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Prevalence and antibiotic resistance pattern of *Staphylococcus aureus* of dairy origin from Udaipur (Rajasthan) region

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Abstract
Staphylococcus aureus is an important food-borne pathogen. Enteropathogenic *S.aureus* in milk should be regarded as a part of the risk analysis of milk and milk products. A total of 60 milk samples were collected from 20 small holder dairy farms having 10 or less than 10 cattle from Vallabhnagar tehsil of Udaipur district, India. The antibiotic susceptibility test for cattle milk samples positive isolates showed high resistance towards penicillin G 36/40 (90%) and ampicillin 30/40 (75%), and showed resistant to methicillin 17/40 (42.5%), trimethoprim 17/40 (42.50%), erythromycin 9/40 (22.5%), co-Trimoxazole 10/40 (25%), tetracycline 8/40 (20%), ceftriaxone 7/40 (17.5%), gentamicin 4/40 (10%), azithromycin 5/40 (12.5%), chloramphenicol 1/40 (2.5%), and ciprofloxacin 1/40 (2.5%).

Keywords: Milk, *Staphylococcus aureus*, antibiotic

Introduction
Food-borne diseases and poisonings are the widespread and great public health concerns in the modern world, where developed and developing countries are largely affected by food-borne infections. Food-borne diseases not only affect human health but also the economy of the country [1]. *Staphylococcus aureus* is an important food-borne pathogen. Enteropathogenic *S.aureus* in milk should be regarded as a part of the risk analysis of milk and milk products. Staphylococcus food poisoning is caused by ingestion of food containing one or more preformed enterotoxins (SEs) produced by *S.aureus*. Staphylococcal food poisoning ranks third among the reported food-borne diseases in the world [2, 3]. Symptoms have a rapid onset and include nausea, vomiting and diarrhoea. This organism has emerged as a major pathogen for both nosocomial and community-acquired infections [4, 5]. *Staphylococcus aureus* is a highly pathogen of humans and animals that causes a different type of the disease. The bacterium is a colonizer of the skin and mucous membrane from where it can invade multiple organs. In livestock, *S. aureus* is an important cause of mastitis, skin and soft tissue infections (SSTI) and to lesser extent infections of the locomotory system [6, 7].

Antimicrobial resistance is a global threat to human and animal health hence a regular monitoring to determine anti biogram should be essential component of surveillance [8, 9]. The extensive variability in the anti biogram patterns exhibited by *S.aureus* from different localities and at different time intervals suggests that this organism is changing its response to different antibiotics very frequently.

Materials and Methods
Sample collection: From July 2016 to December 2016, a total of 60 milk samples were collected from 20 small holder dairy farms having 10 or less than 10 cattle from Vallabhnagar tehsil of Udaipur district, India. The samples were collected in sterilized milk collecting tubes transported to laboratory of the Department of Veterinary Public Health, College of Veterinary and Animal Science, Navania, Vallabhnagar, Udaipur.

Isolation and Identification of *Staphylococcus aureus:*
Enrichment was carried out in Peptone Water (PW) enrichment broth (SRL). Twenty five ml milk sample was homogenized with 225 ml sterile enrichment broth peptone water and enriched for 24 hrs at 37 °C. The medium used for isolation of *S. aureus* was Mannitol salt agar (MSA) (SRL). A loopful of inoculum from enrichment were streaked on MSA and

incubated for 48 hours at 37 ˚C. Characteristic appearance of golden yellow colour was considered to be presumptive *S. aureus*. The pure cultures were streaked on Nutrient agar (SRL) and incubated for 24 hours at 37 ˚C and were further characterized by biochemical tests.

Morphological characteristics
The Gram stained smear was observed under microscope which 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, Urease test, Motility test, and growth in TSI.

Results
The present study revealed that the prevalence of *S. aureus* was 66.66% (40/60) in the cattle milk samples collected from Vallabhnagar tehsil of Udaipur district. Antibiotic resistance patterns of the isolates of *S. aureus* in the present study showed highest resistance to Penicillin-G (90.00%) followed by Ampicillin (75.00%) while 42.50% isolates were resistant to Trimethoprim and Methicillin, 17.50% to Ceftriaxone, 22.50% to Erythromycin, 25.00% to Co-Trimoxazole, 20% to Tetracycline, 12.5% to Azithromycin, 10% to Gentamicin and 2.5% isolates were still less resistant to Chloramphenicol and Methicillin. (Table-1 and Figure-1).

