

Isolation and identification of *Staphylococcus aureus* from milk and milk products and their drug resistance patterns in Anand, Gujarat

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Abstract

Aim: The study was carried out with aim to isolate *Staphylococcus aureus* from milk and milk products (pedha and curd) and determine antibiogram pattern of *S. aureus* isolates.

Materials and Methods: During 9 months duration of study a total of 160 milk and milk product samples (pedha and curd) were collected from different places in and around Anand city such as milk collection centre of Co-operative milk dairies, cattle farms, 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 viz. Baird-Parker Agar. The presumptive *S. aureus* isolates were identified by biochemical tests. Antibiogram pattern of *S. aureus* to antimicrobial agents were evaluated by disk diffusion method.

Results: Analysis of result revealed that out of total 160 samples of milk (100) and milk products i.e. curd (30) and pedha (30) resulted in the isolation of 10 isolates (6.25 %) of *S. aureus*. In the present study *S. aureus* isolates were found variably resistant to the antibiotics tested. The *S. aureus* isolates showed highest sensitivity towards cephalothin (100.00 %), co-trimoxazole (100.00 %), cephalexin (100.00 %) and methicillin (100.00 %) followed by gentamicin (90.00 %), ciprofloxacin (80.00 %), oxacillin (70.00 %), streptomycin (60.00 %) and ampicillin (60.00 %). The pattern clearly indicated that the overall high percent of *S. aureus* isolates were resistant to Penicillin-G (100.00 %) followed by ampicillin (40.00 %), oxytetracycline and oxacillin (20.00 %) and streptomycin and gentamicin (10.00 %)

Conclusions: Results clearly suggested 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: antibiogram pattern, milk products, *S. aureus*, 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 [1].

Milk and milk products are the prime habitat 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. The occurrence of these pathogenic bacteria in milk and

milk products can cause severe health hazards to people as they are highly susceptible to variety of microorganism because of high nutritive value and complex chemical composition [2].

Many contaminants find their way to raw milk, from which they gain access to dairy products [3, 4]. Chapaval [5] found production of staphylococcal enterotoxins in milk when milk was stored at temperatures of 37 °C to 42 °C or when exposed to variations in temperature.

Staphylococcal food poisoning include symptoms such as sudden onset of nausea, vomiting, abdominal cramps and diarrhea [6].

On heating at normal cooking temperature, the bacteria may be killed but the toxins remains active [7]. Staphylococcal enterotoxins are highly heat resistant and are thought to be more heat resistant in foodstuffs than in a laboratory culture medium [8].

Besides these, enterotoxins producing *S. aureus* are most dangerous and harmful for the human health. About 50 % strain of this organism are able to produce enterotoxins associated with food poisoning [9]. Illness

Table-1. Morphological and culture characteristics of *S. aureus*

Isolated bacteria	Gram staining	Culture characteristics on selective media
<i>Staphylococcus aureus</i>	Gram positive cocci (in clusters)	BPA: Typical jet black colonies surrounded by halo zone

Table-2. Biochemical characterisation of *S. aureus*.

Biochemical test	Reaction
Catalase	Positive
Coagulase	Positive
DNase	Positive
Acetoin production	Positive
Oxidase	Negative
D-mannitol fermentation	Positive

Figure-1. Colonies of *S. aureus* on Baird-Parker agar showing typical jet black colonies surrounded by halo zone.

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 septicaemia [2]. Especially in India, rate of infection is still higher because of warm and humid climate [10]. For many years, *S. aureus* was the only staphylococcal species known to produce enterotoxins [11].

