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Prevalence and antibiotic resistance pattern of *Staphylococcus aureus* isolated from milk in Udaipur (Rajasthan)

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Abstract
Staphylococcus aureus is one of the most important food borne pathogen. The presence of enterotoxigenic *S. aureus* in milk is a serious concern for human health. Drinking contaminated milk with preformed toxins of *S. aureus* causes rapid onset of food borne illness. A total of 100 milk samples including pooled milk (n=25), vendors milk (n=25), pasteurized market milk (n=25) and individual cow milk (n=25) were collected randomly from different parts of Udaipur city, Rajasthan. The prevalence of *S. aureus* in pooled milk, vendors milk, pasteurized market milk and individual cow milk was 76% (19/25), 36% (9/25), 0% (0/25) and 56% (14/25), respectively. The overall prevalence was 42% (42/100). Antibiotic susceptibility test results revealed that penicillin G and ampicillin showed highest resistance (100%) in bacteria followed by erythromycin (30.95%) and methicillin (21.43%).

Keywords: Milk, *Staphylococcus aureus*, antibiotic, prevalence

Introduction
Foodborne diseases are one of the important causes of concern in developing countries, resulting in several deaths annually and also lead to of economic burden on the nation. According to World Health Organization, approximately 600 million people get infected every year and around 4.2 lakh people die annually due to these food-borne illnesses [1]. Among the various bacterial food borne pathogens, *Staphylococcus aureus* is considered as one of the most important pathogen. Milk is an important constituent of the human diet and raw milk is an ideal growth medium for several microorganisms. Unhygienic milk production, poor handling and storage practices such as the addition of water or other substances can introduce bacteria or germs that cause spoilage. If the food is contaminated with *S. aureus*, the bacteria can multiply in the food and produce toxins that can make people ill. This bacterium can be killed by cooking or boiling, but the toxins are heat resistant and not destroyed by heat treatments and can cause illness. Of more than 20 staphylococcal enterotoxins SEA and SEB are the best characterized and are also regarded as superantigens because of their ability to bind to class MHC 2 molecules on antigen presenting cells. These proteins are resistant to denaturation, which facilitate them to remain alive in food and leads to disease outbreaks [2]. Drinking contaminated milk with preformed toxins of *S. aureus* causes rapid onset (IP = 2-8 hours) of vomiting, nausea, abdominal cramps and diarrhoea. Production of enterotoxins in milk is favoured when milk is stored at temperatures of 37 °C or 42 °C or when exposed to variations of temperatures [3]. Among the variety of infections, *S. aureus* may cause minor skin infections such as impetigo, pimples and other lesions on the skin such as boils, cellulitis, carbuncles, folliculitis, etc. Scalded skin syndrome, abscess, lung infections or pneumonia, meningitis, osteomyelitis, endocarditis are some other important staphylococcal diseases. There are some generalized staphylococcal infections such as life- threatening blood infection or toxic shock syndrome (TSS), bacteraemia, septicaemia in human beings. In domestic animals, staphylococci cause mastitis, tick pyaemia, executive epidermitis, pyoderma and botryomycosis.
The frequent and inappropriate use of antibiotics in livestock for therapeutic and growth promoting purpose, results in the emergence of the antibiotic resistance in *S. aureus*. The

antibiotic resistance can be easily transferred among healthier commensals and to other animals and humans by close interactions [4].

Materials and Methods

Sample collection

A total of 100 milk samples including pooled milk (n=25), vendors milk (n=25), pasteurized market milk (n=25) and individual cow milk (n=25) were collected randomly from different parts of Udaipur city, Rajasthan during the period of September 2019 to December 2019. The samples were collected in sterile container and transported to laboratory of the Department of Veterinary Public Health in College of Veterinary and Animal Science, Navania, Vallabhnagar, Udaipur within 2 hours in chilled conditions by using ice packs.

