Capsular genotyping (*cap5k* and *cap8k*) of *Staphylococcus aureus* isolates from cattle with clinical mastitis

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Abstract. *Staphylococcus aureus* is regarded as the most common pathogen causing all forms of mastitis and its polysaccharide capsule is considered as an important virulence factor. Among 11 capsular serotypes capsular type 5 (CP5) and 8 (CP8) have been found to be most common in *S. aureus* isolates from human sources. However, there is paucity of information on genotypic capsular typing of *S. aureus* from animal sources. In the present investigation 28 *S. aureus* milk isolates obtained from cattle having clinical mastitis were genotypically confirmed by PCR targeting 23S rRNA gene. The detection of capsular gene fragments was investigated by PCR wherein all the isolates were found to possess either *cap5k* or *cap8k* genes responsible for CP5 or CP8 types, respectively. In this study 26 isolates (92.86%) produced amplicons of 361 bp indicating presence of *cap5k* gene and two isolates (7.14%) produced 173 bp amplicon indicating presence of *cap8k* gene.

Key Words: *cap* genes, typing, cattle, clinical mastitis, *Staphylococcus aureus*.

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Introduction

Mastitis is one of the most common diseases of dairy cattle throughout the world causing huge economical losses. Among several etiological agents *Staphylococcus aureus* is a major mastitis-causing pathogen that also poses food safety and antimicrobial resistance threats (Kim et al 2001; Balakrishnan et al 2004; Momtaz et al 2010; Kumar et al 2011). The capsular polysaccharides expressed by *S. aureus* are important in the pathogenesis of staphylococcal infections and promote bacterial colonization and persistence on mucosal surfaces. The clinical isolates of *S. aureus* have been shown to possess capsular polysaccharides (Karakawa et al 1985) and of the 11 CP types, CP5 and CP8 positive comprise the majority of animal mastitis isolates (Poutrel et al 1988; Naidu et al 1991; Upadhyay et al 2010). The capsular antigens are surface associated, limited in antigenic specificity and highly conserved among clinical isolates and CP5 and CP8 offer promise as target antigens for a vaccine to prevent staphylococcal infections (O'Riordan & Lee 2004). In the present investigation *S. aureus* obtained from milk samples from cattle with clinical mastitis were characterized for their capsular types targeting *cap5K* and *cap8K* genes.

Material and methods

Isolation and identification of bacteria

The milk samples collected from Holstein-Friesian crossbred and Rathi (a local breed) cattle with clinical mastitis were processed for isolation and identification of *S. aureus* as per the standard methods (Cowan & Steel 1975; Quinn et al 1994). In the study 13 *S. aureus* isolates from H-F crossbred and 15 isolates from Rathi cattle were included.

Genotypic confirmation of organisms (Ribotyping)

From overnight grown bacterial culture, DNA was isolated (Nachimuttu et al 2001) and DNA quantification was carried out by spectrophotometric measurements (Sambrook et al 1989). The quantified DNA was diluted to a final concentration of 25 ng/µl in TE buffer and ribotyping based on 23S rRNA gene was carried out (Straub et al 1999) using species specific primers i.e. 5'-CGGAGTTACAAGGACGAC-3' (Primer-1) and 5'-AGCTCAGCCTTAACGAGTAC-3' (Primer-2).

Amplification of *cap5K* and *cap8K* genes

The method of Verdier et al (2007) was used for the amplification of *cap5K* and *cap8K* genes following the protocol as mentioned below. The sequences for primers used were 5'-GTCAAAGATTATGTGATGCTACTGAG-3'(Primer-1) and 5'-ACTTCGAATATAAACTTGAATCAATGTTATACAG-3' (Primer-2) for amplification of *cap5K* gene and it were 5'-GCCTTATGTTAGGTGATAAACC-3' (Primer-1) and 5'-GGAAAAACACTATCATAGCAGG-3' (Primer-2) for amplification of *cap8K* gene. The PCR mixture was prepared by mixing primer-1, 0.5 µl (75 pmol/µl), primer-2, 0.5 µl (75 pmol/µl), 3.5 µl 10x Taq buffer A containing 15mM Mgcl2, 1 unit of Taq polymerase (3U/µl), 2.0 µl dNTP mix (10 mM/µl), 21.0 µl deionised water and DNA template 2.5 µl (25 ng/µl).

The denaturation, primer annealing and primer extension was carried out at 94^{0}C, 55^{0}C and at 72^{0}C, respectively, in each cycle and the time given for denaturation, primer annealing and primer extension for cycle 1 was 5 min, 30sec and 60 sec, respectively; for cycle 2-25 it was 30sec, 30 sec and 60 sec, respectively; and for cycle 26 it was 30 sec, 30 sec and 5 min, respectively. The PCR products, after addition of 2 µl of trekking dye were resolved in 1.2 % agarose gels prepared in 0.5 x TBE buffer containing 0.5 µg/ml of ethidium bromide and 100bp DNA ladder was used as molecular marker. The amplification products were electrophoresed for 1h 30 min at 100 V. The gel was then visualized under U.V. transilluminator.

