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STAPHYLOCOCCUS AUREUS in DAIRY ANIMALS and FARM WORKERS in a CLOSED HERD in KARNAL, NORTH INDIA: ASSESSMENT of PREVALENCE RATE and *COA* VARIATIONS

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Abstract: In order to isolate and characterize *S. aureus* from milk of dairy animals and nasal swabs of farm workers, the present study was conducted on a total of 200 milk samples from lactating animals (including 50 Murrah buffaloes, 90 indigenous Sahiwal and 60 crossbred Karan Fries cattle) and 50 nasal swabs of farm workers in an organized dairy farm in Karnal, North India. The collected samples were cultured on mannitol salt agar and presumptive *S. aureus* colonies were confirmed phenotypically (coagulase, catalase and indole test) and genotypically (PCR amplification of nuc gene). Genotypic variation among *S. aureus* isolates from different sources was studied by amplifying the 3' hypervariable region of the coagulase gene. 73.6% of all the samples, 74.5% of the milk and 70% of the nasal swabs were tested positive. Highest prevalence rate was observed in milk from crossbred cattle (88.33%) followed by indigenous cattle (80%) and buffalo (48%). 89.74% of previously treated animals and 53.01% of the healthy animals were tested positive. Amplification of the coagulase gene from the milk isolates produced a single PCR product of 600-bp whereas the nasal swab isolates produced five different PCR products of sizes 600 (10 isolates), 680 (14 isolates), 790 (8 isolates), 950 (1 isolate) and 1000-bp (1 isolate). Absence of variation among the milk isolates shows the importance of maintaining a close herd to prevent the entry of new *S. aureus* strains in the herd. However, the common existence of 600-bp genotype indicates transmission of the isolates between the species.

Keywords: *Staphylococcus aureus*, Prevalence, Mastitis, Coagulase, Genotype.

I. INTRODUCTION

S. aureus is recognized worldwide as a leading pathogen causing many serious diseases in dairy and healthcare surroundings. Approximately 20–30% of human population carries *S. aureus*, in their anterior nares [1]. Both healthy carriers and infected individuals can transmit *S. aureus* directly or indirectly to others. In bovines, *S. aureus* mainly causes mastitis with subsequent contamination of milk and dairy products [2]. Typically, *S. aureus* of human lineages rarely colonize animals, suggesting a host range barrier [3]. Studies have predicted human-to-bovine transmission by recovering *S. aureus* clones from cattle that are closely related to those obtained from humans [4]. Moreover, livestock can also act as a reservoir for the emergence of new human bacterial clones with potential for pandemic spread, highlighting the potential role of surveillance and biosecurity measures in the agricultural setting for preventing the emergence of new human pathogens [5].

Several studies have been conducted worldwide [6] and in India [7] to evaluate the prevalence of *S. aureus* in milk. Studies have also been carried out to determine the relatedness of livestock and human associated isolates of *S.*

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*aureus*in different sectors [8]. However studies on the molecular epidemiology of *S. aureus* collected from different species of bovines and their handlers from different dairy farms in India are still very scarce.

S. aureus isolates can be phenotypically characterized by using a number of methods which include gram staining, typical colony morphology, mannitol fermentation, coagulase test and other biochemical tests. Genotypic identification of *S. aureus* is generally carried out by DNA isolation followed by amplification of a species specific gene or a part of it. For the first time, the method of genotyping with specific-primer targeting *nuc* gene was developed for *S. aureus* by Brakstad et al. [9], where a PCRproduct of 270-bp size was found to be specific for this organism. This method has since been used by various workers for genotypic confirmation of *S. aureus* from different sources [10]. Likewise, amplification of *aroA* gene [11] and 23S rRNA gene [12], specific to *S. aureus* has also used for genotypic identification of this organism.

Control of *S. aureus* infection can be accompanied by epidemiological typing of *S. aureus*. These typing methods may clarify whether colonized strains from the animals or from their handlers are related to those that cause infection and whether isolates from one source belong to one genotype. Molecular typing methods are valuable in demonstrating the evolutionary and clonal relationships between isolates [13]. Among these methods, coagulase (*coa*) gene typing is considered a simple and effective method for typing *S. aureus* isolates from human patients and bovine mastitic milk [14]. Coagulase production is the principal criterion used by clinical laboratories to distinguish pathogenic *S. aureus* from other staphylococci. Moreover, the *coa* gene is highly polymorphic and does not always give similar amplicons in PCR amplification methods due to the presence of varying number of 81-bp tandem repeats at the 3′ end of this gene in different *S. aureus* strains [15]. This property of the *coa* gene has been exploited by researchers in the typing of *S. aureus* strains where different PCR products are obtained from different strains [16].

