Original Research Article

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

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ABSTRACT

Keywords

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

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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.

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., 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 polysaccharide (*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’)

and Primer-R (5’-AGCTCAGCCTTAACGA GTAC-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:

The amplification was carried out in thermocycler with initial denaturation at 94° C for 2 min and 30 cycles of denaturation, annealing and extension at 94° C, 55° C and 72° C for 60 sec respectively. Final extension was done at 72° C for 5 min and reaction is terminated by holding at 4° C. The PCR products (for genus specific PCR) were subjected to electrophoresis at 4 V/cm for 45 minute in 1.5 per cent agarose gel prepared in 1 x TBE buffer containing 0.5 ng/ml of ethidium bromide. The PCR products (5µl) were run along with 100 b.p. DNA ladder (Invitrogen) and amplicons were visualized under UVP Gel Doc Bioimaging System.

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.	<i>cap5K</i>	F-5'-GTCAAAGATTATGTGATGCTACT GAG-3' R-5'-ACTTCGAATATAAACTTGAATCAA TGTTATACAG-3'	361	55
2.	<i>cap8K</i>	F-5'-GCCTTATGTTAGGTGATAAACC-3' R-5'-GGAAAAACACTATCATAGCAGG-3'	173	55

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.	<i>Cap5K</i>	VS6, VS15, VS19, VS24, VS25, VS27	6(20 per cent)	361 bp
2.	<i>Cap8K</i>	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)	-

Fig.1 PCR amplicons of 23S rRNA ribotyping of *S. aureus* isolates

Fig.2 PCR amplicons of *cap5K* and *cap8k* genes of *S. aureus* isolates

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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