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Prevalence and antibiotic resistance pattern of *Staphylococcus aureus* in cattle of gaushalas from Bikaner (Rajasthan) region

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Abstract
This investigation was carried out from August 2018 to December 2018 to determine the prevalence of staphylococcus in milk and animal drinking water and on cattle udder and milkers' hand and rate of contamination (sample were collected) from seven different Gauashla of Bikaner district. The prevalence rate varies from 35.7% to 92.85% among sample collected from different sources (*viz*. cattle milk, swabs from cattle udder, swabs from milkers' hand and animal drinking water samples). The antibiotic susceptibility test was done for all samples, 82.97% isolate found resistant to Trimethoprim (82.97%) whereas moderately resistant towards Amoxyclav (48.93%). From this study, it is considered that staphylococcosis is a potential hazard for the public and contamination rate is high in distribution site which needs improvement of personnel hygiene and farmers should be educated about the prudent use of antibiotics.

Keywords: Milk, cattle udder, milker human hand swab and animal drinking water

Introduction
Staphylococcus aureus is a gram-positive bacterium that colonizes a variety of animal species. *S. aureus* infections in animals are most commonly reported as a cause of mastitis in dairy animals and 'bumble foot' in chickens [1]. The major reservoirs of *S. aureus* are infected udders, teat canals and teat lesions, but these bacteria also have been found on teat skin, muzzles, and nostrils. The bacteria are spread to uninfected quarters by teat cup liners, milker's hands, wash cloths, and flies. Staphylococci do not persiston healthy teat skin, readily colonize in damaged skin and teat lesions. The organisms multiply in infected lesions and result in increased chance of teat canal colonization and subsequent udder infection [2]. *S. aureus* produces several staphylococcal virulence factors, including enterotoxins (SEA TO SEE AND SEG TO SEQ), and other toxins, such as exfoliative toxin A and B, AND toxic shock syndrome toxin (TSST-1) [3]. Mammary glands infected by *S. aureus* are the main cause of milk contamination [4]. Contaminated milking equipment and the hands of the milkers are also common vehicles of transmission [5].

Materials and Methods
Sample collection: A total of 168 samples were collected from August 2018 to December 2018from seven different Gauashlas of Bikaner district, India. Out of them, 70 samples were of cattle milk and 14 animal drinking water and 70 swabs from the surface of cattle udder and 14 swabs from milkers' hand. The samples were collected in sterilized milk collecting tubes and transported to laboratory of the Department of Veterinary Microbiology and Biotechnology, College of Veterinary and Animal Science, Bikaner.
Isolation and Identification of *Staphylococcus aureus*. Enrichment was carried out in Peptone Water enrichment broth and enriched for 24 hrs at 37 ˚C. The medium used for isolation of *S. aureus* was Mannitol salt agar (MSA). A loopful of inoculums 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.

Results

1. Prevalence of staphylococcus aureus

In 168 samples, the prevalence of *S. aureus* were examined for bacteriological status in cattle milk and animal drinking water and on udder surface of cattle and human hand samples and observed as 67.14% (47), 35.7% (5),54.28% (38) and 92.85% (13), respectively (Table 1). Highest prevalence of *S. aureus* (92.85%) was in Human (milkers) hand swab sample.

Table 1: Prevalence of *Staphylococcus aureus*

S. No.	Type of sample	Sample size	No. of isolates	Prevalence
1.	Cattle milk sample	70	47	67.14%
2.	Cattle udder swab sample	70	38	54.28%
3.	Human hand swab sample	14	13	92.85%
4.	Animal drinking water sample	14	5	35.7%
5.	Total	168	103	61.30%

2. Antibiotic sensitivity and resistant pattern of *S. aureus*

***In milk samples*:** In the present investigation, 8 different antibiotics were used in order to obtain antibiogram for 47 isolates of *S. aureus* recovered from cattle milk. The analysis of antibiogram obtained (Table 2) revealed that most effective antibiotic was chloramphenicol (89.36%) followed by Cefepime to which 82.97% of the isolates were sensitive, 70.22% isolates were sensitive to gentamicin, ceftriaxone and tetracycline, 51.33% to ciprofloxacin, 25.53% to Amoxyclav and12.76% to Trimethoprim. Trimethoprim showed highest resistance (82.97%) followed by Amoxyclav (48.93%). While 25.53% isolates were resistant to gentamicin, 19.14% to ciprofloxacin and tetracycline, and other antibiotics were still less resistant.