A rapid and reliable identification method of *S. aureus* colonies and their genetic characterization in cultures from milk and nasal swabs is primary to the control of *S. aureus* infection in bovines and human. However, limited information with respect to the genetic characterization of *S. aureus* in dairy animals and animal workers in India is available. Keeping the above facts in view the current study was designed to (a) detect *S. aureus* in collected milk samples from animals previously treated for mastitis and healthy animals from a dairy farm, (b) isolate *S. aureus* from nasal swabs of dairy animal workers from the same farm, and (c) examine the prevalence of carriage and variation of *S. aureus* strains among the animals and the workers.

II. MATERIALSAND METHODS

A.Clinical samples

1) *Raw milk:* From 32 Murrah buffaloes, 45 indigenous Sahiwal cattle and 40 crossbred Karan-Fries cattle, treated for mastitis (TFM) within a period of nine months (August, 2012 to April, 3013) from an organized farm in Karnal, North India.

2) *Raw milk:* From healthy 18 Murrah buffalo, 45 indigenous Sahiwal cattle and 20 cross-bred Karan-Fries cattle, with no history of mastitis from the same farm.

3) *Nasal swabs:* From 50 apparently healthy animal workers from the same farm.

B.Sample collection

1) *Milk:* Raw first milk from each udder was collected in sterile 15 ml centrifuge tubes from all the TFM and healthy animals under aseptic condition and quickly transported on ice to the Animal genomics laboratory, NDRI. The milk from all four udders of each animal were pooled and stored at 4°C until further processing. All samples were assigned an animal study ID and date.

2) *Nasal swab:* Sample collection was accomplished by using sterile swabs inserted approximately 2 cm into one naris, rotated against the anterior nasal mucosa and repeated with same swab in second naris. The swabs were transported on ice to the Animal genomics laboratory, NDRI and inoculated directly into 2 ml of enrichment broth (10 g tryptone, 75 g NaCl, 10 g mannitol, 2.5 g yeast extract in 1 litre H_2O). The samples were stored at 4°C until further processing. All samples were assigned an animal worker study ID and date.

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C.Isolation and phenotypic identification of bacterial isolates

0.5 ml of pooled milk from individual animal was inoculated into 4.5 ml of Mueller Hinton Broth and incubated along with the nasal swab samples for 24 hours at 37°C in a shaker cum incubator. All the samples were streaked onto Mannitol salt agar plates and then incubated for 24-48 hours at 37°C. The plates were examined for characteristic colony morphology and then subjected to Gram staining and microscopic observation for identification of the *Staphylococcus* genus. Single suspect colony from each sample plate was streaked onto Nutrient Agar slants and further identified by different biochemical tests.

1) *Tube coagulase test:* A loopful of test culture was inoculated into 5 ml of Nutrient Brothand incubated overnight at 37°C. 0.1 ml of the overnight broth culture was added to 0.5 ml of rabbit plasma (1:10 diluted with 0.9% NaCl) and incubated for 4 hours at 37°C. The time taken by each isolate to coagulate the rabbit plasma was observed at an interval of 1 hour. Isolates showing no coagulation after 4 hours were incubated overnight at 37°C. A negative control containing only diluted rabbit plasma was set to check for any false positive result.

2) *Indole test:* A loopful of test culture was inoculated into 5 ml of 2% peptone water media (2 g peptone, 0.5 g NaCl in 100 ml H_2O) and incubated for 48 hours at 37°C. To this was added 0.5 ml of Kovac's reagent (150 ml amyl alcohol, 10 g *p*-dimethyl-aminobenzaldehyde, 50 ml conc. HCL) and gently shaken. Absence of red color at the top of the alcohol layer indicated negative reaction and hence *S. aureus*.

3) *Catalase test:* A loopful of test culture was inoculated into 5 ml of a medium containing 10 g peptone, 10 g yeast extract, 10 g glucose, 10 g NaCl, 1.5 g agar powder in 1 lit H_2O and incubated for 24 hours at 37°C. Then a loopful of the above culture was inoculated into a tube containing 3% hydrogen peroxide and the results were noted. Production of bubbles indicated positive reaction and hence *S. aureus*.

