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Molecular characterization of *Staphylococcus aureus* capsules in Caprine Clinical Mastitis

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Abstract
In this study, we employed PCR with specific primers targeting the cap5k and cap8k genes to conduct molecular characterization of capsule of 34 *Staphylococcus aureus* isolates originated from caprine clinical mastitis cases. The purpose was to identify the presence and distribution of these genes within the bacterial strains. The PCR results revealed that two isolates displayed amplify of 361 base pairs, indicating the presence of the cap5k gene. Additionally, 22 isolates exhibited amplify of 173 base pairs, indicating the presence of the cap8k gene. Furthermore, a subgroup of 10 isolates exhibited both types of amplify, confirming the simultaneous presence of both cap5k and cap8k genes. Consequently, the cap5k gene was found in 12 (35.29%) *S. aureus* and the cap8k gene was identified in 32 (94.11%) isolates. These findings provide valuable insights into the molecular composition and diversity of capsule among *S. aureus* isolates originating from caprine clinical mastitis, possibly aiding in advancing the comprehension of the pathogenesis and epidemiology of this caprine ailment.

Keywords: Capsular typing, *Staphylococcus aureus*, caprine clinical mastitis

Introduction
Mastitis, a prevalent and economically significant infectious disease, poses a considerable challenge to the dairy industry worldwide. Caprine clinical mastitis, specifically affecting goats, is a prominent concern, causing substantial economic losses due to decreased milk production, treatment costs, and potential culling of infected animals (Dore *et al.*, 2014) [6]. *Staphylococcus aureus*, a major etiological agent of mastitis, possesses various virulence factors, including capsules that assume a vital function in both the pathogenesis and evasion of the host's immune response (Altaf *et al*., 2020) [1].

Capsules, a unique and essential component of bacterial cell envelopes, consist of complex polysaccharides that surround certain bacterial species, including *Staphylococcus aureus*. The encapsulation provides a protective shield against phagocytosis and opsonophagocytosis, enabling the bacteria to endure within the host and lead to persistent, chronic infections. Moreover, the composition and expression of capsule genes contribute to variations in virulence and antimicrobial resistance among different strains (Thomer *et al*., 2016) [21].

Given the significance of capsule's polysaccharides in the pathogenicity of *S. aureus*, understanding their genetic diversity and distribution in caprine clinical mastitis isolates becomes crucial. Molecular capsular typing methods, such as PCR targeting specific capsule genes, have emerged as valuable tools for such investigations. This research aims to shed light on the capsular types present in *Staphylococcus aureus* isolates originated from caprine clinical mastitis, thereby enriching our understanding of the disease dynamics and providing insights for effective control measures (Cress *et al*., 2014) [5].

In this study, we focus on two prominent capsule genes, cap5k and cap8k, known to encode key enzymes involved in the synthesis of capsule polysaccharides. The PCR-based approach with specific primers targeting these genes allows for rapid and reliable capsular typing, enabling the differentiation of strains based on their capsule gene content. Through this methodology, we seek to determine the distribution and prevalence of cap5k and cap8k genes among the *Staphylococcus aureus* isolates in our sample set.

The outcomes of this research hold significant implications for veterinary and public health alike. Identifying prevalent capsular types can aid in tailoring specific vaccine strategies to target the most virulent strains and potentially mitigate the impact of caprine clinical mastitis on both animal health and dairy productivity. Furthermore, this study may contribute to our understanding of interspecies transmission and the zoonotic potential of specific *Staphylococcus aureus* capsular types.

Overall, the findings from this investigation will provide valuable insights into the molecular epidemiology of caprine clinical mastitis-associated *Staphylococcus aureus* and pave the way for more targeted and effective control measures to combat this challenging bovine disease.

Materials and Methods

Sample Collection: Under sterile conditions, a total of 57 milk samples, with each sample containing 5–10 ml, were collected from goats exhibiting clinical mastitis. These goats were owned by various farmers in and around the Bikaner city of Rajasthan state.