Isolation and identification of *S. aureus*

An aliquot of milk sample (25 ml) was inoculated in buffered peptone water (SRL) for enrichment and incubated at (37 ºC/24 hrs.). Then the loopful of culture was streaked on mannitol salt agar (MSA) and then incubated overnight at 37 ºC for 24 hours. Next day characteristic golden-yellow bacterial colonies were considered as presumptive *S. aureus*. The pure culture were streaked on nutrient agar and incubated at 37 ºC for 24 hours and further characterized by biochemical tests.

Morphological characteristics

The observation of Gram-stained smear under microscope revealed the presence of irregularly arranged gram positive clusters of cocci resembling to bunch of grapes.

Biochemical examination

Biochemical testing was performed for conformation of presumptive isolates of *S. aureus* using catalase test, coagulase test and motility test.

Results

The present study revealed the prevalence of *S. aureus* in pooled milk, vendors milk, pasteurized market milk and individual cow milk as 76% (19/25), 36% (9/25), 0% (0/25) and 56% (14/25), respectively. The overall prevalence of *S. aureus* in milk was 42% (42/100) in Udaipur city, Rajasthan. The antibiotic resistance pattern revealed that the bacteria showed highest resistant towards penicillin G and ampicillin (100%) followed by erythromycin (30.95%), methicillin (21.43%) and ciprofloxacin (11.91%) while 7.14% isolates were resistant to oxytetracycline, gentamicin and co-trimoxazole. (Table- 1 and Figure-1)

Table 1: Antibiotic resistance pattern of *S. aureus* recovered from milk samples

S. No.	Name of antibiotics	Antibiotic susceptibility pattern (42 *S. aureus* isolates)		
		S	I	R
1.	Penicillin G	0 (0%)	0 (0%)	42 (100%)
2.	Ampicillin	0 (0%)	0 (0%)	42 (100%)
3.	Methicillin	29 (69.05%)	4 (9.52%)	9 (21.43%)
4.	Ceftriaxone	33 (78.57%)	7 (16.67%)	2 (4.76%)
5.	Co-trimoxazole	39 (92.86%)	0 (0%)	3 (7.14%)
6.	Gentamicin	38 (90.48%)	1 (2.38%)	3 (7.14%)
7.	Erythromycin	0 (0%)	29 (69.05%)	3 (30.95%)
8.	Chloramphenicol	33 (78.57%)	7 (16.67%)	2 (4.76%)
9.	Oxytetracycline	38(90.48%)	1(2.38%)	3(7.14%)
10.	Ciprofloxacin	33(78.57%)	4(9.52%)	5(11.91%)
S= sensitive, I= intermediate, R= resistant				