D.Genotypic identification of bacterial isolates

1) *DNA isolation:* The Phenol-Chloroform extraction method (with modifications) was followed to isolate DNA from all the phenotypically positive isolates. A loopful of test culture was inoculated into 5 ml of nutrient broth and incubated overnight at 37°C in a shaker cum incubator. 5 µl of ampicillin (100 mg ampicillin ml^{-1}) was added to the culture and incubated for 1 hour at 37°C. Bacterial cells were collected by centrifuging 4.5 ml of the above culture at 5000 rpm for 5 min and the pellet was washed thrice with 1 ml NaCl:EDTA (30 mM:2 mM). Finally the pellet was re-suspended in 100 µl of NaCl:EDTA and transferred to a 2 ml centrifuge tube. To this was added 100 µl of lysozyme (10 mg lysozyme ml^{-1}) and 4 µl of RNaseA (100 µg of RAaseA ml^{-1}) and incubated for 1 hour at 37°C. Then 50 µl of 10% SDS in NaCl:EDTA was added to the above suspension and incubated for 1 hour at 55°C. Equal volume of Tris-saturated phenol (pH- 7.8) was added and mixed properly by inverting the centrifuge tube a number of times. The suspension was centrifuged at 10,000 rpm for 10 min at room temp° and the upper aqueous phase was transferred to a new 2 ml centrifuge tube. DNA was purified by adding equal volume of Phenol:Chloroform:Isoamyl alcohol (25:24:1) to the collected upper phase and mixed properly by inverting the centrifuge tube a number of times. The suspension was centrifuged at 10,000 rpm for 10 min at room temp° and the upper phase was transferred to a new 2 ml centrifuge tube. DNA was precipitated by adding 0.8 volumes of chilled isopropanol and 0.3 M sodium acetate to the collected phase and the suspension was mixed properly until it turned clear. The suspension was centrifuged at 10,000 rpm for 10 min at 4°C and the pellet obtained was washed twice with 70% ice cold ethanol. Finally the pellet was air-dried and dissolved in 50 µl of 1X TE (Tris-EDTA) and incubated at 55°C for 15 min. Concentration and purity of the isolated DNA was determined spectrophotometrically (2 µl) by taking the ratio of absorbance at 260 nm (A_{260}) and 280 nm (A_{280}). The integrity of the isolated DNA was determined by visualizing it in 0.8% TAE (1X Tris-acetate-EDTA)-agarose gel containing 0.5 µg/ml ethidium bromide [17]. The gel was then photographed under UV illumination in a Gel doc XR imaging system. DNA suspension was stored at -20°C until further use.

2) *nucgene amplification:* To determine the presence of the *nuc* gene (present only in *S. aureus*), polymerase chain reaction (PCR) was performed on all the phenotypically positive isolates using `5′-GCGATTGATGGTGATACGGTT-3′` as the forward primer and `5′-AGCCAAGCCTTGACGAACTAAAGC-3′` as the reverse primer (Product size- 270-bp) [18]. PCR was performed in a 25-µl volume with 2.5-µl of 10X Standard Taq Reaction Buffer containing 3 mM $MgCl_2$, 2.5-µl of 2 mM dNTP mix, 0.5-µl each of 10 µM forward and reverse primers, 0.2-µl of Dream Taq DNA polymerase,

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0.5-µl of template DNA (as prepared above) and ddH_2O up to 25-µl. Thermocycling conditions in the GeneAmp 9600 thermocycler were as follows: 94°C for 4 min (initial denaturation), followed by 35 cycles of 94°C for 1 min (denaturation), 62°C for 30 s (annealing), 72°C for 1 min (extension), with a final extension at 72°C for 4 min.
Electrophoresis at 100 V for 30 min was performed to visualize the PCR amplicon on a 1.5% TAE-agarose gel containing 0.5 µg/ml ethidium bromide [17]. The gel was then photographed under UV illumination in a Gel doc XR imaging system. PCR control organism was used with each batch of samples and included *Staphylococcus aureus* ATCC 29213 (*nuc* positive). Master-mix without DNA template was used as negative control. The size of the amplicon was determined by comparing it with a 100-bp DNA ladder.

E.Coagulase gene-based typing of the bacterial isolates

To determine the presence of the *coa* gene, PCR was performed on all the phenotypically and genotypically positive isolates using 5'-ATAGAGATGCTGGTACAGG-3' as the forward primer and 5'-GCTTCCGATTGTTCGATGC-3'as the reverse primer [16].PCR was performed in a 25-µl volume with components similar to that used for amplification of *nuc* gene except for the primers. Thermocycling conditions in the GeneAmp 9600 thermocycler (Applied Biosystems) were same as above except for the annealing temperature being 60°C instead of 62°C. The amplicon was visualized and its size was determined as mentioned above for the *nuc* gene.

