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Detection of staphylococci in raw milk and milk products and evaluation of their antibiotic sensitivity: a report from Southern Assam, India

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Abstract: *The objective of this study was to detect the presence of staphylococci in raw milk and milk products collected from various regions in and around Southern Assam and to evaluate their antibiotic sensitivity patterns. Out of 117 samples analysed, 35 milk samples and 21 milk product samples were positive for staphylococci. Staphylococci were isolated from 22 cow milk samples, 8 goat milk samples and 5 buffalo milk samples. Antibiotic sensitivity test of the isolates showed maximum resistance for penicillin (87.5%).Resistance for oxacillin and vancomycin were also observed in 5 and 2 isolates respectively. 92.86% of the isolates were positive for at least one of the antibiotics used in this study.The high level of staphylococcal isolates found in raw milk samples and milk products in this study suggest poor quality of these food products and health risk for the consumers.Antibiotic resistance exhibited by the isolates in this study is alarming as such isolates are considered a threat to public health.*

Key words: *antibiotics, milk, milk products, Southern Assam, staphylococci, vancomycin,*

I. Introduction

Milk is an important component of human diet and is highly popular as a good source of nutrition. Conversely, it can serve as a vehicle for pathogenic bacteria like staphylococci [1]. This poses health risk to the consumers given that many strains of *Staphylococcus spp.* are capable of producing thermostable enterotoxins, the ingestion of which may lead to staphylococcal food poisoning (SFP). SFP is considered one of the most common food-borne illness worldwide with high occurrence [2]. Staphylococci can gain access to milk by direct excretion from infected udders or by contamination from fodder, equipments, air or milk handlers [3]. Many milk products are prepared from raw milk and hence contamination of milk products by staphylococci can not be ignored. Milk and dairy products are among the most common sources of SFP [4 5 6].

Apart from pathogenicity, one more consideration in the context of food safety and health concern is the presence of transmissible antibiotic resistance markers. Staphylococci isolated from food are frequently resistant to one or more antibiotics [7 8 9] and hence could act as agents for spreading of antibiotic resistance genes [10]. Besides, antibiotic residues in foods of animal origin affect the nutritive value of these foods. Recently there has been an enormous increase in the isolation of Methicillin-resistant *Staphylococcus aureus* (MRSA) strains resulting from wide spread and prolonged use of methicillin in clinical settings and food animal production facilities [11 12]. With increase in methicillin resistance,vancomycin became the drug of choice but in the last decade, many cases of vancomycin resistance have been reported. For long, the pathogenicity of coagulase negative staphylococci was ignored but of late, there have been many reports on their enterotoxigenicity and antibiotic resistance [13 14 15 16]. Given the pathogenic nature of staphylococci and the indiscriminate use of antibiotics, determination of antibiotic resistance patterns is crucial as it might provide information about specific control measures [17]. In light of these findings, the quality assessment of milk thus becomes imperative and a detailed knowledge about the biochemical characteristics and antibiotic resistance profiles becomes necessary for determining their clinical significance and epidemiology. To our knowledge, there is limited literature available on the properties of *Staphylococcus spp.* isolated from milk and milk products of this region. In the present study, an attempt has been made to detect staphylococci in milk and dairy products collected from various regions in and around Southern Assam and to evaluate their antibiotic sensitivity patterns.

II. Materials And Methods

2.1. Collection of samples and screening of isolates

A total of 117 samples including raw milk and milk products (locally made sweets from various local sweet stalls) were collected from various regions in and around Southern Assam and were immediately transported to the laboratory in ice-cold conditions. Of the 117 samples, 69 were milk samples from cows, goats, buffaloes and 48 were milk product samples. The samples were cultured on Mannitol Salt Agar (MSA) and Baird-Parker Agar (BPA) with an incubation period of 24-48 hours at 37°C following incubation in *Staphylococcus* enrichment broth for 24-48 hours at 37°C. Screening of staphylococcal isolates was done on the basis of

morphology, Gram's stain, catalase, coagulase and mannitol fermentation tests according to standard protocols. 56 staphylococcal isolates were obtained from 117 samples of which 35 were from milk samples and remaining 21 from milk products.

2.2. Biochemical characterization of the isolates

Isolates supposed to belong to *Staphylococcus* species based on their morphology (creamy or yellow colonies on Mannitol Salt Agar, black colonies on Baird-Parker Agar) were tested for the following biochemical aspects for characterization.