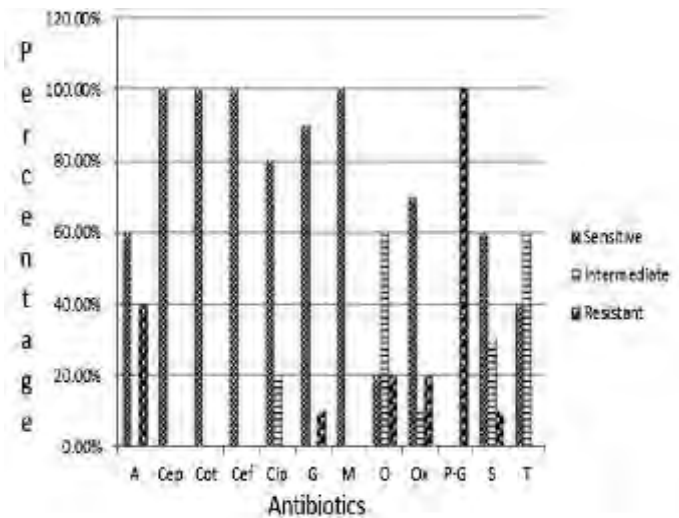
Thus, the objective of this study was to investigate the occurrence of *S. aureus* in milk and milk products (pedha and curd) and determine antibiogram pattern of *S. aureus* isolates.

Materials and Methods

Sample collection: From February 2011 to October 2011, a total of 160 milk and milk product samples which includes raw milk (100 samples), curd (30 samples) and pedha (30 samples) were collected from different places in and around Anand city such as milk collection centres of Co-operative milk dairies, cattle farms, 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 laboratory of the Post Graduate Department of Veterinary Public Health, College of Veterinary Science & Animal Husbandry, AAU, Anand for further processing and microbiological analysis.

Isolation and Identification of *Staphylococcus aureus*:

Isolation of *S. aureus* was attempted according to Singh and Prakash [12] with slight modification. Enrichment was carried out in Peptone Water (PW) enrichment broth (HiMedia Pvt. Ltd.). 10 ml or 10 g sample was homogenized with 90 ml sterile enrichment broth peptone water and enriched for 24 hrs at 37 °C.

Figure-2. Antibiogram of *S. aureus* isolates

The selective medium used for isolation of *S. aureus* was Baird Parker Agar (BPA) (HiMedia Pvt. Ltd.). A loopful of inoculum from enrichment were streaked on BP agar and incubated for 48 hours at 37°C. Characteristic appearance of jet black colonies surrounded by a white halo were considered to be presumptive *S. aureus* (Fig-1). The pure cultures were streaked on Nutrient agar (HiMedia Pvt. Ltd.) and incubated for 24 hours at 37°C and were further characterized by biochemical tests.

Morphological characteristics: The smear was prepared from the isolated culture on clean grease free microscopic glass slide and stained with Gram's method of staining. The stained smear was observed under microscope. Smear revealed Gram positive, spherical cells arranged in irregular clusters resembling to bunch of grapes.

Biochemical examination: Biochemical tests were performed to confirm *S. aureus* using Catalase test, Coagulase test, DNase test, Acetoin production, Oxidase test and D-mannitol fermentation.

Antibiogram pattern of the isolated *S. aureus* to some antimicrobial agents: The susceptibility of isolates to different anti-microbial agents was done by disk diffusion method using commercial disks [13] procured from HiMedia Pvt. Ltd. and almost all antimicrobial agents were having expiry date after 5-6 months of completion of research.

The antimicrobial agents tested were the following: ampicillin (10µg), cephalothin (30µg), co-trimoxazole (1.25/23.75µg), cephalexine (30 µg), ciprofloxacin (5µg), gentamicin (10µg), methicillin (5µg), oxy-tetracycline (30µg), oxacillin (1µg), penicillin-G (10units), streptomycin (10µg) and tetracycline (30µg).

Results

Analysis of result showed that out of total 160 samples of milk (100) and milk products i.e. curd (30) and pedha (30) resulted in the isolation of 10 isolates (6.25 %) of *S. aureus*.

Of the 100 milk samples, 30 pedha and 30 curd samples, 6(6.00%), 3(10.00%) and 1 (3.33%) respectively were found to be positive for *S. aureus* according to morphological and cultural characteristics (Table-1) and biochemical tests (Table-2).