F.Statistical analysis

A Chi-square test was used to determine the significance of the prevalence rates of the organism in a host specific group by use of Microsoft Excel Worksheet, 2013. The nominal P value for statistical significance was 0.05.

III. RESULTS

A.Isolation and phenotypic identification of bacterial isolates

On the basis of colony morphology on Mannitol salt agar plates, Gram staining data and different biochemical tests (Fig. 1; Tab. 1), 184 suspected *S. aureus* isolates were identified from a total of 250samples including 200 milk and 50 nasal swabs from animal workers (Tab. 2).

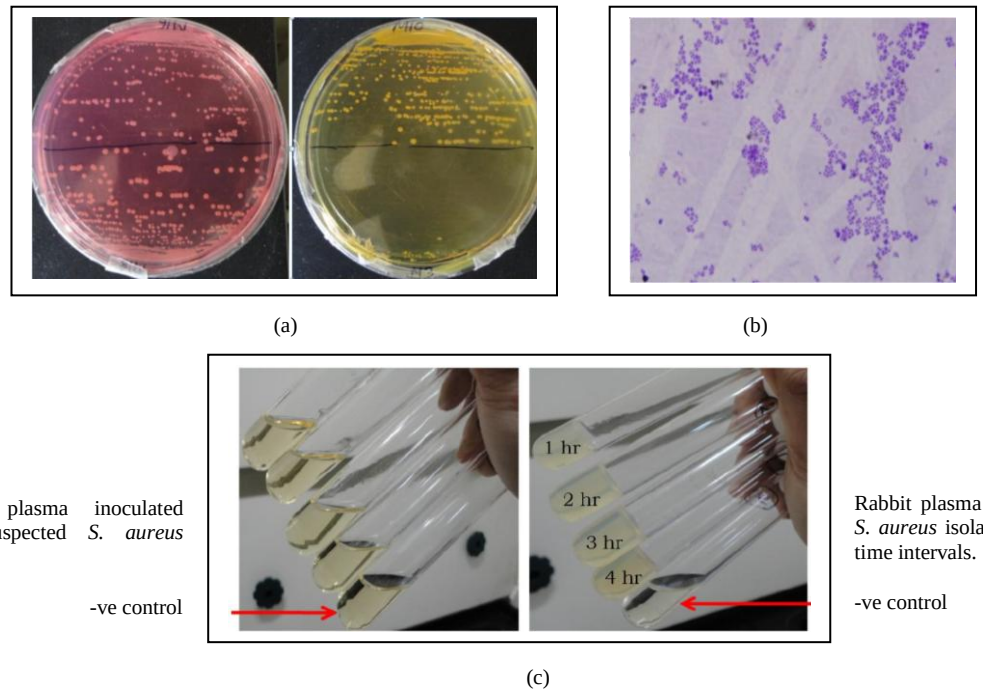

Figure 1. Colony morphology on Mannitol salt agar (Yellow positive; Pink negative) (a), Gram positive cocci in clusters (b), Coagulase test (c).

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Table 1.Number of isolates coagulating rabbit plasma at different time intervals.

Incubation period	Murrah	Sahiwal	Karan-Fries	Animal workers
1 hr	20	46	37	35
2 hr	2	20	8	0
3 hr	1	0	0	0
4 hr	0	4	0	0
Overnight	1	2	8	0

Table 2.Prevalence of *S. aureus* milk and nasal samples from different sources.

	Sources							
	Murrah		Sahiwal		Karan-Fries		Animal workers	Total
History	TFM[a]	Healthy	TFM	Healthy	TFM	Healthy	Healthy	
Subjects screened	32	18	45	45	40	20	50	250
Subjects positive	23	1	43	29	39	14	35	184
Percentage	**72%**	**6%**	**96%**	**64%**	**98%**	**70%**		
Total isolates	24/50		72/90		53/60		35/50	184/250
Percentage	**48%**		**80%**		**88%**		**70%**	**74%**

[a]Animals previously treated for mastitis

Thus the overall prevalence rate of *S. aureus* in the farm was 73.6%(184/250). Out of this 74.5% (149/200) of the milk samples and 70% (35/50) of the nasal swab samples were tested positive. The highest prevalence rate was observed in milk samples from crossbred cattle (Karan-Fries) (88.33%) followed by indigenous cattle (Sahiwal) (80%) and buffalo (Murrah) (48%). Among the TFM, an overall prevalence rate of 89.74% (105/117) and individual prevalence rate of 71.88%, 95.56% and 97.5% was observed in the milk samples of buffalo, indigenous and crossbred cattle respectively. Whereas among the healthy animals an overall prevalence rate of 53.01% (44/83) and individual prevalence rate of 5.56%, 64.44% and 70% was observed in the milk samples of buffalo, indigenous and crossbred cattle respectively.