Table 1: Antibiotic resistance patterns of positive isolates of *S. aureus* from cattle milk samples.

| S. No.	Antibiotic	Antibiotic resistance Pattern		
		Sensitive	Intermediate	Resistant
1.	Chloramphenicol	65% (26)	32.5% (13)	2.5% (1)
2.	Methicillin	37.5% (15)	20% (8)	42.5% (17)
3.	Ciprofloxacin	62.5% (25)	35% (14)	2.5% (1)
4.	Azithromycin	65% (26)	22.5% (9)	12.5% (5)
5.	Erythromycin	42.5% (17)	35% (14)	22.5% (9)
6.	Co-Trimoxazole	47.5% (19)	27.5% (11)	25% (10)
7.	Ampicillin	25% (10)	0% (0)	75% (30)
8.	Ceftriaxone	40% (16)	42.5% (17)	17.5% (7)
9.	Gentamicin	85% (34)	5% (2)	10% (4)
10.	Penicillin-G	10% (4)	0% (0)	90% (36)
11.	Trimethoprim	37.5% (15)	20% (8)	42.50% (17)
12.	Tetracycline	72.5% (29)	7.5% (3)	20% (8)
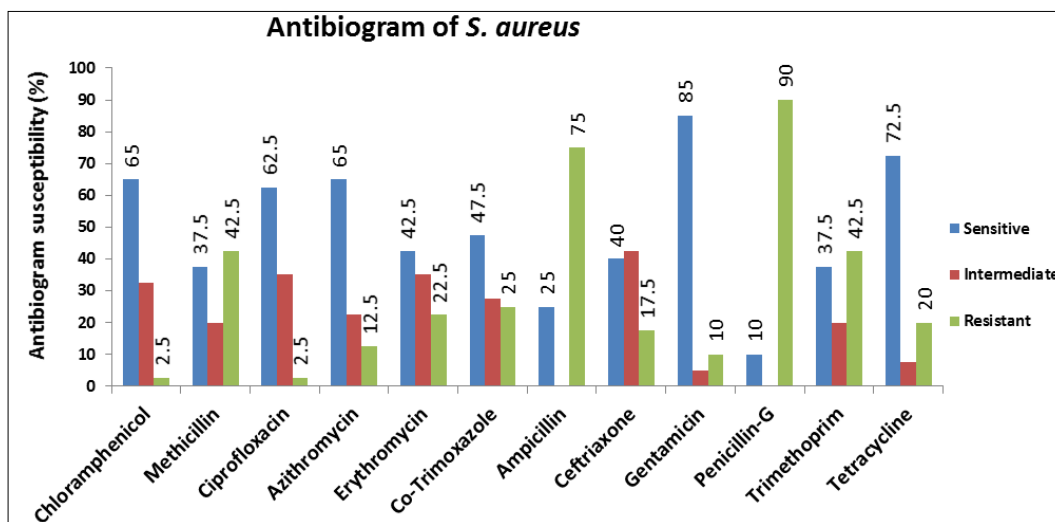
Fig 1: Antibiogram of *S.aureus* isolates from cattle milk

Discussion
In the present study, the prevalence of *S. aureus* was recorded as 66.66% in cattle milk samples. Similar results were also reported by Sarkar *et al.,* (2014) who recorded 74.5% prevalence of *S. aureus* in milk samples [10]. However, lower prevalence was reported by Abdel All *et al.,* (2010); Samaha *et al.,* (2012); El-Jakee *et al.* (2013) and Gwida and El-Gohary (2013) from the raw milk which was 43.1%, 44%, 41.2% and 56%, respectively [11, 12, 13, 14].

In the present study high prevalence of *S. aureus* is a cause of concern for public health but it is supposed that the prevalence will get decreased after heat treatment of milk. Similar findings were observed in the study conducted by Akindolire *et al.* (2015), who recorded the prevalence of *S. aureus* in pasteurized milk (13%) which was comparatively low when compared with that of raw milk (75%) [15].

In the present study high resistance towards Penicillin-G, Ampicillin and Trimethoprim was recorded in the *S. aureus* isolates recovered from dairy ecosystem. Similar findings, were observed by Thaker *et al.,* (2013) who indicated that the overall high percent of *S. aureus* isolates were resistant to Penicillin- G (100.00%) and Ampicillin (40.00%) [16]. Also it was reported by Sudhanthiramani *et al.,* (2015) that the antibiotic susceptibility test of positive isolates showed high resistance towards Penicillin- G 37/43 (86.04%) and Ampicillin 32/43 (74.42%) [17].

High prevalence rate of MRSA was recorded by Helal *et al.,* (2015) who reported a prevalence rate of 66.6% in human

nasal swab samples and 77.7% in mastitic milk samples [18]. Slightly, high prevalence of MRSA was recorded by Nusrat sss*et al.,* (2015) who reported prevalence of MRSA as 58.82% in raw milk cheese samples [19].While a lower prevalence of MRSA was observed in the findings of Kumar *et al.,* (2011), Sudhanthiramani *et al., (*2015) and Thaker *et al.,* (2013) as 13.1%, 13.95% and 0% respectively [16, 17, 20].

