Capsular Typing of *Cap5k* and *Cap8k* Genes in *S. aureus* Isolates from Wound Samples of Cattle

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**A B S T R A C T**

The present study was implemented to evaluate *S. aureus* for their 2 major capsular polysaccharide genes namely *cap5k* and *cap8k*. Capsule is an important virulence factor of *S. aureus* which help the organism in its survival by evading host immune system specially phagocytosis. Thirty isolates of *S. aureus* were obtained from skin wounds of cattle in Bikaner, Rajasthan which were first identified by conventional cultural and biochemical properties and then confirmed by ribotyping based on 23S rRNA gene segment. All the 30 isolates were subjected to capsular typing by duplex PCR targeting *cap5K* and *cap8K* genes with specific primers. Capsular typing revealed 9 (30 per cent) isolates with *cap5K* and 15 (50 per cent) isolates with *cap8K* gene.

**Keywords**

Cattle, Capsular polysaccharide, *cap5k* and *cap8k* genes, *Staphylococcus aureus*

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**Introduction**

Capsular polysaccharides (CP) produce by *Staphylococcus aureus* play an important role in its colonization, pathogenesis and confer resistance to phagocytosis by masking of surface proteins, the typical antigens that trigger an adaptive immune response and prolong its persistence in the bloodstream of the host (Keinhörster *et al.*, 2019). Many bacterial species have been shown to produce CP, and a single species can produce numerous, structurally distinct CP, forming a basis for serotyping isolates (Visansirikul *et al.*, 2020). Of the 11 capsular polysaccharides types, the majority of the human and animal staphylococci are of *cap5* and *cap8* genotypes (O'Riordan and Lee, 2004; Verdier *et al.*, 2007). *S. aureus* strains which produce a high amount of capsular polysaccharides are relatively more resistant to nonspecific opsonophagocytic killing indicating that encapsulation might be an important immune evasion strategy for the organism (Nanra *et al.*, 2019).
al., 2013). The gene cluster for CP5 and CP8 type contains 16 open reading frames (cap5A through cap5P and cap8A through cap8P, respectively) the four of which are located in the central region (H-K) and are type specific. These 16 open reading frames encode for CP5 or CP8 biosynthesis, O-acetylation, transport and regulation (O’Riordan and Lee, 2004; Weidenmaier and Lee, 2017; Rausch et al., 2019). CP enhances virulence in abscess formation (Portoles et al., 2001), surgical wound infection (McLoughlin et al., 2006), and bovine mastitis (Zaatout et al., 2020).

The objective of the present investigation was to carry out genotypic characterisation of S. aureus obtained from skin wounds in cattle from Bikaner, Rajasthan for capsular polysachharide (cap5k and cap8k) genes.

Materials and Methods

Sample collection

A total of 63 swabs from wound samples with pus were collected from cattle, belonging to different veterinary clinics in and around Bikaner (Rajasthan). All samples were collected aseptically with sterile absorbent cotton swab soaked in nutrient broth from skin wounds in cattle and brought to the laboratory immediately over ice and processed for isolation and identification of S. aureus as per standard conventional methods (Cowan and Steel, 1975; Quinn et al, 1994).

Ribotyping targeting 23S rRNA

Extraction of S. aureus genomic DNA was carried out by method described by Nachimuttu et al., (2001) and quantification as per Sambrook et al., (1989). Further, the isolates were genotypically confirmed as per the method described by Straub et al., (1999) using species specific primers i.e. Primer-F (5’-ACGGAGTTACAAAGGACGA C-3’)

Cap5k and cap8k gene amplification

The method of Verdier et al., (2007) was used for the amplification of cap5K and cap8K gene in duplex PCR with some modification. The sequences for 2 primers used for cap5k and cap8k gene according to Table 1:

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Results and Discussion

Genotypic confirmation

In the present investigation, all the 30 isolates from wound samples of cattle after their identification by conventional microbiological procedures were subjected to 23S rRNA based ribotyping for genotypic confirmation. The ribotyping produced an amplicon of 1250 bp in all the isolates (Fig.1) confirming them to be S. aureus.

Similar genotypic method based on 23S rRNA ribotyping for S. aureus identification has been used by other workers from the same laboratory during the previous year viz. Upadhyay et al., (2010); Rathore et al., (2012); Khichar and Kataria (2014); Yadav et
al., (2015a), Bhati et al., (2018), Singh et al., (2018) and Nirwan (2020) from the same study area successfully and Suleiman et al., (2012), Vazquez et al., (2013), Widianingrum et al., (2016), Hamid et al., (2017) and Sundareshan et al., (2017) from elsewhere.