B.Genotypic identification of bacterial isolates

DNA was isolated from all the isolates by phenol chloroform extraction method (Fig. 2) and the presence of the *nuc* gene was detected by polymerase chain reaction. The *nuc* gene amplicon obtained from all the 184 *S. aureus* isolated from milk and nasal samples was approximately 270-bp in size (Fig. 3).

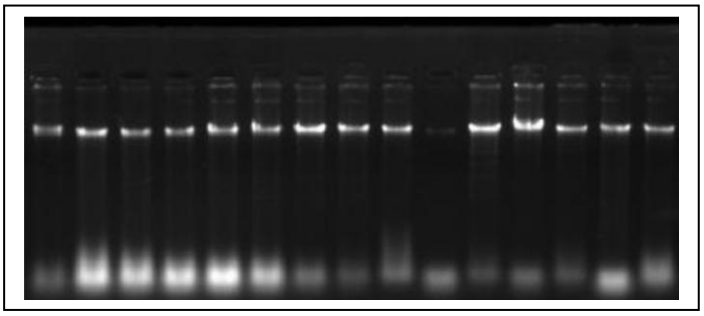

Figure 2. Agarose gel showing DNA isolated by phenol chloroform extraction method.

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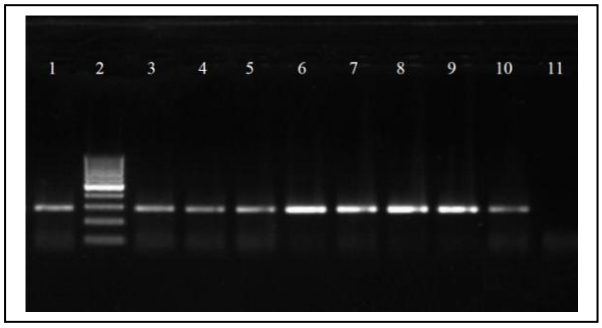

Figure 3. Agarose gel showing PCR-amplified product (270-bp) of nuc gene.
Lane 2, 100-bp DNA ladder. Lane 1, S. aureus ATCC 29213 control. Lane 3 and 4, murrah isolates.
Lane 5 and 6, sahiwal isolates. Lane 7 and 8, KF isolates. Lane 9 and 10, human isolates. Lane 11, reagent control.

C.Coagulase gene-based typing of the bacterial isolates

The presence of the *coa* gene was detected by polymerase chain reaction. The *coa* gene amplicon obtained from all the 149 *S. aureus* isolated from milk was approximately 600-bp in size (Fig. 4). Whereas 34 out of 35 human isolates generated five classes of bands, based on size, ranging from 600-1000-bp (Fig. 5), one isolate did not give any amplification product. The molecular masses of the *coa* PCR products, corresponding number of repeats as well as the number of isolates belonging to each group are summarized in Table 3.

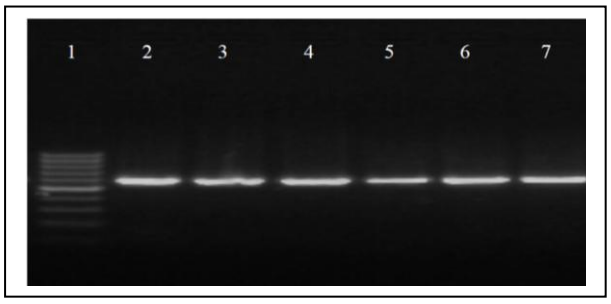

Figure 4. Agarose gel showing PCR-amplified product (600-bp) of *coa* gene from animal isolates.
Lane 1, 100-bp DNA ladder. Lane 2 and 3, murrah isolates. Lane 4 and 5 sahiwal isolates. Lane 6 and 7, KF isolates.

