RESEARCH ARTICLE

Capsular Genotyping of Methicillin-Resistant *Staphylococcus aureus* (MRSA) from Human and Animal Sources Using Duplex PCR

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Abstract

Capsular polysaccharides, particularly types 5 and 8 encoded by the *cap5K* and *cap8K* genes, are key virulence factors in *Staphylococcus aureus*, contributing to immune evasion through resistance to phagocytosis. This study aimed to determine the prevalence and distribution of these capsule-associated genes among 78 methicillin-resistant *S. aureus* (MRSA) isolates obtained from various human and animal sources in Western India. Duplex PCR targeting *cap5K* and *cap8K* was employed for genotypic characterization. Among the isolates, 75 (96.15%) were typeable, while 3 (3.85%) were non-typeable (NT). The *cap5K* genotype was the most prevalent (44.87%), followed by *cap8K* (26.92%), and dual gene presence was 24.36%. Notably, dual gene carriage was more common in human isolates (50.00%), whereas *cap5K* alone predominated in animal isolates, particularly in unprocessed meat (60.00%) and animal pus (48.00%). The NT strains were exclusively identified in animal pus samples. A Chi-square test revealed a statistically significant association between sample type and capsule gene distribution ($\chi^2 = 17.87$, $p = 0.037$). These findings highlight the dominance of *cap5K* in this region and the influence of host source on capsule gene variation, underlining the importance of ongoing molecular surveillance to understand the epidemiological and pathogenic dynamics of MRSA.

Key words: *cap5K, cap8K,* Capsular polysaccharide, Duplex PCR, MRSA, *Staphylococcus aureus.*

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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) remains a significant threat to both human and veterinary public health due to its antimicrobial resistance, zoonotic potential, and widespread environmental persistence (Li *et al.,* 2022; Elbehiry *et al.,* 2023). Among its numerous virulence factors, the capsular polysaccharide (CP), or capsule, is particularly important as it facilitates immune evasion by inhibiting opsonophagocytosis (Ouyang *et al.,* 2021). The capsule forms an essential component of the bacterial cell wall and contributes significantly to colonization, survival, and persistence within the host (Gao *et al.,* 2024). Although *S. aureus* has been reported to possess 11 capsular serotypes, types 5 and 8 are the most commonly associated with clinical infections in both humans and animals, accounting for over 80-90% of encapsulated isolates globally (Touaitia *et al.,* 2025). These capsular types are encoded by the *cap5K* and *cap8K* genes, respectively, and have been implicated in disease severity, modulation of the immune response, and vaccine design strategies (Alkatheri *et al.,* 2022). Interestingly, the prevalence and distribution of these capsular types vary considerably depending on host species, infection site, and geographical region. This variation suggests that local environmental pressures, host specificity, and clonal diversity may shape the capsular genotype profiles of MRSA strains.

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With the increasing concern over the zoonotic transmission of MRSA, understanding the molecular epidemiology of virulence genes such as *cap5K* and *cap8K* across human, animal, and food sources is critical for tracking transmission dynamics and developing targeted interventions. Therefore, the present study was undertaken to characterize the capsular genotypes of MRSA isolates

from diverse sources in Western India using a duplex PCR assay targeting the *cap5K* and *cap8K* genes. This approach not only facilitates rapid detection but also provides insights into the strain diversity and potential host adaptations among circulating MRSA isolates. A duplex PCR to detect both capsular genes (*cap5K* and *cap8K*) in single PCR reaction and to detect variability in capsular genes (*cap5K* and *cap8K*) among 78 MRSA isolates was carried out.

Materials and Methods

Collection of Samples

The present study included a total of 203 samples collected from various human and animal sources in and around Bikaner, Rajasthan (India). A total of 45 human pus samples were collected from PBM Hospital, Bikaner. Animal pus samples (n=60) obtained from different species including dogs (15), cattle (15), camels (15), and horses (15), were collected at the Teaching Veterinary Clinical Complex (TVCC) of the College in Bikaner. Additionally, 38 mastitis milk samples were collected from bovines presented at TVCC, comprising 19 samples from cattle and 19 from buffalo. Lastly, 60 unprocessed meat samples were included, consisting of 30 each of poultry meat swabs and goat meat swabs, collected from local meat shops in Bikaner. The samples were collected in sterile HiMedia hiculture collecting device and immediately transferred to the laboratory on ice for further processing.

Isolation and Identification of MRSA

Methods of Quinn *et al*. (1994) were used for isolation and identification of *S. aureus*. Biochemical identification of presumptive Gram-positive MRSA isolates was carried out using VITEK® 2 automated system (bioMerieux, France). All isolates were identified based on cultural and biochemical characteristics, followed by molecular confirmation through 23S rRNA ribotyping (Straub *et al*., 1999) (Table 1) and *nuc* gene amplification (Brakstad *et al*., 1992) via polymerase chain reaction (PCR) and MRSA was confirmed by the presence of *mec*A gene (Mehrotra *et al.,* 2000). The confirmed isolates were used for further processing.