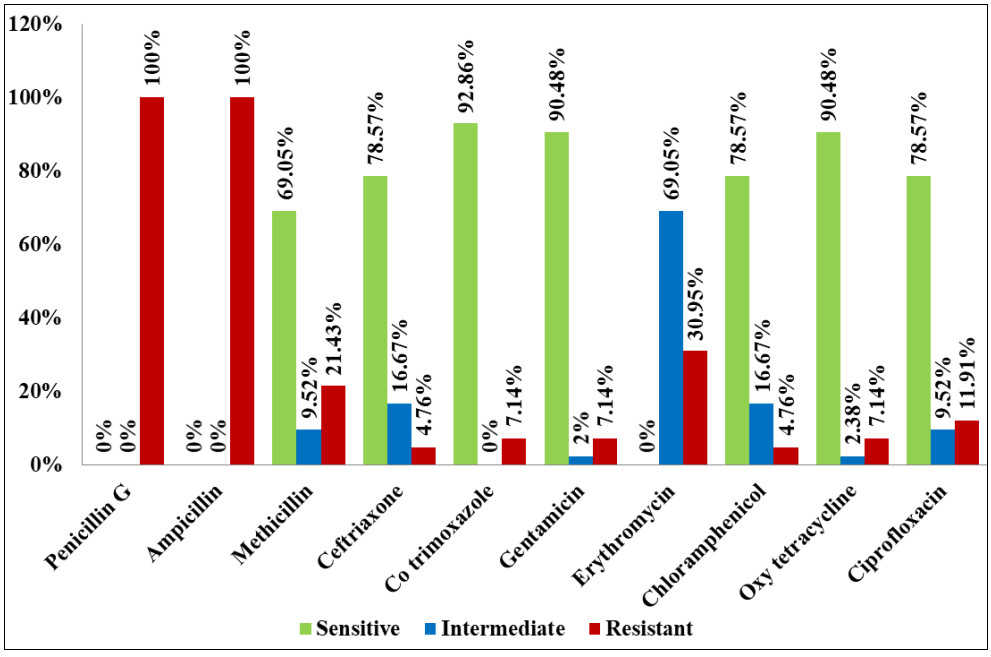

Fig 1: Antibiogram of *S. aureus* isolates recovered from milk samples

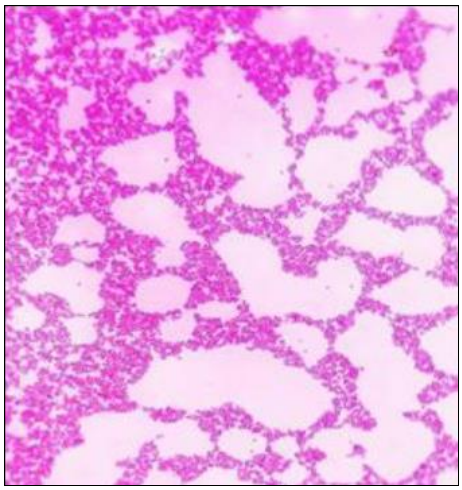

Fig 2: Gram staining of the isolates

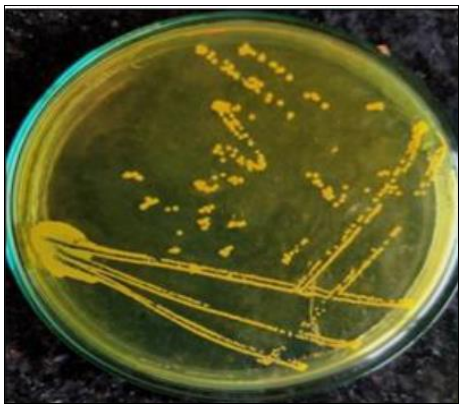

Fig 3: Characteristic golden-yellow colonies of *S. aureus* on mannitol salt agar