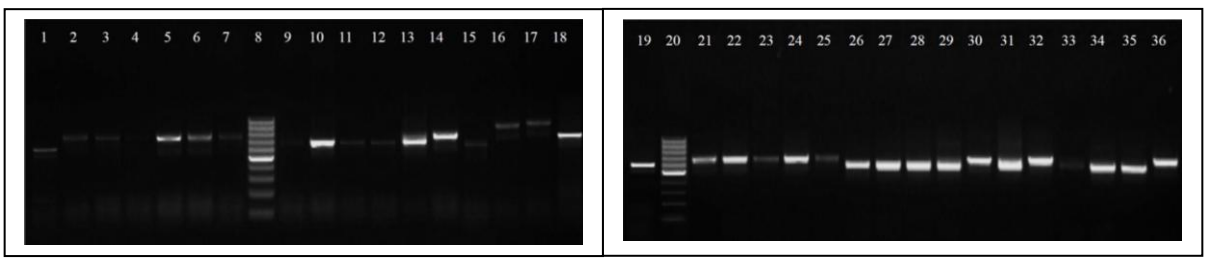

Figure 5. Agarose gel showing PCR-amplified product of *coa* gene from human isolates.
Lane 8 and 20, 100-bp DNA ladder. Lane 1, 19, 26, 27, 28, 29, 31, 33, 34 and 35, 600-bp. Lane 9, 10, 11, 12, 13, 15, 21, 22, 23, 24, 25, 30, 32 and 36, 680-bp. Lane 2, 3, 4, 5, 6, 7, 14 and 18, 790-bp. Lane 16, 950-bp. Lane 17, 1000-bp.

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Table 3: Molecular masses of different *coa* PCR products, corresponding number of repeats and the number of isolates belonging to each subgroup.

Source	Molecular masses of PCR products (bp)	No. of isolates	Repeats
Animal milk	600	149	4
Nasal swabs	600	10	4
	680	14	5
	790	8	6
	950	1	7
	1000	1	8

IV. DISCUSSION

In this study the overall prevalence of *S. aureus* in the farm detected was 73.6%. Source wise, the difference in the prevalence rate between milk (74.5%) and nasal swab (70%) was insignificant ($p<0.05$). A prevalence rate of 40.6% was reported from samples of cow's milk obtained from Hawassa area farms (South Ethiopia) [19]. Prevalence rates of 62% and 84% were reported from bulk tank milk and herd respectively from Minnesota dairy farms (USA) [20]. Variations observed in the prevalence rate of *S. aureus* in milk samples may be a result of variation in the size and geographic region of the area sampled [21]. Variations were also observed when milk samples from different region within a country were studied. In North Karnataka (South India), 53.57% of the milk samples collected randomly from Holstein Freshein (H.F), Jersey, Dharwari and Murrah breeds over a period six months were found positive for *S. aureus* [7]. A prevalence rate of 21.73% in milk samples of dairy animals from various dairy farms in Meerut region (North India), over a period of one year was reported [22]. 62.34% of milk samples collected from mastitic Sahiwal and Karan Fries cattle, and Murrah buffaloes maintained in the institutional experimental herd of NDRI, Karnal, Haryana (North India) was detected positive for *S. aureus* [23]. Although conducted in the same study area as the present study, a difference of approximately 12% is observed in the prevalence rate. Thus farm to farm variations in the prevalence rate of *S. aureus* should be taken into consideration before formulating control measures.

Carriage of *S. aureus* in the anterior nares plays a key role in the epidemiology and pathogenesis of infection. The rate of prevalence (70%) among the animal workers observed in this study could not be compared due to lack of any such earlier studies in India. However a number of studies conducted among the human health care workers are available. In such a study nasal swabs of 96.7% of the health care workers in a hospital (Yavatmal, India) were found to be *S. aureus* positive [24]. When compared to the prevalence rates detected by other workers worldwide, we found that alike the prevalence rate of *S. aureus* in milk, the prevalence rate of *S. aureus* in the anterior nares of animal worker population varies depending upon the size, geographical area and management practices of the herd under study. Using 16S rRNA and *coa* gene amplification, hands of 82.6% and 49.61% contact dairy workers respectively, of Aswan governorate, Egypt were detected positive for *S. aureus* [25]. A 13.2% prevalence rate of *S. aureus* from nasal swabs of 68 dairy farm workers at four different farms around Addis Ababa [26], 41% and 40% among industrial and antibiotic free livestock operational workers respectively in North Carolina [8] and 21.6% from hog slaughter and processing plant workers in the same study area were reported positive for *S. aureus* [27].

Now, considering that the milk samples in the present study were collected and immediately put to test with minimal exposure and negligible chances of contamination from external sources, it seems that bacterial contamination of the milk of most of the animals resulted from excretion of the organisms present in their udder. This is also true in case of the animal workers, most of them carrying the organism on their nasal mucosal membrane. This is important because it has been reported that poor hygienic and farm management practices also contribute to a high percentage of these organisms in milk [28]. Moreover, similar prevalence rate in the milk and the nasal swabs indicated that the animals and the workers were equally exposed to the threat of *S. aureus* infection.

