



Prevalence of *Staphylococcus aureus* Isolated from Mastitic Milk, Udder Surfaces and Milkers' Hands from Different Farms in Bikaner, Rajasthan

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Received: 06 Aug., 2018

Revised: 25 Sept., 2018

Accepted: 05 Oct., 2018

ABSTRACT

Staphylococcus aureus is recognized worldwide as one of the most important contagious mastitis pathogen and is frequently isolated from mastitic milk and extramammary sites. The present study was undertaken to study prevalence of *S. aureus* strains isolated from mastitic milk, udder surfaces and milkers' hands from organized (n=5) and unorganized dairy farms (n=2). For this, a total of 197 samples (80 mastitic milk samples, 66 udder swabs and 51 swabs of milkers' hands) were collected from different places. A total of 107 isolates including 51 from mastitic milk samples, 35 from udder and 21 from milkers' hands, were presumptively identified as *S. aureus* on the basis of cultural and biochemical properties and then genotypically confirmed using 23S rRNA ribotyping followed by PCR amplification of *nuc* gene. An overall recovery rate of *S. aureus* was 54.3% with highest (63.8%) recovery from mastitic milk samples followed by udder (53.0%) and milkers' hands (41.2%). The unorganized dairy farm showed highest prevalence (65.4%) of *S. aureus* than that in other farms.

Keywords. Mastitis, *nuc* gene, ribotyping, *Staphylococcus aureus*

Mastitis is one of the most complex and multifactorial disease affecting dairy animals. *Staphylococcus aureus* is one of the chief causative agent of clinical mastitis in bovines in India (Padhy *et al.*, 2014; Kutar *et al.*, 2015; Bhagat *et al.*, 2017; Choudhary *et al.*, 2018) and worldwide (Abdel-Tawab *et al.*, 2016; Hanon, 2017). The milk from infected mammary glands serves as the primary reservoir of the bacterium which may be transferred to other animals in the herd during milking (Capurro *et al.*, 2010). In addition human handlers, milking equipment, environment, udder and teat skin of infected animals are possible sources of infection to the animals. In dairy workers, the hands may be considered the most important site of *S. aureus* because the hands are in intimate contact with the udder.

Although various PCR based detection systems have been developed so far for identification of *S. aureus* but not found sufficiently reliable to detect all strains of *S.*

aureus. A PCR system developed by Straub *et al.* (1999) based on amplification of species-specific 23S rRNA allows specific detection of all strains of species. Thus this method is extensively used throughout the world for genotypic identification and confirmation of *S. aureus* from various clinical and subclinical infections (Salasia *et al.*, 2004; Upadhyay *et al.*, 2010; Khichar *et al.*, 2014; Yadav *et al.*, 2015).

Furthermore the molecular diagnosis of *S. aureus* infection is also carried out by amplification of *nuc* gene (~270 bp) that encodes for thermonuclease enzyme (Kateete *et al.*, 2010; Vremera *et al.*, 2011; Nazir *et al.*, 2017).

Staphylococcus aureus is a contagious mastitis pathogen and its transmission is thought to occur from animal to animal principally through the mastitic milk, udder surfaces, milkers' hands, milking utensils, or milking machine (Radostits *et al.*, 2006). However, its epidemiological behaviour is not definite with strains



signifying infectious and/or environmental transmission patterns (Fernandez *et al.*, 2013). Hence the present study was undertaken to study the molecular epidemiology of *S. aureus* strains in and around Bikaner, Rajasthan, India by 23S rRNA ribotyping and amplification of *nuc* gene.

MATERIALS AND METHODS

Animals and sample collection

In the present study, a total of 197 samples were collected from seven different locations (five organized and one unorganized dairy farm in and around Bikaner, Rajasthan, India and one unorganized dairy farm in Bhiwani, Haryana). Animals of selected locations were diagnosed with clinical mastitis by clinical symptoms. From each location, three types of samples were collected which comprised of milk samples from cows with clinical mastitis, swabs from udder of infected cows and swabs of milkers' hands who were working in that farm or location. A total of 80 mastitic milk samples, 66 udder swabs and 51 swabs of milkers' hands were collected in the morning hours and were immediately transported on ice to the laboratory for further processing as per standard procedures (Quinn *et al.*, 1994). All the procedures have been carried out in accordance with the guidelines laid down by the Institutional Ethics Committee and in accordance with local laws and regulations.