Table 2: Antibiogram of *S. aureus* isolates from cattle milk

S. No.	Antibiotic	Percent (%)		
		Sensitive	Intermediate	Resistant
1.	Amoxyclav	25.53%(12)	25.53%(12)	48.93%(23)
2.	Gentamicin	70.22%(33)	4.25%(2)	25.53%(12)
3.	Ceftriaxone	70.22%(33)	19.14%(9)	10.63%(5)
4.	Cefepime	82.97%(39)	8.51%(4)	8.51%(4)
5.	Chloramphenicol	89.36%(42)	6.38%(3)	4.25%(2)
6.	Ciprofloxacin	51.06%(24)	29.78%(14)	19.14%(9)
7.	Tetracycline	70.22%(33)	10.63%(5)	19.14%(9)
8.	Trimethoprim	12.76%(6)	4.25%(2)	82.97%(39)

On cattle udder surface: The antimicrobial resistance profile of the tested 38 isolates of *S. aureus* obtained in cattle udder surface swabs to 8 antibiotics (Table3). Profile revealed that 39.47% isolates were sensitive to tetracycline while 21% isolates were sensitive to Ciprofloxacin, 7.89% isolates were sensitive to Gentamicin, Ceftriaxone, Chloramphenicol and rest antibiotics (Amoxyclav, Cefepime and Trimethoprim) were not effective.It was also reported that isolates were found resistant to Amoxyclav and Trimethoprim (100%), while 68.42% isolates were found resistant to cefepime, 63.15% isolates to gentamicin, 47.36% isolates to ceftriaxone, 39.47% isolates to Ciprofloxacin and 18.42% isolates to Chloramphenicol.

Table 3: Antibiogram of *S. aureus* isolates from cattle udder samples

S. No.	Antibiotic	Percent (%)		
		Sensitive	Intermediate	Resistant
1.	Amoxyclav	-	-	100%(38)
2.	Gentamicin	7.89%(3)	28.94%(11)	63.15%(24)
3.	Ceftriaxone	7.89%(3)	44.73%(17)	47.36%(18)
4.	Cefepime	-	31.57%(12)	68.42%(26)
5.	Chloramphenicol	7.89%(3)	73.68%(28)	18.42%(7)
6.	Ciprofloxacin	21.05%(8)	39.47%(15)	39.47%(15)
7.	Tetracycline	39.47%(15)	39.47%(15)	21.05%(8)
8.	Trimethoprim	-	-	100%(38)

On human hand (milker's hand): Thirteen isolated of *S. aureus* were found in milkers' hand swab samples. Antibiogram against these 13 isolates of *S. aureus* were studied and results are presented in Table3.Out of the 8 antibiotics only one namely Cefepime was detected effective against 30.76% isolates, followed by Gentamicin, Ceftriaxone and Tetracycline (23.07%) whereas Ciprofloxacin showed 15.38% efficacy. But Amoxyclav, Chloramphenicol and Trimethoprim were not effective. Highest resistance was shown towards Amoxyclav and Trimethoprim (100%), then towards gentamicin and tetracycline (53.84%) and 46.15% isolates were resistance to Cefepime and ciprofloxacin and other antibiotics were still less resistant.

Table 4: Antibiogram of *S. aureus* isolates from human hand swab samples

S. No.	Antibiotic	Percent (%)		
		Sensitive	Intermediate	Resistant
1.	Amoxyclav	-	-	100%(13)
2.	Gentamicin	23.07%(3)	23.07%(3)	53.84%(7)
3.	Ceftriaxone	23.07%(3)	53.84%(7)	23.07%(3)
4.	Cefepime	30.76%(4)	23.07%(3)	46.15%(6)
5.	Chloramphenicol	-	100%(13)	-
6.	Ciprofloxacin	15.38%(2)	38.46%(5)	46.15%(6)
7.	Tetracycline	23.07%(3)	23.07%(3)	53.84%(7)
8.	Trimethoprim	-	-	100%(13)

In animal drinking water: Five isolates were obtained in 14 drinking water samples from 7 different gaushalas and antibiogram against these 5 isolates of *S. aureus* were studied (Table 5).Out of the 8 antibiotics, Cefepime was found to be most effective (60%) against *S. aureus* isolates that were obtained from animal drinking water samples. And 20% of the isolates were sensitive to chloramphenicol, ciprofloxacin and tetracycline. But Amoxyclav, Gentamicin, Ceftriaxone and Trimethoprim are not effective. Highest resistance (100%) was shown towards Amoxyclavfollowed by Chloramphenicol, Tetracycline and Trimethoprim (80%) while 60% isolates were resistant to gentamicin and 20% isolates were resistant to Cefepime, Ceftriaxone and Ciprofloxacin.

