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Capsular typing of *Staphylococcus aureus* isolates from cattle and goat mastitis by PCR targeting *cap5K* and *cap8K* genes

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ABSTRACT

The capsular typing of *Staphylococcus aureus* of bovine and caprine origin was carried out by polymerase chain reaction using primers specific for *cap5K* and *cap8K* genes. From cattle mastitis 60% of *S. aureus* were found to possess *cap5K* gene responsible for production of CP5 capsule and 20% of the isolates possessed *cap8K* gene responsible for biosynthesis of CP8 capsule. The rest 20% isolates did not produce amplicon with these primers and were considered non-CP5 and non-CP8 isolates. From goats, 30% of the isolates showed presence of *cap5K* gene and 20% of the isolates exhibited presence of *cap 8K* gene whereas 50% of the isolates were non-typable for *cap5K* or *cap 8K* genes.

No polymorphism was observed in *cap5K* and *cap8K* genes observed by restriction fragment length polymorphism (RFLP) and by polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP) analysis suggesting that the K genes of *cap5* and *cap8* gene cluster were highly conserved.

Key words: Capsular typing, *cap* genes, Mastitis, PCR- SSCP, RFLP, *Staphylococcus aureus*

Mastitis continues to be recognized as one of the major disease problems of dairy industry throughout the world and has been singled out as the most significant cause of economical loss in the dairy industry (Sordelli *et al*. 2000). Out of many different etiological agents, *Staphylococcus aureus* has been demonstrated as the most important pathogen in the causation of mastitis in cattle (Balakrishnan *et al.* 2004, Karimuribo *et al*. 2005) as well as in caprine (Moroni *et al*. 2005, Mørk *et al*. 2005, da Silva *et al*. 2006). The pathogenicity of the organism is largely determined by its ability to coordinately produce a plethora of extracellular toxins, enzymes, and surface antigens in bovine, ovine and caprine mastitis (Poutrel *et al*. 1988, Luong *et al*. 2003, O'Riordan and Lee 2004).

As many as 11 different capsular types (1 through 11) have been demonstrated by Sompolinsky *et al.* (1985) in *S. aureus* isolates obtained from different sources. However, most clinical isolates from both human and bovine infection produce either capsular polysaccharide type5 (CP5) or capsular polysaccharide type8 (CP8) and considerable geographical variations exist in the prevalence of these types in bovine isolates (Sordelli *et al*. 2000, Tollersrud *et al*. 2000). Watts *et al*. (2005) revealed that capsulated isolates were more virulent than the capsule negative mutant. The antibody specific for capsular polysaccharides protect against *S. aureus* infection in murine model (Lee *et al.* 1997, Fattom *et al*. 2004) and these capsular polysaccharides offer promise as target antigens for a vaccine to prevent staphylococcal infections (O'Riordan and Lee 2004). The information regarding capsular types is important for the rational design of vaccine for the prevention of staphylococcal mastitis (Sordelli *et al.* 2000).

The present paper puts on record genotyping of *S. aureus* regarding capsule type possessed by them.

MATERIALS AND METHODS

Samples: A total of 59 milk samples from cattle (41 indigenous of non-descript breed and 18 H-F crossbreds) and 41 samples from goats (Marwari) were collected for isolation of *S. aureus*. These animals with clinical mastitis were presented to Veterinary clinical complex, College of Veterinary and Animal Science, Bikaner.

Isolation and identification of bacteria: The organisms were isolated and identified as described by Cowan and Steel (1975) and Quinn *et al*. (1994). Genotypic confirmation of all the *S. aureus* isolates was done by ribotyping for 23S rRNA as per Straub *et al*. (1999) using the two primers, Staur4 and Staur6 as used by them.

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Amplification of cap5K and cap8K genes: The method of Verdier *et al*. (2007) was followed for amplification of *cap5K* and *cap8K* genes using the same primer sequences as used by them. The PCR mixture (total volume 30 µl) was prepared by mixing primer–1, 0.5 µl (75 pmol/µl) primer–2, 0.5 µl (75 pmol/µl), 2.5 µl 10×buffer, 3 µl $Mgcl_2$ (2.5 µM/µl), 1 unit of *Taq* polymerase (3U/µl), 0.5 µl dNTP mix (10 mM/ µl), deionised water (20 µl) and DNA template 2.5 µl (25 ng/µl). The PCR was performed in Biometra Thermocycler and the PCR products, after addition of 2 µl of trekking dye were resolved in 1.2% agarose gels prepared in 0.5×TBE buffer containing 0.5µg/ml of ethidium bromide and 100 bp DNA ladder was used as molecular marker. The amplification products were electrophoresed for 2 h 30 min at 100 V and the gel was then visualized under U.V. transilluminator.

RFLP of cap5K and cap8K gene product

Restriction fragment length polymorphism of *cap5K* and *cap8K* gene product digested by *Alu*I was carried out as per Hookey *et al*. (1998).

Single strand conformation polymorphism in cap5K and cap8K gene: The method of Orita *et al.* (1989) was followed for SSCP for *cap5K* and *cap8K* gene products and silver staining of the gel was carried out as per Caetano-Anollés and Gresshoff (1997).