Isolation and identification of bacteria

The samples were inoculated in nutrient broth over night and then swabbed on nutrient agar followed by overnight incubation at 37 °C. Bacterial colonies were closely observed for their morphology, color and consistency. Gram's staining was used as primary identification test and suspected colonies were streaked on mannitol salt agar and incubated for 24 h at 37 °C under aerobic conditions.

Molecular identification of *S. aureus*

23S rRNA Ribotyping

The isolates were genotypically confirmed by 23S rRNA species specific PCR using forward primer-1 (5'-ACGGAGTTACAAAGGACGAC-3') and reverse

primer-2 (5'-AGCTCAGCCTTAACGAGTAC-3') (Straub *et al.*, 1999).

nuc gene typing

The amplification of *nuc* gene was carried out using forward primer (5'-GCG ATT GAT GGT GAT ACG GTT-3') and reverse primer (R-5'-ACG CAA GCC TTG ACG AAC TAA AGC-3') as described by Brakstad *et al.* (1992). The 25.0 µl reaction mixture for *nuc* gene consisted of 5.0 µl 5X Go Taq® Flexi buffer, 3.0 µl MgCl₂ (25mM), 1.0 µl dNTP mix (25mM each), 1.0 µl Forward Primer (10 pM/µl), 1.0 µl Reverse Primer (10 pM/µl), 0.2 µl Taq DNA polymerase (5U/µl), 3.0 µl DNA template (30 ng/µl) and 10.8 µl nuclease free water to make 25.0 µl. Amplification was carried out in a Veriti thermal cycler (Applied biosystem) and consisted of PCR cycle of pre denaturation at 94°C for 5 min, followed by 34 cycles of amplification (denaturation at 94°C for 60 s, primer annealing at 55°C for 60 s and primer extension at 70°C for 60 s), and final extension at 72°C for 10 min. The PCR products, were resolved on 1.2% agarose gels prepared in 1.0X TBE buffer containing 0.5 µg/ml of ethidium bromide and 50 bp DNA ladder were used as molecular marker. The amplification products were electrophoresed for 50-60 min at 100 Volts. The gels were then visualized under gel documentation system (ENDURO GDS).

RESULTS AND DISCUSSION

Details of samples and recovery of *S. aureus* isolates in samples collected from different locations of Bikaner have been depicted in Table 1. On the basis of cultural and biochemical properties, out of 197 samples, 107 *S. aureus* isolates were presumptively identified of which 51 isolates were from mastitic milk samples, 35 from udder and 21 from milkers' hands.

In the present investigation, all the 107 isolates produced an amplicon of 1250 bp following 23S rRNA based ribotyping (Fig. 1) and an amplicon of 270 bp upon PCR amplification of *nuc* gene confirming them to be *S. aureus* (Fig. 2). The overall recovery rate was 54.3%. Highest prevalence of *S. aureus* was observed in mastitic milk samples being 63.8% followed by 53% and 41.2% prevalence in udder and milkers' hands, respectively.

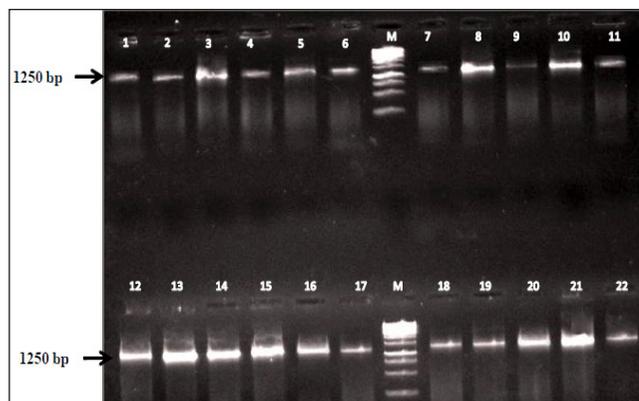


Fig. 1: Agarose gel (1.2%) showing 1250 bp PCR products of 23S rRNA of *S. aureus* in all Lanes (1-22); M=Ladder (1Kbp)