Table 5: Antibiogram of *S. aureus* isolates from animal drinking water samples

S. No.	Antibiotic	Percent (%)		
		Sensitive	Intermediate	Resistant
1.	Amoxyclav	-	-	100%(5)
2.	Gentamicin	-	40%(2)	60%(3)
3.	Ceftriaxone	-	80%(4)	20%(1)
4.	Cefepime	60%(3)	20%(1)	20%(1)
5.	Chloramphenicol	20%(1)	-	80%(4)
6.	Ciprofloxacin	20%(1)	60%(3)	20%(1)
7.	Tetracycline	20%(1)	-	80%(4)
8.	Trimethoprim	-	20%(1)	80%(4)

Discussion

In the present study, the prevalence of *S. aureus* was recorded as 67.14% in cattle milk sample. Similar results were also reported by Akriti *et al.*, (2019) [6] who recorded 66.66% prevalence of *S. aureus* in milk samples. It was also reported by Abdel All *et al.*, (2010) [7]; and El-Jakee *et al.*, (2013) [8] that the prevalence of *S. aureus* from the raw milk was 43.1%, and 41.2% respectively. Similarly in our study, the prevalence of *S. aureus* in human hand swab samples was recorded as 92.85% which was in accordance with the findings of El-Gohary *et al., (*2016) [9] who reported a prevalence of 67.5% in hand swab samples. However, results of study results at Bikaner region showed prevalence of 67.14% (47/70) which were very high compared to other studies conducted by Thaker *et al*., (2013) [10]; who reported 6.25% in Gujarat region of North India. Sarkar *et al.*, (2014) [11]; documented 74.5% (149/200) of the milk samples were positive for *S. aureus* from the Karnal, North India, it is higher than our study. However, lower prevalence has been previously reported by Fagundes *et al*. (2011) [13] [10.8%] from São Paulo state, Brazil, Ayano *et al*. (2013) [14] [13.8%] from Holeta, Ethiopia, Ekici *et al*. (2004) [18.18%] from Turkey. From all these study results of above mentioned indicates prevalence of *S. aureus* is varied from place to place and regions to regions around the world and it highlights that hygienic practice of milking and selling influence the prevalence of *S. aureus* in milk.

In the present study high resistance towards Trimethoprim (82.97%) and Amoxyclav (48.93%) was recorded in the *S. aureus* isolates recovered from dairy ecosystem. The finding of Alian *et al.*, (2012) [12] indicated the resistance to Trimethoprim (17.4%).

Conclusions

Staphylococci are expected to be among the organisms that contaminate animal udder by handlers. The order of severity of prevalence *S. aureus* was observed as Gaushala worker>cattle milk>cattle udder>animal drinking water.It is indicative of poor hygienic measures. Present study revealed that the prevalence rate of *S. aureus* in Gaushala worker hand and cattle milk sample were 92.85% and 67.14%, respectively. Gaushala workers are usually the main contamination source. Therefore, *S. aureus* is a good indicator for the personal hygiene of workers at the Gaushala. *S. aureus* which is one of the causes of mastitis in cows could have its source from milk handlers. Thus, it is evident that the resistance against the commonly used drugs is increasing day by day. Therefore, the antibiotic should be used judiciously to treat livestock disease. Also, the farmers should educated about the prudent use of antibiotics.

The presence of *S. aureus* in animal drinking water can deteriorate the overall microbiological quality of potable water and causes long term persistence of bacteria. The presence of pathogenic bacteria in milk is of immense public health significance. Milk can be contaminated by *S. aureus* when there is infection of mammary gland or by bad hygiene habits like not washing hands during and after milking, where, human activity is responsible for the contamination. The animal itself is possible source of milk contamination by pathogenic bacteria. 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. Contamination with *S. aureus* may also come through improper sanitary management of farm animals, dirty udder of the milking animals, milking vessels, and the milk handlers.

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