Conclusion

In this preliminary study, *S. aureus* were isolated from 66.66% (40/60) of raw milk samples collected from small holder dairy farm, which clearly indicated that there is a possibility of potential public health threat through consumption of milk and milk products. All these isolates of milk presented multiple drug resistance for more than 2 drugs. This kind of practice may lead to serious public health issues like food-borne intoxication, or transfer of antibiotic resistant *S. aureus* to the human population. Antimicrobial resistance is becoming an extremely serious global health problem. Methicillin resistant *Staphylococcus aureus* (MRSA) is one of the highest ranking pathogens worldwide and represents a real challenge to the clinical practice with significant public health concern.

References

1. Odeyemi OA. Public health implications of microbial food safety and food-borne diseases in developing countries. Food & Nutrition Research, 2016, 60.
2. Kadariya J, Smith TC, Thapaliya D. *Staphylococcus aureus* and staphylococcal food-borne disease: an ongoing challenge in public health. BioMed research international, 2014.
3. Zouharova M, Rysanek D. Multiplex PCR and RPLA Identification of *Staphylococcus aureus* enterotoxigenic strains from bulk tank milk. Zoonoses and Public Health. 2008; 55(6):313-9.
4. Vysakh PR, Jeya M. A comparative analysis of community acquired and hospital acquired methicillin resistant *Staphylococcus aureus*. Journal of Clinical and Diagnostic Research: JCDR. 2013; 7(7):13-39.
5. Valaperta R, Tejada MR, Frigerio M, Moroni A, Ciulla E, Cioffi S *et al. Staphylococcus aureus* nosocomial infections: the role of a rapid and low-cost characterization for the establishment of a surveillance system. New Microbiologica. 2010; 33(3):223-32.
6. Castelani L, Santos AF, dos Santos Miranda M, Zafalon LF, Pozzi CR, Arcaro JR. Molecular typing of mastitis-causing *Staphylococcus aureus* isolated from heifers and cows. International Journal of Molecular Sciences. 2013; 14(2):4326-33.
7. Courter RD, Galton MM. Animal staphylococcal infections and their public health significance. American Journal of Public Health and the Nations Health. 1962; 52(11):1818-27.
8. Kumar M. Multidrug-resistant *Staphylococcus aureus*, India, 2013–2015. Emerging Infectious Diseases. 2016; 22(9):1666.
9. Hiramatsu K, Katayama Y, Matsuo M, Sasaki T, Morimoto Y, Sekiguchi A, Baba T. Multi-drug-resistant *Staphylococcus aureus* and future chemotherapy. Journal of Infection and Chemotherapy. 2014; 20(10):593-601.
10. Sarkar P, Mohanta D, Debnath C. *Staphylococcus aureus* in dairy animals and farm workers in a closed herd in Karnal, North India: Assessment of prevalence rate and COA variations. International Journal of Innovative Research in Science, Engineering and Technology. 2014; 3(4):10962-72.
11. Abdel All, AABashandy MM, Yasin MH, Ibrahim AK. Assessment of Conventional and Molecular Features of *Staphylococcus aureus* Isolated from Bovine Milk Samples and Contact Dairy Workers. Global Veterinaria. 2010; 4(2):168-175, ISSN 1992-6197.
12. Samaha HA, Haggag YN, Nossair MA, Mohammad HS. Using some recent techniques in diagnosis of some zoonotic bacterial diseases transmitted through milk. Alexandria Journal of Veterinary Sciences. 2012; 35(1):11-21.
13. El Jakee J, Marouf SA, Ata NS, Abdel Rhaman EH, Abd El Moez SI, Samy AA *et al*. Rapid method for detection of *Staphylococcus aureus* enterotoxins in food. Global Veterinaria. 2013; 11(3):335-341.
14. Gwida MM, El Gohary FA. Zoonotic bacterial pathogens isolated from raw milk with special reference to Escherichia coli and *Staphylococcus aureus* in Dakahlia Governorate, Egypt. Open Access Sci. Rep. 2013; 2(4):705-8.
15. 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(9):10254-75.
16. Thaker HC, Brahmbhatt MN, Nayak JB, Thaker HC. Isolation and identification of *Staphylococcus aureus* from milk and milk products and their drug resistance patterns in Anand, Gujarat. Veterinary World. 2013; 6(1):10-3.
17. Sudhanthiramani S, Swetha CS, 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(4):478.
18. Helal GT, El Enbaawy MI, Nasef SA. Genetic Expression of MecA Gene in Methicillin Resistant *Staphyococcus aureus* (MRSA) Strains of Animal and Human Samples. Journal of Microbiology Research. 2015; 5(3):77-83.
19. Nusrat J, Ifra TN, Mrityunjoy A. Detection of methicillin-resistant *Staphylococcus aureus* within raw milk and cheese samples. International Food Research Journal. 2015; 22(6):2629.
20. Kumar R, Yadav BR, Singh RS. Antibiotic resistance and pathogenicity factors in *Staphylococcus aureus* isolated from mastitic Sahiwal cattle. Journal of biosciences. 2011; 36(1):175-88.