Isolation and identification of *S. aureus*: The conventional methods described by Cowan and Steel (1975) [4], as well as Quinn *et al.* (1994) [15], were utilized for isolating and identifying *S. aureus* from the collected milk samples. To confirm the isolates phenotypically, they were subjected to genotypic confirmation through 23S rRNA ribotyping, following the procedure outlined by Straub *et al.* (1999) [20].

Capsular gene amplification: To characterize the capsule of *S. aureus* isolates originated from caprine clinical mastitis cases, we targeted the cap5K and cap8K genes using the amplification technique described by Verdier *et al.* (2007) [23]. The reaction mixture was comprised the following components; Green master mix: 12.5 µl; Forward primer (20 pmol): 1 µl; Reverse primer (20 pmol): 1 µl; Template DNA: 3 µl; Nuclease free water: Up to 25 µl. Optimum PCR cyclic conditions for capsular genes amplification in *S. aureus* isolates are given in the Table 1 and PCR product subjected to horizontal electrophoresis.

Table 1: Optimum PCR cyclic conditions for capsular genes amplification in *S. aureus* isolates

Primer	Initial denaturation (°C/ min)	Denaturation (°C/sec)	Annealing (°C/sec)	Extension (°C/sec)	Final Extension (°C/ min)	No of Cycles
cap5K	94/5	94/30	55/30	72/60	70/5	25
cap8K	94/5	94/30	55/30	72/60	70/5	25

Results and Discussion

The pathogenicity of *Staphylococcus aureus* heavily relies on the ability of its capsule to evade the host immune system, particularly phagocytosis. Previous studies conducted by Sompolinsky *et al.* (1985) [18] and Poutrel *et al.* (1988) [13] have consistently demonstrated that capsular types 5 and 8 predominate in both animal and human isolates of this bacterium. The genetic clusters responsible for CP5 and CP8 consist of 16 ORFs, named cap5A through cap5P and cap8A through cap8P, respectively. Within these clusters, four are type-specific and are situated in the central region (H-K).

Considering the significance of capsular proteins in *S. aureus*' virulence, there has been a growing interest in the development of a new generation of vaccines. Researchers such as Bergonier *et al.* (2003) [2] and O'Riordan and Lee (2004) [11] have highlighted the potential of utilizing capsular proteins as promising candidates for future vaccine development. This direction of research holds promise in enhancing our ability to combat *Staphylococcus aureus* infections effectively.

In this study, all 34 *Staphylococcus aureus* isolates were subjected to molecular characterization of capsular using PCR was performed, targeting specific primers of the cap5k and cap8k genes. The PCR results revealed distinctive banding patterns on agarose gels, providing valuable information about the presence and distribution of the two capsule genes in the isolates.

Fig. 1 displays the PCR amplification of the cap5k gene, where two isolates exhibited 361 bp amplicon, indicating the presence of the cap5k gene in these isolates. Fig. 2 illustrates the PCR amplification of the cap8k gene, with 22 isolates showing 173 bp amplicon, confirming the existence of the cap8k gene in these isolates.

Additionally, Table-2 presents a comprehensive summary of the capsular typing results, showing that 10 isolates displayed both types of amplicon, indicating the simultaneous presence of both the cap5k and cap8k genes in these particular isolates.

The analysis revealed that the cap5k gene was detected in 12 (35.29%) isolates, while the cap8k gene was found in 32 (94.11%) isolates. These findings provide valuable insights into the molecular characterization of capsular types in the *Staphylococcus aureus* isolates originated from cases of caprine clinical mastitis. Understanding the distribution of these capsule genes is essential for studying the pathogenicity and potential virulence of these isolates in goats and may have implications for future control and prevention strategies for caprine mastitis.

Table 2: Capsular Typing Results of *S. aureus* Isolates from Caprine Clinical Mastitis

S. No.	Target Gene	Isolate ID	Total isolates (%)
1.	cap5K	GM11, GM24	2 (5.88%)
2.	cap8K	GM1, GM2, GM4, GM6, GM8, GM9, GM10, GM13, GM14, GM15, GM18, GM19, GM20, GM22, GM25, GM27, GM28, GM29, GM30, GM32, GM33, GM34	22 (64.70%)
3	Both	GM3, GM5, GM7, GM12, GM16, GM17, GM21, GM23, GM26, GM31	10 (29.4%)

The amplicons observed in our current investigation were consistent with the findings of previous studies conducted by many researchers [9, 22, 23, 24]. In our study, all isolates were observed to harbor either one or both of the cap5k and cap8k genes, consistent with earlier findings reported by Ikawaty *et al.* (2010) [8], Khichar and Kataria (2014) [9], Proietti *et al.* (2010) [14], and Salimena *et al.* (2016) [16].