Among the seven groups under study, the overall highest prevalence of *S. aureus* was found in group no. 5 (unorganized dairy farm) being 65.4% followed by group no. 7 (60.0%), group no. 3 (58.3%), group no. 2 (55.3%), group no. 4 (55.0%), group no. 1 (40.0%) and group no. 6 (35.7%). Further, all the groups showed higher occurrence of *S. aureus* in mastitic milk than in extramammary sites (Table 1).

Genotypic confirmation by 23S rRNA ribotyping using similar primers has been reported by Sanjiv *et al.* (2008); Upadhyay *et al.* (2010); Khichar *et al.* (2014); Yadav *et al.* (2015); Choudhary *et al.* (2018) from the same laboratory. Likewise, Salasia *et al.* (2004); Bhandari *et al.* (2009); Momtaz *et al.* (2010); Yang *et al.* (2012); Parth *et al.* (2016); Hamid *et al.* (2017) obtained species-specific amplicon of 1250 bp in genotypic identification of this organisms from mastitic milk samples from different geographical locations.

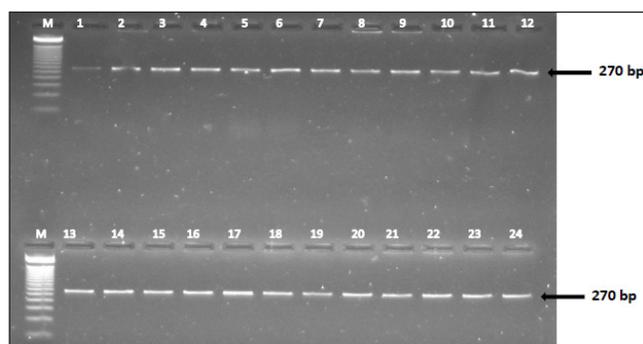


Fig. 2: Agarose gel (1.2%) showing 270 bp PCR amplicons of *nuc* gene of *S. aureus* in all Lanes (1-24); M=Ladder (50 bp)

Kateete *et al.* (2010) considered identification of *S. aureus* using PCR amplification of the *nuc* gene as a gold standard method. Detection of *nuc* gene for the identification of *S. aureus* strains isolated from intramammary infections has been adopted by many workers in India and from other parts of the world. Similar prevalence of 100% *nuc* gene carrying *S. aureus* isolates has been reported in previous studies on intramammary infections in cattle by Kalorey *et al.* (2007), Sarkar *et al.* (2014), Xu *et al.* (2015), Nazir *et al.* (2017) and Javid *et al.* (2018) in different parts of the world.

The overall occurrence of *S. aureus* (54.3%) from all the locations in present study is similar to that reported by Souza *et al.* (2012) who collected 446 samples from several sites in dairy farms and recovered 244 (54.7%) *S. aureus* isolates. Sarkar *et al.* (2014) also reported 73.6% prevalence of *S. aureus* in the farm from mastitic milk samples and nasal swabs of farm workers similar to present study.

Nathiya *et al.* (2018) reported recovery of 47.45% *S. aureus* isolates from milk of cows with clinical mastitis from same area of work as in present study. Similarly, Kutar *et al.* (2015) detected higher (56%) incidence of *S. aureus* in clinical mastitis cases in Uttar Pradesh, India. Likewise, Parth *et al.* (2016) found that 54.29% of clinical mastitic milk samples yielded *S. aureus* (cows 61.90% and buffaloes 42.85%) in Gujrat, India. A high prevalence of 63.8% of *S. aureus* isolates from mastitic milk samples observed in present study is similar to the findings of Sori *et al.* (2011) who isolated 86 (52.4%) *S. aureus* strains from 164 high CMT score milk samples in Ethiopia. Hanon, (2017) reported incidence of 47.69% *S. aureus* from bovine mastitis in Iraq and Baloch *et al.* (2018) isolated 46.2% *S. aureus* strains from bovine mastitic milk in China.