Among the animal sources prevalence rate in crossbred (88.33%) and indigenous cattle (80%) was significantly higher ($p<0.05$) and they seem to be at a greater risk than buffalo (48% prevalence rate). The above result is in accordance to a study reporting buffaloes to be less susceptible to mastitis than cattle [29]. Another study conducted in the institutional experimental herd of NDRI, Karnal, Haryana (North India), indicated a prevalence rate of 69.19% (crossbred cattle), 54.87% (indigenous cattle) and 63.43% (buffalo) [23]. Thus within a herd the prevalence rate can vary among different species and breeds.

During the study period of nine months a total of 117 animals (32 buffalo, 45 indigenous and 40 crossbred cattle) could be identified in the farm which were subjected to treatment for mastitis (TFM). The prevalence rate of *S. aureus* in

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themilk of these animals was 71.88%, 95.56% and 97.5% respectively. This shows that even after treatment the organism persisted in the udder of a major percentage of the animals (sub-clinically) and the rate of persistence was significantly higher in indigenous and crossbred cattle as compared to buffaloes ($p<0.05$). This is presumably due to induction and persistence of antibiotic resistance in biofilm producing *S. aureus* isolates [30].

Also during the study a total of 83 animals (18 buffalo, 45 indigenous and 20 crossbred cattle) were identified in the farm which were healthy. The prevalence rate of *S. aureus* in the milk of these animals was 5.56%, 64.44% and 70% respectively. These bacteria are also of immense importance as they are known for causing over 25% of intra-mammary infections and adversely affecting the quality of milk in a large number of sub-clinical cases [31]. This shows that the chances of getting an intra-mammary infection and hence mastitis was significantly higher in indigenous and crossbred cattle as compared to buffalo ($p<0.05$). Moreover the findings also suggest that among the cattle breeds the crossbred animals are slightly more susceptible to mastitis than the indigenous ones. However, it has been reported in a study that crossbred animals can be as much as 2.55 times more susceptible to mastitis than the indigenous ones [32].

The above data also suggests that the overall and species wise prevalence rate of *S. aureus* in the milk of TFM (overall- 89.74%; species wise- 71.88%, 95.56%, 97.5%) was significantly higher than that of healthy animals (overall- 53.01%; species wise- 5.56%, 64.44%, 70%) ($p<0.05$). Therefore it seems that it is much easier for *S. aureus* to thrive and maintain a constant bacterial load sub-clinically in carrier animals as compared to infecting healthy animals. This is mainly because after decolonization (antibiotic therapy), persistent carriers often become re-colonized with their prior *S. aureus* strain, whereas non-carriers resist colonization [33]. These differences in persistent and non-persistent carriage patterns are critical in determining the risk of subsequent infection and may thus influence the nature of response to potential candidate vaccines [34].

In the present study, *coa* gene amplification from 149 bovine milk *S. aureus* isolates revealed a single PCR amplification product of 600-bp indicating the presence of four repeats. This suggests the predominance of a single *coa* genotype in the herd. Using the same set of primers, the presence of *coa* gene was investigated in 15 *S. aureus* isolates from bovine subclinical mastitis in different herds located in Buenos Aires, Argentina. The PCR amplification yielded 400, 500, 600, 900 and 1000-bp amlicons with 900-bp being the most predominant [35]. 600-bp amplicon as obtained in the present study was obtained from three isolates only. *coa* gene typing (with similar primers), of *S. aureus* isolated from bovine sub-clinical mastitis from a herd in Bikaner city (India) revealed the presence of single 600-bp *coa* genotype and thus was considered as location specific [36].

The absence of any other genotype in the herd could be explained by the fact that being a close herd the entry of any other genotype was restricted and the single genotype was being transmitted among the animals of different species and breed within the herd [37]. However, in a study involving a closed and an open herd prevalence rate of *S. aureus* was found to be equally high due to inefficacy of the management practices (contaminated milking machine, post-milking tit dips, etc.) [38]. This probably explains the absence of variation and high prevalence rate of *S. aureus* in the present study.