However, it is noteworthy that several researchers have reported a variable number of isolates as non-typable for these genes. As an example, Naidu *et al.* (1991) [10] reported that 28% of the *Staphylococcus aureus* isolates derived from bovine mastitis showed non-typability for the cap5 and cap8 genes. Upadhyay *et al.* (2010) [22] found that 20% of the *Staphylococcus aureus* isolates from both bovine and caprine

mastitis cases were absence of CP5 or CP8. Similarly, Yadav *et al*. (2015) [24] encountered 9.37% of non-typable isolates.

In a separate investigation, Sordelli *et al*. (2000) [19] revealed that a significant proportion (86.15%) of *S. aureus* isolates bovine mastitis origin lacked both the CP5 and CP8 genes. Conversely, only 3.70% of the *Staphylococcus aureus* isolates from caprine mastitis were non-typable, as studied by Nathawat *et al*. (2015) [11]. Guidry *et al*. (1998) [7] also observed a low rate of untypeable isolates (2%) from cattle mastitis milk. These diverse findings suggest that the capsular typing results may vary depending on the geographical location, host species, and other factors, warranting further investigation to better understand the underlying reasons for the non-typable isolates.

In our study, we observed a higher frequency of the cap8 gene (94.11%) compared to the cap5 gene (32.29%). These findings align perfectly with the observations made by Ikawaty *et al*. (2010) [8], who determined the cap8 gene (96.05%) of their 76 *Staphylococcus aureus* isolates from mastitis cases in bovines, while only 3.94% of the isolates positive for cap5 gene.

Similarly, Singhal *et al*. (2021) [17] conducted duplex PCR on 30 isolates and found that 30% of the isolates were carried the cap5K gene, and 50% of the isolates were carried the cap8K gene, using appropriate primers for capsule typing. Contrasting observations have been reported by other researchers, wherein a higher occurrence of the cap5 gene was observed. For instance, Proietti *et al*. (2010) found that out of 170 *S. aureus* isolates, 82.35% positive cap5 gene from bovine milk sample of 7 dairy farms. Camussone *et al*. (2012) [3] reported that 52.9% of 157 *Staphylococcus aureus* isolated from mammary infections were positive for the cap5 gene, while 11.4% of isolates Posses the cap8 gene.

Similarly, Khichar and Kataria (2014) [9] identified the cap5K gene in 92.85% of *S. aureus* isolates originated bovine mastitis, and the cap8K gene was found in 7.14% of the isolates. Researcher [24] reported 68.75% of strains positive for the cap5K gene and 21.87% positive for the cap8K gene in isolates from bovine mastitis. In Salimena *et al*. (2016) [16] studied, 80% of 159 *S. aureus* isolates from bovine mastitis found to be positive for the cap5 gene, while 20% for the cap8 gene.

These significant variations in the presence and type of genes among the *Staphylococcus aureus* isolates may be attributed to differences in the sources of isolation, geographical locations, and the time period of isolation. These factors may influence the prevalence and distribution of capsule types in different populations of *S. aureus*, and highlight the importance of considering regional variations in understanding the molecular epidemiology of this pathogen.

Conclusions

In conclusion, the results showed a higher prevalence of the cap8k gene (94.11%) compared to the cap5k gene (32.29%) in the isolates analyzed. These findings are supported with previous research, supporting the presence of both cap5k and cap8k genes in the majority of the strains, as reported by other studies. However, there were variations in the capsular types among *Staphylococcus aureus* isolates across different studies, suggesting the influence of factors such as geographical location, host species, and time period of isolation on the distribution of capsular types. This research contributes to a better understood of the molecular epidemiology of *Staphylococcus aureus* and may have implications for future control and prevention strategies for caprine mastitis.