However some workers have reported low prevalence *viz.* 6.6% (Lee *et al.*, 2012), 19.9% (Anderson *et al.*, 2012), 15.5% (Mekuria *et al.*, 2013) and 12% (Leigue *et al.*, 2017) with similar study pattern of isolating *S. aureus* from mastitic milk samples and extramammary sites. While some workers have reported a low prevalence of *S. aureus* from mastitic milk samples collected from different farms *i.e.* 22.7% (El-Jakee *et al.*, 2008); 29.16% (Abdeen *et al.*, 2015); 22.5% (Hamid *et al.*, 2017); and 28.1% (Srednik *et al.*, 2018).

**Table 1:** Detail of samples and recovered *S. aureus* isolates from samples collected from different locations of Bikaner

Group No.	Place of sampling	Total No. of samples	Source of samples	No. of Isolates	Total No. of isolates Included in study (%)
1	Bhiwani (Haryana)	20	Mastitic milk (n=06)	03	08 (40)
			Udder (n=07)	03	
			Milkers' hands (n=07)	02	
2	Phalodi	38	Mastitic milk (n=18)	11	21 (55.3)
			Udder (n=10)	06	
			Milkers' hands (n=10)	04	
3	LRS, Kodamdesar	24	Mastitic milk (n=06)	04	14 (58.3)
			Udder (n=08)	04	
			Milkers' hands (n=10)	06	
4	LRS, Beechwaal	20	Mastitic milk (n=08)	05	11 (55.0)
			Udder (n=06)	03	
			Milkers' hands (n=06)	03	
5	Sarvodya basti, Bikaner	52	Mastitic milk (n=26)	19	34 (65.4)
			Udder (n=20)	13	
			Milkers' hands (n=06)	02	
6	LRS, Chandan	28	Mastitic milk (n=10)	05	10 (35.7)
			Udder (n=10)	03	
			Milkers' hands (n=08)	02	
7	Local Dairy, Bikaner	15	Mastitic milk (n=06)	04	09 (60.0)
			Udder (n=05)	03	
			Milkers' hands (n=04)	02	
Total		197		107	107 (54.3)

Abbreviations: n=number of sample; LRS= Livestock research station.

In the present study, the occurrence of *S. aureus* in mastitic milk, udder skin and milkers' hands was found to be different in all groups owing to the difference in management practices of maintaining animal health as well hygiene of the farm and farm workers.

Similar to the present research work of isolating *S. aureus* from mastitic milk samples and extramammary sites, studies have been conducted in other parts of India (Sarkar *et al.*, 2014) and world reporting the prevalence of *S. aureus* in milk, body sites and environment in varying degrees (Capurro *et al.*, 2010; Souza *et al.*, 2012; Mekuria *et al.*, 2013; Schmidt *et al.*, 2017). When compared to the prevalence rates detected by other workers worldwide, it was found that alike the prevalence rate of *S. aureus* in milk, the prevalence rate of *S. aureus* on the udder skin and in the hands of animal worker population varies depending upon the size, geographical area and management practices of the herd under study.

In conclusion, the results of the present study demonstrate that genotyping methods involving 23S RNA ribotyping and *nuc* gene amplification provides easy identification of *S. aureus* isolates in bovine mastitis. Furthermore this study also substantiates the previous reports of *S. aureus* being most prevalent pathogen causing clinical mastitis in dairy animals revealing higher prevalence in samples of bovine origin as compared to human samples. The isolation of *S. aureus* from milkers hands in fairly good numbers indicate transmission of this pathogen between animals and man on a farm. Hence it may be suggested from the present study that stringent management practices should be adopted to prevent the spread of these bacteria and of mastitis in a herd of animals.

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