Fig 4: Catalasest

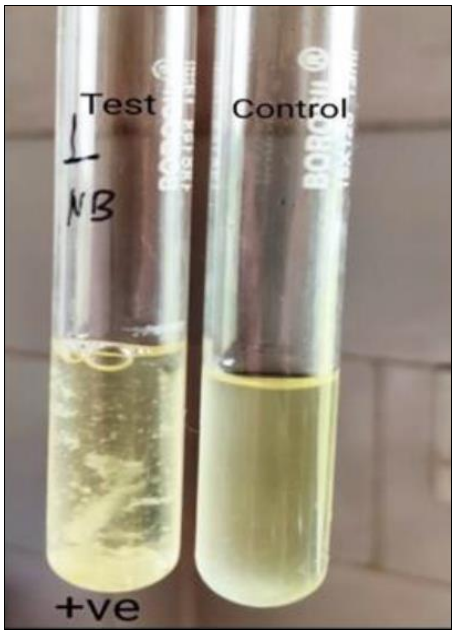

Fig 5: Tube coagulase test

Discussion

In present study, prevalence of *S. aureus* was found in 76% (19/25) pooled milk samples collected from dairy shops. Akindolire *et al.* (2015) reported similar prevalence as 75% in pooled milk samples [5]. While, higher prevalence was reported by Jørgensen *et al.* (2005) who found 96.2% of samples being positive for *S. aureus* [6]. On the other hand, Cortimiglia *et al.* (2015), Muehlherr *et al.* (2003) and Chu *et al.* (2012) have reported lower prevalence as 43.1%, 31.7% and 16.75%, respectively [7, 8, 9]. Sudhanthiramani *et al.* (2015) found 39.09% samples of milk sold by local milk vendors as positive for *S. aureus* which is similar to the prevalence of *S. aureus* (36%) found in our study [10]. Lower and higher prevalence rates were also reported by Kumar and Prasad (2010) and Munsi *et al.* (2015) as 26% and 96.43%, respectively [11,12]. None of the samples (n=25) of pasteurized milk analysed in our study were found to be contaminated with *S. aureus*. As opposed to this, some researchers like Santos *et al.* (1981) and Akindolire *et al.* (2015) have found the prevalence of *S. aureus* in pasteurized milk as 6% and 13%, respectively [5, 13]. Individual cow milk samples showed the prevalence of *S. aureus* in 56% of the samples in our study. These findings are in agreement with the findings reported by Chaalal *et al.* (2016), who observed the prevalence as 55.26%. Higher prevalence rates were observed by Bharathy *et al.* (2015) who reported the contamination of the milk samples with *S. aureus* to be 68% [14, 15]. While, Wang *et al.* (2018) and Thaker *et al.* (2013) observed lower prevalence rates as 46.2% and 6%, respectively [3, 16]. In our study, *S. aureus* isolates were found to be resistant to penicillin G, ampicillin and erythromycin as 100%, 100% and 30.95%, respectively. Similar findings were reported by Parisi *et al.* (2016) who found all the isolates of *S. aureus* resistant towards ampicillin and penicillin [17]. Slightly lower resistance rates among *S. aureus* isolates were reported by Zhang *et al.* (2016) who found 90.40% *S. aureus* isolates to be resistant towards both ampicillin and penicillin [18]. On the other hand, lower resistance percentages were reported by Alian *et al.* (2012) towards ampicillin and penicillin as 39.1% and 23.9%, respectively [19].

Conclusion

In present study, the prevalence of *S. aureus* in pooled milk, vendors milk, pasteurized market milk and individual cow milk was 76% (19/25), 36% (9/25), 0% (0/25) and 56% (14/25), respectively. The difference in the prevalence rate of *S. aureus* is due to the difference in their method of production, storage and handling. Staphylococcal food poisoning is a major public health concern worldwide as they pose a high risk of food poisoning. Thus, hygienic measures should be implemented along the milk production chain to improve the bacteriological quality of milk and milk products. The antibiotic resistance pattern revealed that the bacteria showed highest resistant towards penicillin G and ampicillin (100%) followed by erythromycin (30.95%), methicillin (21.43%) and ciprofloxacin (11.91%). Methicillin resistant *Staphylococcus aureus* (MRSA) can cause a number of life-threatening human diseases. MRSA are potential vehicles of colonization or infections for humans [20]. These antibiotic resistant isolates are transmitted to humans by the consumption of food products containing the resistant bacteria. Antibiotic resistance development among the bacteria poses a problem of concern. If the necessary action against the indiscriminate use of antibiotics is not taken as an early stage, it may lead to serious human health hazards.

Thus, there is a need for continuous monitoring and surveillance of antibiotic therapy.