In the present study, *S. aureus* isolates were found variably resistant to the antibiotics tested. The *S. aureus* isolates showed highest sensitivity towards cephalothin (100%), co-trimoxazole (100%), cephalexin (100%) and methicillin (100%) followed by gentamicin (90%), ciprofloxacin (80%), oxacillin (70.00%), streptomycin (60%) and ampicillin (60%). The pattern clearly indicated that the overall high percent of *S. aureus* isolates were resistant to Penicillin-G (100%) followed by ampicillin (40%), oxytetracycline and oxacillin (20%) and streptomycin and gentamicin (10 %).

Also intermediate sensitivity of *S. aureus* isolates was highest towards oxytetracycline and tetracycline (60%), followed by streptomycin (30%), ciprofloxacin (20%) and oxacillin (10 %) (Fig. 2).

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* spp. may be present in milk when it leaves the udder [14].

Negligence of hygienic condition such as improper cleaning of bulk tank, dirty udder, milking equipments, milk handling technique and improper storage will increase the proportion of Gram-positive and Gram-negative bacteria in the bulk tank milk [15,16].

Food products serve not only as a source of nutrition but also as substrates for the growth of microorganisms. The growth of microorganisms causes food spoilage. It may result in food-borne illness. In tropical countries raw milk and milk products are responsible for many outbreaks of gastrointestinal tract. It is also reported that immunocompromised individuals are prone to food-borne infection [17].

Of the 100 milk samples, 30 pedha and 30 curd samples, 6 (6%), 3 (10%) and 1 (3.33 %) respectively were found to be positive for *S. aureus*. The highest incidence of *S. aureus* was from pedha (10%), followed by milk samples (6%) and curd (3.33 %).

The present overall isolation rate in milk and milk products was 6.25% which seems to be similar to the findings of 7.3% by Fagundes [18], 8.3 % by Shah [19] and 6.6% by Kumar and Prasad [20]. As compared to present findings higher level of incidence of *S. aureus* have been reported by Tambekar and Bhutda [21], Ekici [22], Santana [23], Zakary [24] and Lingathurai [25] who found 17.39 %, 18.18 %, 18.80 %, 40 % and

61.70 % incidence respectively.

The findings of present study are in accordance with the findings 8.3 % Shah [19], 9.50 % Ekici [22], 12.80 % Normanno [26], 10.34 % Singh and Prakash [12], 6.6 % Kumar and Prasad [20] and 10% Addis [27].

The incidence of *S. aureus* in curd in the present study was 3.33%. While lower incidence 0% was reported by Singh and Prakash [12] and higher incidence 6.66% was reported by Kumar and Prasad [20].

The incidence of *S. aureus* in pedha in the present study was 10.00 % seems to be correlated with findings reported by Tambekar and Bhutda [21] 17.39%. While higher incidence of 20% was reported by Kumar and Prasad [20].

The difference in the prevalence rates of *S. aureus* between milk and milk products may originate from the method of manufacture, storage and handling.

This study presents the sensitivity of the *S. aureus* isolates towards life saving drugs, viz., cephalothin, cephalexin and co-trimoxazole followed by gentamicin and ciprofloxacin thus indicating the safety of food products. However, few numbers of isolates exhibited resistance towards ampicillin, oxytetracycline and oxacillin.

Antibiotic resistance development among the bacteria poses a problem of concern. Effectiveness of current treatments and ability to control infectious diseases in both animals and humans may become hazardous.

Conclusion

Staphylococcal food poisoning is of major concern in public health programs worldwide. *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 milk and milk based products available in the market were contaminated with *S. aureus*, posing a high risk of food poisoning. Thus more hygienic preventive measures are required to reduce the bacterial contamination, so as to increase the wholesomeness of these milk and milk based products.

The indiscriminate use of antibiotics/anti-microbials 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* with a view to selecting appropriate therapy.

Authors' contributions

All authors contributed equally in the collection, analysis and processing of samples as well as preparation of

manuscript for publication. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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