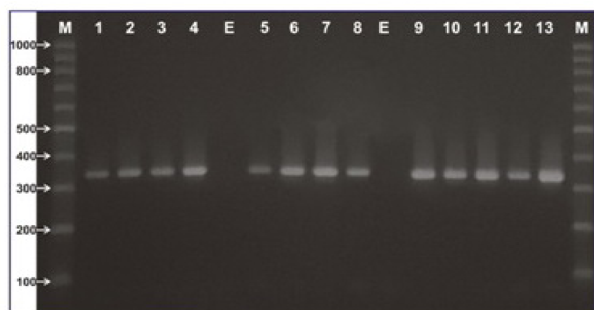
Results and Discussion

23S rRNA gene based confirmation. In the present investigation all the 28 isolates which were subjected to PCR amplification targeting 23S rRNA gene produced a species specific amplicon of 1250 bp size.
In the phenotypic identification of *S. aureus* colony pigmentation, mannitol fermentation and production of coagulase are considered as important criteria. However, there may be variations in the phenotypic properties of this organism making the identification of the organism uncertain. In order to overcome this problem a PCR method was developed by Straub et al (1999) which was a 23S rRNA gene based system essentially identifying *S. aureus*. Later similar studies were conducted by various other workers and found this method foolproof for genotypic confirmation of *S. aureus* strains isolated from bovine mastitis (Stephan et al 2001; Salasia et al 2004; Sanjiv et al 2008; Momtaz et al 2010; Upadhyay et al 2010).

cap genotyping

All the isolates in the present study were typable for either CP5 (Fig. 1) or CP8 capsules (Fig. 2). The PCR products obtained with primers for *cap5K* and *cap8K* were 361 bp and 173 bp, respectively. Out of the 28 isolates 26 isolates (92.86%) produced amplicons of 361 bp indicating presence of *cap5K* gene and two isolates (7.14%) showed presence of 173 bp amplicon indicating presence of *cap8K* gene (Table1).

Fig. 1 : PCR amplicons of *cap5* gene of *S.aureus* isolated from H-F cattle with mastitis



M= Molecular marker (100 bp DNA ladder)
1-13 = Isolate numbers
E = Lane without *S. aureus cap5K* amplicon

The encapsulation of *S. aureus* has been demonstrated to be very important trait for survival of this organism in the host system as the capsulated organism are able to protect themselves from being phagocytosed. There have been 11 different capsular types demonstrated on the basis of serological or agglutination reaction with monospecific antisera. Among 11 capsular serotypes capsular type 5 and 8 have been found to be most common in *S. aureus* isolates from human sources. However, there seems to be great variation in capsular types from animal sources and information concerning the geographical distribution of capsular serotypes is important for the rational design and use of vaccine against S. aurues causing mastitis based on capsular typing (Tollersrud et al 2000).

Fig. 2 : PCR amplicons of *cap8* gene of *S.aureus* isolated from Rathi cattle with mastitis.

M= Molecular marker (100 bp DNA ladder)
14-28 = Isolate numbers

Table 1: Capsular typing of *S. aureus* isolates obtained from cattle with clinical mastitis

S. No	Capsular type	Amplicon size (bp)	Isolate numbers	Total isolates
1	CP5	361	1-13 (H-F isolates)	13
		361	14-26 (Rathi isolates)	13
2	CP8	173	27, 28 (Rathi isolates)	2

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The gene cluster for CP5 and CP8 type contains 16 ORFs designated as cap5A through cap5P and cap8A through cap8P, respectively. The four of the ORFs located in the central region (H through K) for cap5 and cap8 are different to each other and are type specific.

In the present investigation all the isolates (100%) were typable either for CP5 or CP8 capsule types. However, other workers had demonstrated lower percentage of CP5/CP8 possessing isolates (Naidu et al 1991; Guidry et al 1998; Upadhyay et al 2010) from different geographical locations. Out of the 28 isolates 26 isolates (92.86%) produced amplicons of 361 bp indicating presence of *cap5K* gene and two isolates (7.14%) showed presence of 173 bp amplicon indicating presence of *cap8K* gene. From the same area in the previous study by Upadhyay et al (2010) only 60% bovine isolates were found to be CP5 positive and only 20% positive for CP8. The comparison of the two studies revealed higher recovery of CP5 positive *S. aureus* in the present study. Sordelli et al (2000) in genetic and epidemiological analysis for capsule expression by bovine isolates of *S. aureus* reported that there are remarkable differences in the prevalence of CP5 and CP8 among bovine strains of *S. aureus* from different geographical sources. A wide variation in results in regards to CP5 or CP8 and typability of the isolates have also been reported by different workers (Han et al 2000; Sordelli et al 2000). Our results are almost similar to those obtained by Hata et al (2006) who recorded that 89.6% of *S. aureus* isolates from bovine mastitic milk expressed serotypes 5 and 8 capsules. The results for cap8 are almost similar to those of Reinoso et al (2008) who did not record any of the isolates to possess cap8 gene in their studies on genotypic characterization of bovine isolates for cap5 and cap8 genes.

Conclusion

All the isolates were typable by the PCR based method targeting specific genes (*cap5k* and *cap8k*) with the predominance of CP5 positive *S. aureus* isolates from area of study.

Acknowledgement

We are thankful to Dean, College of Veterinary and Animal Science, Bikaner for the facilities provided to accomplish the work.

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Citation	Khichar V, and Kataria AK. Capsular genotyping (*cap5k and cap8k*) of *Staphylococcus aureus* isolates from cattle with clinical mastitis. HVM Bioflux 2014;6(1):30-33.
Editor	Stefan C. Vesa
Received	23 October 2013
Accepted	22 December 2013
Published Online	12 March 2014
Funding	None reported
Conflicts/ Competing Interests	None reported