2.2.1. Gram's staining

The suspected cultures of *Staphylococcus* species were subjected to Gram's staining and observed under the light microscope. Gram positive cocci occurring in grape like irregular clusters were taken as presumptive *Staphylococcus* species.

2.2.2. Catalase test

A single colony from a pure culture plate was picked using a sterile loop and mixed with 3% H_2O_2 on a clean glass slide. Liberation of oxygen in the form of bubbles within a few seconds was taken as a positive test. The catalase positive cultures were taken to be staphylococci.

2.2.3. Coagulase test

The tube coagulase test was performed in sterile tubes by adding 0.5 ml of broth culture of the selected isolates to 0.5 ml of citrated rabbit plasma. After mixing the contents, the tubes were incubated at 37°C along with a negative control tube containing a mixture of 0.5 ml of uninoculated sterile broth and 0.5 ml of citrated rabbit plasma. The tubes were monitored for clot formation at 30 minutes interval for the first 4 hours and then after 24 hours incubation. The reaction was considered positive if a clot was visible within the tube and negative if no degree of clotting was visible.

2.2.4. Mannitol fermentation

The isolates grown on MSA were classified as either positive or negative for mannitol fermentation depending on their ability to ferment mannitol resulting in change of pH of the medium. This pH change is indicated in the form of discolouration of the medium from red to yellow. The isolates growing on MSA indicated the growth of salt tolerant staphylococci. Discolouration of the medium from red to yellow was taken as positive result while no change in medium colour was recorded as negative result for mannitol fermentation.

2.3 Antibiotic sensitivity

Antibiotic sensitivity was performed using the Kirby-Bauer disk diffusion method according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS). The screened staphylococcal isolates were tested with a panel of 7 antibiotics namely penicillin G (10 units), oxacillin (1 mcg), chloramphenicol (30 mcg), ciprofloxacin (30 mcg), erythromycin (15mcg), tetracyclin (25mcg) and vancomycin (30mcg), Results were recorded after 24 hours of incubation at 35°C on Mueller Hinton agar and interpreted as per NCCLS (2009) [18] standards.

III. Results

A total of 56 (47.86%) staphylococcal isolates were obtained from a pool of 117 isolates, 62.5% from milk and remaining 37.5% from milk products. Of the milk samples, the majority of the staphylococcal isolates were isolated from cow milk (22) followed by goat milk (8) and buffalo milk (5) (Fig 1).

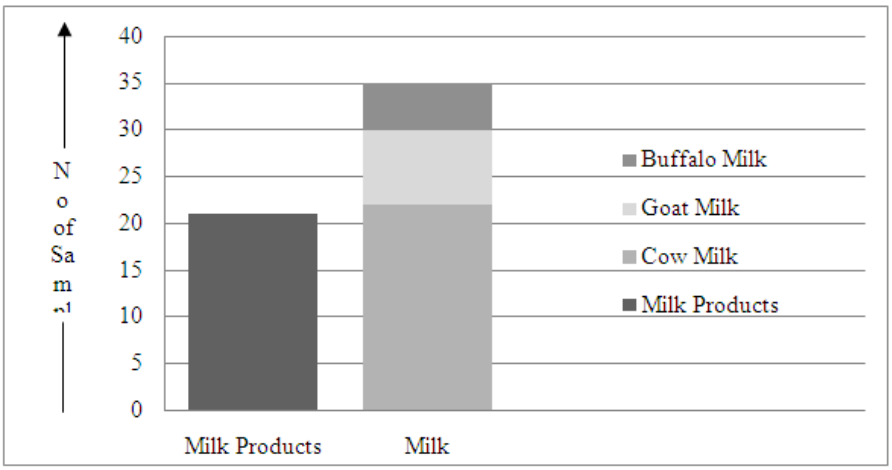

Fig 1: Prevalence of staphylococci in collected samples

All the 56 isolates were positive for Gram's test and catalase but there was variation in the results of coagulase and mannitol fermentation. The percentage of coagulase negative *Staphylococcus* was higher (75%) than that of coagulase positive *Staphylococcus*(CPS)(25%)(Fig 2).