Cap typing

In the present study, all the 30 isolates were subjected to detection of gene fragment in the K region for cap5K and cap8K genes by duplex PCR using specific primers. The result revealed that six (20 per cent) isolates produced amplicons of 361 bp indicating presence of cap5K gene, 12 (40 per cent) isolates produced amplicon of 173 bp suggesting presence of cap8K gene, three (10 per cent) isolates were detected with both types of amplicons suggesting presence of both the gene and nine (30 per cent) isolates were non typable (Table-2, Fig.2).

The amplicons obtained in the present study were similar to those obtained by Verdier et al., (2007), Upadhyay et al., (2010), Khichar and Kataria (2014) and Yadav et al., (2015b). In the present study, nine isolates were detected non typable for both of the genes which corroborated the earlier observations of Naidu et al., (1991) who recorded 28 per cent of the S. aureus of bovine mastitis origin as non-typeable for cap5 and cap8. Upadhyay et al., (2010) recorded 20 per cent of the S. aureus of bovine and caprine mastitis origin as non-CP5 or non-CP8. Yadav et al., (2015b) reported 9.37 per cent non-typable isolates for cap 5 or 8 genes and Ambroggio et al., (2018) found that none S. aureus isolates of Argentina carrying any of the cap genes.

Table.1 Primers used for PCR for S. aureus isolates from wound samples of cattle

| S. No. | Gene  | Primer sequence (5´ to 3´)                     | Size (bp) | Annealing Temp. (°C) |
|-------|-------|-----------------------------------------------|-----------|----------------------|
| 1.    | cap5K | F-5´-GTCAAAGATTATGTGATGCTACT GAG-3’          | 361       | 55                   |
|       |       | R-5´-ACTTCGAATATAAAACTTGAATCAA TGTTATACAG-3’ |           |                      |
| 2.    | cap8K | F-5´-GCCTTATGTGTTAGGTGATAAACC-3’            | 173       | 55                   |
|       |       | R-5´-GGAAAAACACTATCATAGCAGG-3’              |           |                      |

Table.2 Capsular typing by PCR targeting cap5K and cap8K genes in S. aureus isolates from wound samples of cattle

| S. No. | Type     | Isolate numbers | Total isolates (per cent) | Amplicon size(bp) |
|--------|----------|-----------------|--------------------------|-------------------|
| 1.     | Cap5K    | VS6, VS15, VS19, VS24, VS25, VS27 | 6(20 per cent) | 361 bp |
| 2.     | Cap8K    | VS1, VS5, VS8, VS9, VS10, VS11, VS13, VS14, VS16, VS20, VS22, VS23 | 12(40 per cent) | 173 bp |
| 3      | Both     | VS4, VS21, VS28 | 3(10 per cent) | 361 bp, 173 bp |
| 4      | Non typable | VS2, VS3, VS7, VS12, VS17, VS18, VS26, VS29, VS30 | 9(30 per cent) | - |

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However, a variable percentage of isolates were detected to possess one or both of the genes which corroborated the earlier observations of Ikawaty et al., (2010), Proietti et al., (2010), Khichar and Kataria (2014) and Salimena et al., (2016).

In our study, we recorded higher frequency of *cap8K* (40 per cent) as compared to *cap5K* (20 per cent) gene which is in complete agreement to observations of Ikawaty et al., (2010) who detected *cap8* gene in 96.05 per cent isolates and *cap5* gene in 3.94 per cent isolates from clinical mastitic case of bovine. Ote et al., (2011) recorded 68.6 per cent isolates from bovine mastitis positive for *cap8* gene and 33.6 per cent isolates positive for *cap5* gene.

On the contrary, a higher frequency of *cap5* gene was detected by other scientists. Salimena et al., (2016) recorded *cap5* gene in 80 per cent and *cap8* gene in 20 per cent isolates. Khichar et al., (2016) demonstrated that among 18 (90 per cent) typable *S. aureus* isolates, 11 (55 per cent) were found to harbour *cap5K* gene and seven isolates (35 per cent) carried *cap8K* gene, while two isolates (10 per cent) were non-typable for either of the 2 genes.
In conclusion, there was considerable recovery of *S. aureus* from wound samples of cattle and all the isolates were typable using 23S rRNA ribotyping and could be identified with high specificity. An overall high prevalence of virulence genes *cap5K* and *cap8K* was observed. Hence pathogenic strains were recovered from wound samples of cattle in the present study.

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