References

1. World Health Organization. WHO Estimates of the Global Burden of Foodborne Diseases. Food safety. Fact sheet N; c2015. p. 399.
2. Pinchuk IV, Beswick EJ, Reyes E. Staphylococcal Enterotoxins. Toxins. 2010;2:2177-2197.
3. Thaker HC, Brahmbhatt MN, Nayak JB. Isolation and identification of *Staphylococcus aureus* from milk and milk products and their drug resistance patterns in Anand, Gujarat. Veterinary World. 2013;6(1):10-13.
4. Sharma V, Sharma S, Dahiya DK, Khan A, Mathur M, Sharma A. Coagulase gene polymorphism, enterotoxigenecity, biofilm production, and antibiotic resistance in *Staphylococcus aureus* isolated from bovine raw milk in North West India. Annals of Clinical Microbiology and Antimicrobials. 2017;16:65.
5. Akindolire MA, Babalola OO, Ateba CN. Detection of Antibiotic Resistant *Staphylococcus aureus* from Milk: A Public Health Implication. International Journal of Environmental Research and Public Health. 2015;12:10254-10275.
6. Jorgensen HJ, Mork T, Hogasen HR, Rorvik LM. Enterotoxigenic *Staphylococcus aureus* in bulk milk in Norway. Journal of Applied Microbiology. 2005;99:158-166.
7. Cortmigilia C, Bianchini V, Franco A, Caprioli A, Battisti A, Colombo L *et al*. Short communication: Prevalence of *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* in bulk tank milk from dairy goat farms in Northern Italy. Journal of Dairy Science. 2015;98:2307-2311.
8. Muehlherr JE, Zweifel C, Corti S, Blanco E, Stephan R. Microbiological Quality of Raw Goat's and Ewe's Bulk-Tank Milk in Switzerland. Journal of Dairy Science. 2003;86:3849-3856.
9. Chu C, Yu C, Lee Y, Su Y. Genetically divergent methicillin-resistant *Staphylococcus aureus* and sec-dependent mastitis of dairy goats in Taiwan. BMC Veterinary Research. 2012;8:39.
10. Sudhanthiramani S, Swetha SC, Bharathy S. Prevalence of antibiotic resistant *Staphylococcus aureus* from raw milk samples collected from the local vendors in the region of Tirupathi, India. Veterinary World. 2015;8:478-481.
11. Kumar R, Prasad A. Detection of *E. coli* and *Staphylococcus* in Milk and Milk Products in and around Pantnagar. Veterinary World. 2010;3(11):495-496.
12. Munsi MdN, Sarker NR, Khatun R, Alam MK. Identification and antibiogram study of bacterial species isolated from milk samples of different locations in Bangladesh. Asian Journal of Medical and Biological Research. 2015;1(3):457-462.
13. Santos EC, Genigeorg C, Farver TB. Prevalence of *Staphylococcus aureus* in Raw and Pasteurized Milk Used for Commercial Manufacturing of Brazilian Minas Cheese. Journal of Food Protection. 1981;44(3):172-176.
14. Chaalal W, Aggad H, Zidane K, Saidi N, Kihal M. Antimicrobial Susceptibility Profiling of *Staphylococcus aureus* Isolates from Milk. British Microbiology Research Journal. 2016;13(3):1-7.
15. Bharathy S, Gunaseelan L, Porteen K, Munnisamy BM. Prevalence of *Staphylococcus aureus* in raw milk: Can it be a potential public health threat? International Journal of Advanced Research. 2015;3(2):801-806.
16. Wang W, Lin X, Jiang, Zixin T, Peng, Xu J, *et al*. Prevalence and Characterization of *Staphylococcus aureus* Cultured From Raw Milk Taken From Dairy Cows With Mastitis in Beijing, China. Frontiers in Microbiology 2018;9:1123.
17. Parisi A, Caruso M, Normanno G, Latorre L, Sottili R, Miccolupo A, *et al*. Prevalence, antimicrobial susceptibility and molecular typing of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in bulk milk from southern Italy. Food Microbiology. 2016;58:36-42.
18. Zhang L, Li Y, Bao H, Wei R, Zhou Y, Zhang H, Wang R. Population structure and antimicrobial profile of *Staphylococcus aureus* strains associated with bovine mastitis in China. Microbial Pathogenesis. 2016;97:103-109.
19. Alian F, Rahimi E, Shakerian A, Momtaz H, Riahi M, Momeni M. Antimicrobial Resistance of *Staphylococcus aureus* Isolated from Bovine, Sheep and Goat Raw Milk. Global Veterinaria. 2012;8(2):111-114.
20. EFSA. Assessment of the Public Health significance of the methicillin resistant *Staphylococcus aureus* (MRSA) in animals and foods. EFSA Journal. 2009;993:1-73