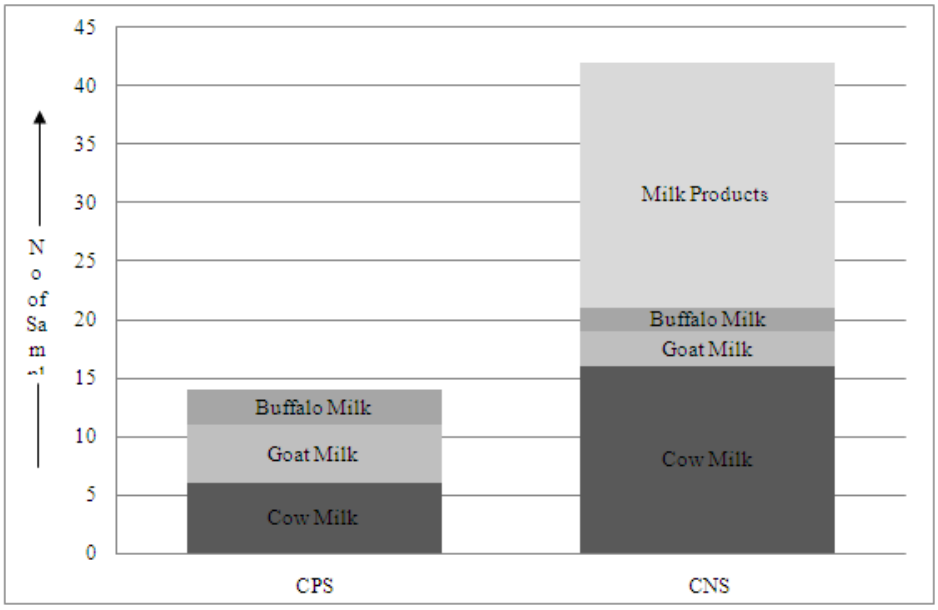

Fig 2 Distribution of CNS and CPS in the collected samples

While all the coagulase positive *Staphylococcus* fermented mannitol (100%), the percentage of mannitol fermenting CNS in this study was found to be 52.38% (TABLE 1, Fig 3).

Table 1 showing the distribution of staphylococcal isolates from various sources from which they are obtained along with the prevalence of CPS, CNS and mannitol fermenting CNS from these sources.

	Staphylococci-positive samples		CPS		CNS		Mannitol Fermenting CNS	
Source	No	%	No	%	No	%	No	%
Cow Milk	22	39.29	6	42.86	16	38.10	12	54.55
Goat Milk	8	14.29	5	35.71	3	7.14	1	4.55
Buffalo Milk	5	8.93	3	21.43	2	4.76	1	4.55
Milk Products	21	37.50	0	0.00	21	50.00	8	36.36
Total	56	100	14	100.00	42	100.00	22	100.00

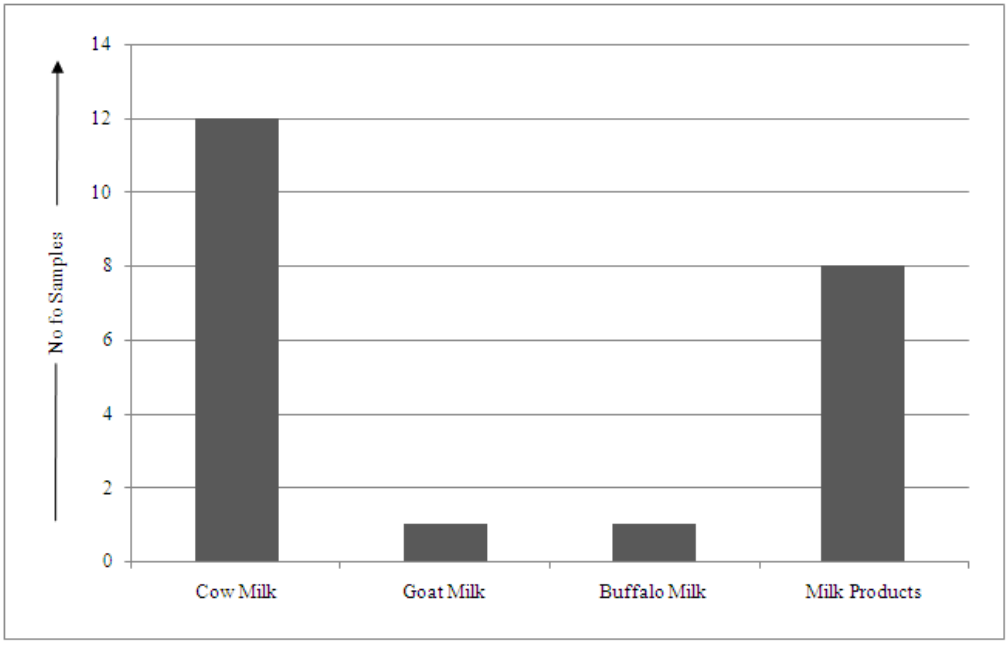

Fig 3 showing mannitol fermenting CNS isolated from collected samples

The staphylococcal isolates detected in this study showed the following frequencies of resistance to the 7 antibiotics used. 52 out of the 56 isolates (92.86%) were resistant to at least one of the antibiotics used. High rate of resistance was observed for penicillin with 87.5% isolates showing resistance. Among the 56 isolates, 49, 5, 3, 5, 11, 9 and 2 were resistant to penicillin, oxacillin, chloramphenicol, ciprofloxacin, erythromycin, tetracycline and vancomycin respectively (TABLE 2).

