillin and streptomycin respectively. The pattern clearly indicated that the overall high percent of *S. aureus* isolates were 100.00 % resistant to Penicillin-G, metronidazole, methicillin, cephoxitin, ampicillin and oxytetracycline followed by intermediate sensitivity with, ciprofloxacin (20.00%) and carbenicillin (10.00 %).

**Discussion**

A total of 60 i.e 40 milk samples, 10 Ice cream and 10 Pedha samples, 10 (25.00%), 7 (70.00%) and 4 (40.00 %) were found to be positive for *S. aureus*. The highest incidence of *S. aureus* was from Ice cream (70.00%), followed by Pedha (40.00 %) and milk samples (35.00%)

The present overall isolation rate in raw milk and milk products was 35% which seems to be higher than the findings of 6.25 % by Thaker et al 2010 in Gujarat, India. As compared to present findings in raw milk higher level of incidence of *S. aureus* have been reported by Lingathurai et al 2011 in Madurai, India and Oleiverira et al 2011 in Brazil who found 61.70 %, 68.00% incidence in raw milk respectively.

The incidence of *S. aureus* in icecream in the present study was 70% and higher than incidence 63.3% was reported by Deepa et al 2015 in Karnataka, India. The incidence of *S. aureus* in pedha in the present study was 40.00 % seems to be higher with findings reported by Tambekar and Bhutda 2010 in Amravati, India, kumar and Prasad north India who
reported 17.39 % and 20.00 % respectively. The difference in the incidence rates of *S. aureus* between milk and milk products may origin from the method of manufacture, storage and handling.

This study presents the sensitivity of the *S. aureus* isolates towards life saving drugs, viz., cephalothin, cephalxin, co-trimoxazole, tetracycline, and erythromycin followed by clindamycin and ciprofloxacin thus indicating the safety of food products. However, few numbers of isolates exhibited resistance towards penicillin G, ampicillin, oxytetracycline, oxacillin, metronidazole, and methicillin. Whereas Thaker *et al* 2012 reported methicillin 100% sensitive towards *S. aureus*. Antibiotic resistance development among the bacteria poses a problem of concern. The effectiveness of current treatments and ability to control infectious diseases in both animals and humans may become hazardous.

**Conclusion**

*S. aureus* may be present in milk and milk products as a result of milk collected from the animal suffering from disease condition and excreting *S. aureus* in milk or due to unhygienic conditions during production, processing, storage and handling of milk products, which are the main causes of food borne diseases. Results clearly indicated that compared to raw milk the milk based products available in the market were highly contaminated with *S. aureus*, posing a high risk of food poisoning. The proper surveillance and also the application of Hazard Analysis Critical Control Point principles in the production place can minimize the microbial load. The indiscriminate use of antibiotics/antimicrobials agents for prophylactic as well as other therapeutic purpose could be the reasons for increased antimicrobial resistance of *S. aureus*. A relatively high number of strains are also resistant to the antibiotics commonly used in the therapeutic protocols of many human and animal infections. This study highlights the need for continuous surveillance of antibiotic sensitivity pattern of *Staphylococcus aureus*.

**References**


Thaker, HC., Brahmbhatt, MN. and Nayak, JB. (2013) Isolation and