RESULTS AND DISCUSSION

In the present study *S. aureus* isolates were first identified by conventional methods using phenotypic and biochemical characteristics and then confirmed genotypically by PCR using specific primers targeted against 23S rRNA gene. With the specific primer an amplicon of 1250 bp was obtained with all the 30 isolates from cattle and goat which was specific to *S. aureus* (Fig.1). This method was developed by Straub *et al.* (1999) and was used by various workers (Salasia *et al*. 2004, Sanjiv *et al*. 2008) for identification of *S. aureus* from bovine mastitis.

Most of the *S. aureus* possess one of the 11 different capsular types identified by reaction of their capsules with specific antibodies. Whereas there is general agreement that CP5 and CP8 are the predominant serotypes in human *S. aureus*, evaluation of capsule production among *S. aureus* strains from ruminants shows varying results (Tollersrud *et al*. 2000).

Experiments have been conducted and validated by various workers proving role of CP5 and CP8 possessing *S. aureus* in immunoprophylaxis. However, the vaccines produced with inclusion of these 2 types of CPs do not protect the host from staphylococcal infections. This has been proposed that non-typable *S. aureus* isolates also do play a role in causation of infection. From non-typable *S. aureus* isolates an antigen named as 336 antigen is regarded as an important vaccine component for immunoprophylaxis and it has been recorded that if in a vaccine CP5, CP8 and 336 antigens are incorporated the vaccine proves almost 100% immunoprophylectic.

In an effort to know the capsular *S. aureus* types, in the present study 20 isolates from cattle and 10 isolates from goats were subjected to genotyping using specific primers for *cap5K* and *cap8K* genes responsible for coding of CP5 and CP8, respectively. The amplification of *cap5K* (Fig.2) and *cap8K* (Fig.3) genes with specific primers revealed PCR products of 361 bp and 173 bp, respectively. The amplicon sizes obtained in our study are similar to those obtained by Verdier *et al.* (2007).

In this investigation 60% of the cattle isolates were CP5 positive and 20% were CP8 positive whereas 20% of the isolates were non-typable by these 2 primers and were regarded as non-CP5 and non-CP8 *S. aureus*. The prevalence of these capsular types in this study is similar to that of Poutrel *et al*. (1988) who reported that capsular serotypes 5 and 8 accounted for 69.4% of bovine isolates and type 5 was predominant in *S. aureus* strains from bovine sources (51.4%). Similarly 70% *S. aureus* from bovine with mastitis were reported to possess CP5 or CP8 by Naidu *et al.* (1991). Guidry *et al*. (1997, 1998) evaluated the prevalence of serotype 5 and 8 *S. aureus* strains in milk from bovines in US and Europe and found that 41% of the US isolates and 70% of the isolates from Europe were CP5 and CP8 positive.

Contrary to the above findings very low (14%) prevalence of CP5 and CP8 *S. aureus* isolates was reported by Sordelli *et al.* (2000) who recorded 7.1% serotype5 and 6.6% serotype 8. Surprisingly very high percentage (86.2%) of non-typable isolates was detected by them by serotyping. Similarly, Sompolinsky *et al*. (1985) reported 17.5% of *S. aureus* isolates to possess CP5 or CP8 capsules.

In the present study only 30% of *S. aureus* isolates from goats possessed *cap5K* genes and 20% for *cap8K* whereas 50% isolates were non-typable. In contrast to present observations higher prevalence (81.5%) of capsular type 5 and 8 were reported by Poutrel *et al*. (1988) in goats from France. They reported 13.0% CP5 and 68.5% CP8 *S. aureus* and 18.5% non-typeable isolates. The wide variations in the capsular types may be results of differences in geographical areas and typing methods.

Genetic and serological evaluation of capsule production by bovine mammary isolates of *S. aureus* by Tollesrud *et al.* (2000) revealed a considerable variability that existed in the prevalence of serotype 5 and 8 capsules among bovine mammary isolates of *S.aureus* from different countries. They also found that the 50 isolates that failed to react with capsular antisera possessed *cap* genes required for production of CP5 or CP8 suggesting a possibility of point mutation in one or more of the 16 genes involved in capsule expression.

In order to detect any difference within same capsular types, the amplicons obtained in the present study were digested with endonuclease *Alu*I and subjected to RFLP analysis. No difference was observed in the RFLP patterns

among *S. aureus* isolates possessing CP5 or CP8 capsule type.

Single-strand conformation polymorphism (SSCP) analysis is a rapid, simple and effective method in which a mutated sequence is detected by a change in mobility during polyacrylamide gel electrophoresis caused by its altered folded structure (Hayashi 1991) and the technique is able to detect even single base pair change (single nucleotide polymorphism) in the gene. In the present investigation no polymorphism was recorded in *cap5K* and *cap8K* gene products.

The similarity in the RFLP and SSCP patterns suggested that these gene fragments responsible for synthesis of capsular polysaccharide were highly conserved in the *S. aureus* organisms. The present findings corroborated the earlier observations of O'Riordan and Lee (2004) who demonstrated that CP5 and CP8 are limited in antigenic specificity and are highly conserved among clinical isolates.

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