Table 2 showing antibiotic sensitivity patterns of the staphylococcal isolates

Antibiotics	Disc Content (mcg/disc)	Resistant		Sensitive	
		Number	%	Number	%
Penicillin	10 units	49	87.50	7	12.50
Oxacillin	1	5	8.93	51	91.07
Chloramphenicol	30	3	5.36	53	94.64
Ciprofloxacin	30	5	8.93	51	91.07
Erythromycin	15	11	19.64	45	80.36
Tetracyclin	25	9	16.07	47	83.93
Vancomycin	30	2	3.57	54	96.43

IV. Discussion

Staphylococci have been frequently isolated from milk and dairy products with reports of significant toxin production implicated in food poisoning cases. In the present study we detected staphylococci in milk and milk products with prevalence rates of 62.5% and 37.5% respectively. Many researchers have reported the presence of staphylococci in milk and milk products with varying prevalence rates. Addis *et al.*,[19] reported 46% prevalence rate of staphylococci in raw milk. Kumar and Prasad [20] in their study reported the presence of staphylococci in 19% of the dairy products analysed. In our study, we isolated staphylococci from 8 goat milk and 5 buffalo milk samples which support the findings of other authors who isolated staphylococci from these sources. Sindhu *et al.*,[21] reported prevalence of staphylococci in buffalo milk while studies conducted by Ebrahimi *et al.*,[22] and Valle *et al.*,[14] threw light on the abundance of staphylococci in goat milk with the latter reporting enterotoxigenic strains of staphylococci in goat milk. The percentage of CNS was significantly higher than that of CPS which is in accordance with the findings of Addis *et al.*, [19] who demonstrated prevalence of CNS over CPS in raw milk. The high number of CNS isolated in this study could be justified by the

fact that CNS are found abundantly in the normal teat skin flora and mucosa of humans and animals while some are free living in the environment [19]. Contamination might have been a result of direct excretion of staphylococci into milk from infected udders or by human manipulation. Although mannitol fermentation by coagulase negative *Staphylococcus* is often disregarded, we, in our study found 52% CNS fermenting mannitol. Similar property of CNS was reported by Shittu *et al.*,[23] although the percentage of such isolates reported by them was only 18%. Antibiotic sensitivity test of the isolates revealed maximum resistance for penicillin which is in accordance with previous studies by Lee *et al.*,[24] Mohan *et al.*,[25], Aslantas *et al.*,[26]. Resistance for oxacillin was observed in 9% of the isolates. In our study two of the isolates, one from cow milk and the other from milk product showed resistance for both oxacillin and vancomycin. Similar finding has been reported by Kamal *et al.*, [27] who detected MRSA isolates from milk and dairy products showing oxacillin and vancomycin resistance. In our study the isolates that showed resistance for oxacillin and vancomycin were coagulase negative staphylococcal isolates obtained from cow milk and milk products. This finding supports earlier reports by Silva *et al.*,Yardukul *et al.*, and Cicconi-Hogan *et al.*,[15 16 28] who detected oxacillin and vancomycin resistance in coagulase negative staphylococci isolated from milk samples. The isolates showed high susceptibility for chloramphenicol and ciprofloxacin as reported earlier by Umaru *et al.*,[29] and Lee [24]. Such a trend of resistance to antibiotics can be attributed to random use of antibiotics, dry period treatments, different treatment choices in farms of this region [26].

V. Conclusion

In our study we isolated a good number of staphylococcal isolates (both coagulase positive and coagulase negative *Staphylococcus*) from both milk and milk products. This indicates improper handling and storage of these food products and hence necessitates better hygienic practices. Since staphylococci possess the potential of producing toxins responsible for food poisoning, their presence in these popular food items is a matter of major health concern. Hence further investigation regarding the identity of the isolates and their toxin-producing potential is important for a better understanding of these pathogens.

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