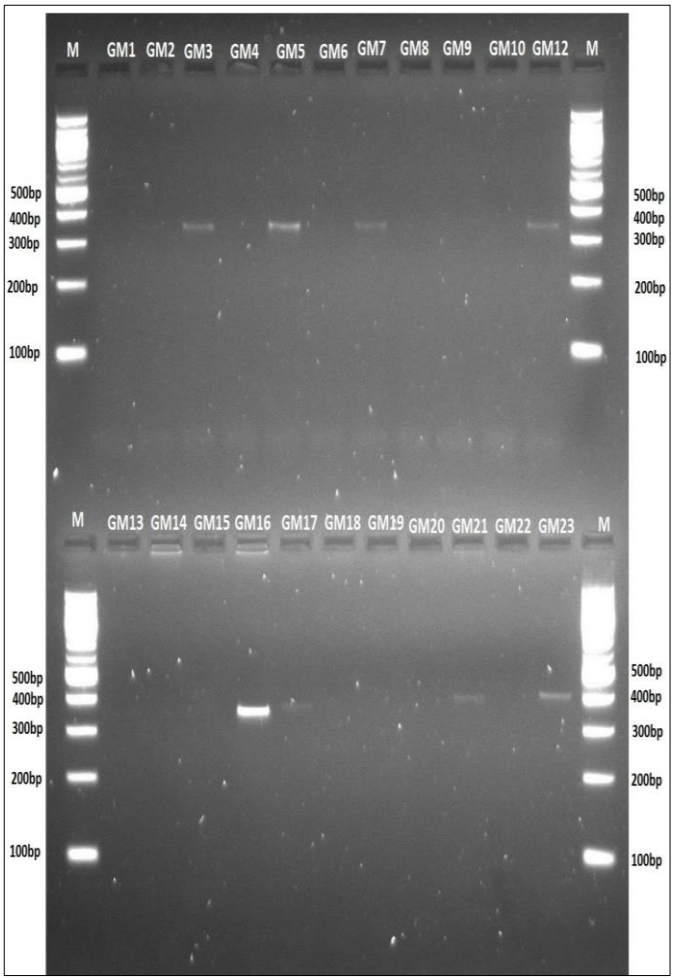

Fig 1: Gel electrophoresis of cap5k gene Amplicon in *staphylococcus aureus* isolates originated caprine clinical mastitis

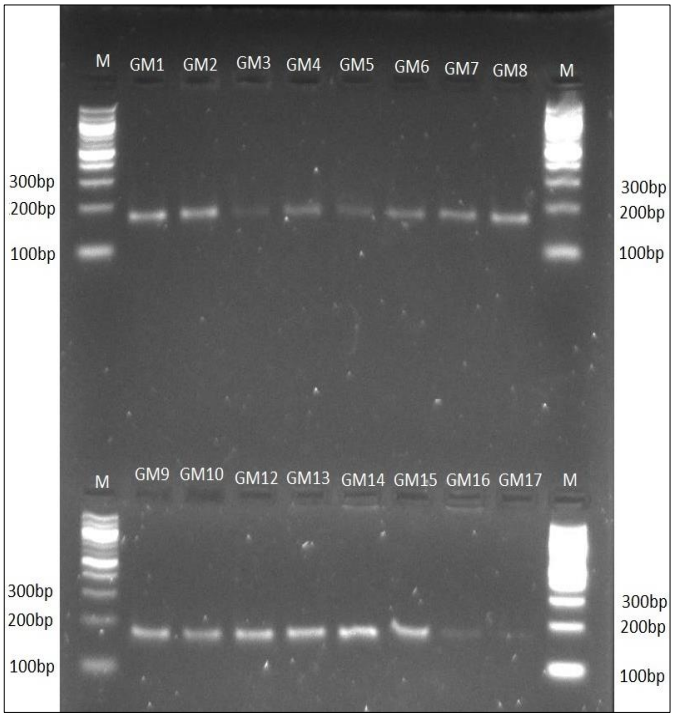

Fig 2: Gel electrophoresis of cap8k gene amplicons in *Staphylococcus aureus* isolates originated caprine clinical mastitis

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