Estimation of the predominance and variation of nasal *S. aureus* isolates can give us an idea about the putative *S. aureus* strains colonized at other sites of infection helping the development of preventive strategies against subsequent infections ([39], [40]).The *coa* gene amplification from 34 out of 35 nasal swab *S. aureus* isolates generated a single PCR amplification product of five different sizes. This suggests that the animal handlers were carrying five different *coa* genotypes. 600-bp amplicon (four repeats) was present in 28.57%, 680-bp amplicon (five repeats) was present in 40%, 790-bp amplicon (six repeats) was present in 22.86% and 950-bp amplicon (seven repeats) and 1000-bp amplicon (eight repeats) was obtained from 2.86% isolates. 680-bp amplicon was found to be the most predominant genotype among the nasal swab isolates. Using similar primers 600 ± 20-bp and 700 ± 20-bp amplicons were obtained from *S. aureus* in infected patients [38]. 85% of the isolates gave a 700 ± 20-bp amplicon similar to the present study. Using the same set of primers PCR amplification of *coa* gene in 15 *S. aureus* isolates from human infections (blood, catheter tips, respiratory tract, surgical wound, urine and skin) in different private clinics in Buenos Aires, Argentina yielded 400, 500, 600, 700, 800 and 1000-bp amlicons with 700-bp being the most predominant [35]. Thus, the findings of the present study suggest that the *coa* genotypes 600, 700 (680 in the present study), 800 (790 in the present study) and 1000-bp can colonize anterior nares beside the above mentioned sites of human infection. Moreover in both the studies the most predominant genotypes obtained were almost similar in their sizes (680 ± 20-bp). A size difference of 10-20 base pairs for PCR products of *coa* gene of *S. aureus* has been suggested [16]. The same study also showed the presence of 500 and 900-bp *coa* genotypes among seven *S. aureus* isolates obtained from anterior nares of apparently

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healthy persons. Thus it seems that apart from the genotypes obtained in the present study, 500 and 900-bp *coa* genotypes can also colonize the anterior nares of apparently healthy persons. 700 and 790-bp *coa* gene amplicons were obtained from 26 *S. aureus* isolates from human infected skin and urine samples [41] using same set of primers as used in this study. However, unlike our observation where the 790-bp *coa* genotype was isolated from anterior nares, in this study the same genotype could be detected only in urine. The 950-bp *coa* genotype identified in this study has not been previously reported (using the same primers).One of the isolates did not give any amplification product in spite of being phenotypically positive. That *S. aureus* may be *coa* gene-deficient was reported in a study [36]. Moreover, phenotypically positive but genotypically negative isolate was also reported [42] as found in the present study.

Comparison between the milk and the nasal swab isolates revealed that only a single genotype was common to the animals and the workers (600-bp). Now as this is the only genotype prevalent among all the animals, it seems probable that the animal workers have acquired this genotype from the animals in their contact. This is important because recent studies employing multilocus sequence typing (MLST) and whole-genome sequencing (WGS) have identified several *S. aureus* sequence types (ST) that are associated with multiple host species, implying either zoonotic transmission or a recent common ancestor [43].

Two or more animal workers were found to carry isolates with similar genotypes indicating that the *S. aureus* isolates were being transmitted among them. However, only a single isolate being studied from each individual, it could not be confirmed whether a single individual carried two or more genotypes or not.This is important because co-colonization of *S. aureus* strains, at least with minor frequency variants have been recently reported by in U.K. [44]. The presence of other four genotypes among the animal workers indicates that they might have acquired their load of *S. aureus* not only from the animals they are in contact with but also from some other sources. This is in accordance to the findings that *S. aureus* strains colonizing dairy cows and humans from one farm can differ in their coagulase genotype [45]. This makes the animal workers carrier of these *S. aureus* strains which may be subsequently picked up by the animals in the herd due to persistent exposure [46]. This seems probable because except the 950-bp *coa* genotype identified in this study, the other four *coa* genotypes identified in the animal workers in this study have been frequently reported to be present in different bovine species ([35],[47], [48]).

V. CONCLUSIONS

High prevalence rate of *S. aureus* in the milk and the nasal swab samples collected from the farm is an important finding of this study. Among the animals, crossbred and indigenous cattle were found to be significantly more susceptible to *S. aureus* infection that buffaloes indicating that within a herd the prevalence rate can vary among different species and breeds. It was also concluded that even after treatment the organism persisted in the udder of a major percentage of the animals (sub-clinically), the rate of persistence being significantly higher in indigenous and crossbred cattle as compared to buffaloes. Moreover, it seemed much easier for the organism tothrive and maintain a constant load sub-clinically in carrier animals as compared to infecting healthy animals. Regarding the variations in strains, the milk isolates belonged to a single genotype whereas the nasal swab isolates belonged to five different genotypes. The absence of variation among the milk isolates was the result of the closed herd maintained by the farm. Only a single genotype commonly existed among the animals and the workers and it seemed that the animal workers have acquired this genotype from the animals in their contact. In conclusion, successful strategies to control human and bovine *S. aureus* infections would require efforts directed against highly virulent clones causing diseases in either species. It is therefore important to isolate and characterize *S. aureus* strains associated with bovines as well as the humans in their surroundings.

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