DNA Extraction and Amplification

DNA was isolated by QIAamp DNA Mini Kit (Qiagen, USA). PCR reaction mixture (total volume 25 µL) was prepared by mixing the ingredients: GENETAQ Green Master Mix, 2X-12.5 µL, *cap5K* Primer-F (10 pM/µL) 0.4 µL, *cap5K* Primer-R (10 pM/µL) 0.4 µL, *cap8K* Primer-F (10 pM/µL) 0.4 µL, *cap*8K Primer-R (10 pM/µL) 0.4 µL, DNA template 3.0 µL, nuclease free water to make 25.0 µL (Table 1). PCR was performed in Veriti Thermal Cycler (Applied biosystem) using the cycling parameters, pre-denaturation at 94°C for 5.0 min, followed 30 cycles each of denaturation 94°C for 30 s, annealing 55°C for 30 s, and extension 72°C for 60 s, and final extension at 72°C for 5 min. Amplified products were separated by electrophoresis in a 1.5% agarose gel in 1X TBE buffer at a constant voltage of 4 V/cm, stained using ethidium bromide (0.5 µg/mL) and 100 bp DNA ladder was used as a molecular marker. The gel was then visualized under a gel documentation system (ENDURO GDS).

Results and Discussion

Out of the 203 isolates analyzed, 78 were confirmed as *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA). Among these 78 MRSA isolates, 75 (96.15%) were successfully genotyped for capsule-associated loci (*cap5K* or *cap8K*) using PCR (Fig. 1), while 3 isolates (3.85%) remained non-typeable (NT). The high genotyping success rate highlights the utility of PCR in detecting capsular genes in *S. aureus* strains. Among the typeable isolates, the *cap5K* genotype (361 bp amplicon) was the most prevalent, detected in 35 isolates (44.87%). The *cap8K* genotype (173 bp amplicon) was identified in 21 isolates (26.92%), whereas 19 isolates (24.36%) co-expressed both *cap5K* and *cap8K* (Table 2). Only three isolates were non-typeable and were exclusively recovered from animal pus samples, specifically from cattle, camel, and horse. A distinct distribution pattern of capsular genotypes was observed across sample types. Isolates harbouring both *cap5K* and *cap8K* were more common in human samples (8 out of 16; 50.00%) compared to animal-derived samples (Table 2). In contrast, *cap5K*-only strains were more frequently found in animal isolates; for

Table 1: Primers used for PCR of MRSA isolates from animals and humans

S. No.	Gene	Primer sequence (5´ to 3´)	Size (bp)	Annealing Temp. (°C)	Reference
1.	23S rRNA	F-5′-ACGGAGTTACAAAGGACGAC-3′ R-5′-AGCTCAGCCTTAACGAGTAC-3′	1250	64	Straub *et al.* (1999)
2.	*nuc*	F-5′-GCG ATT GAT GGT GAT ACG GTT-3′ R-5′-ACG CAA GCC TTG ACG AAC TAA AGC-3′	280	55	Brakstad *et al*. (1992)
3.	*mec*A	F-5′-ACTGCTATCCACCCTCAAAC-3′ R-5′-CTGGTGAAGTTGTAATCTGG-3′	163	57	Mehrotra *et al.* (2000)
4.	*cap5K*	F-5′-GTCAAAGATTATGTGATGCTACT GAG-3′ R-5′-ACTTCGAATATAAACTTGAATCAA TGTTATACAG-3′	361	55	Verdier *et al.* (2007)
5.	*cap8K*	F-5′-GCCTTATGTTAGGTGATAAACC-3′ R-5′-GGAAAAACACTATCATAGCAGG-3′	173	55	Verdier *et al.* (2007)

Table 2: Detection of Antiphagocytosis (Capsule) factor-associated genes among MRSA isolates

S. No	Sample type	No. of MRSA isolates	Capsule associated genes (%)			
			cap5K **(361 bp)**	***cap8K*** **(173 bp)**	**Both**	**NT**
1.	Pus of human	16	4 (25.00)	4 (25.00)	8 (50.00)	0
2.	Pus of animals	25	12 (48.00)	8 (32.00)	2 (8.00)	3 (12.00)
	Dog	6	5 (83.33)	1 (16.67)	0	0
	Cattle	7	1 (14.29)	3 (42.86)	2 (28.57)	1 (14.29)
	Camel	6	3 (50.00)	2 (33.33)	0	1 (16.67)
	Horse	6	3 (50.00)	2 (33.33)	0	1 (16.67)
3.	Mastitis milk	17	7 (41.18)	4 (23.53)	6 (35.29)	0
	Cattle	9	4 (44.44)	3 (33.33)	2 (22.22)	0
	Buffalo	8	3 (37.50)	1 (12.50)	4 (50.00)	0
4.	Unprocessed Meat	20	12 (60.00)	5 (25.00)	3 (15.00)	0
	Chicken	7	5 (71.43)	1 (14.29)	1 (14.29)	0
	Goat	13	7 (53.85)	4 (30.77)	2 (15.38)	0
	Total	**78**	**35 (44.87)**	**21 (26.92)**	**19 (24.36)**	**3 (3.85)**

example, 12 out of 25 animal pus isolates (48.00%) and 12 out of 20 unprocessed meat isolates (60.00%) as compared to 4 out of 16 (25.00%) human pus isolates. Notably, *cap8K*-only strains showed a moderate presence across both human and animal samples.

The Chi-square analysis yielded a Chi-square value of 17.87 with 9 degrees of freedom, and a p-value of 0.037. This indicates a statistically significant relationship between sample source and the distribution of *cap* genes in MRSA isolates. The predominance of *cap5K* in our study aligns with previous findings in the same geographical region. Various authors (Upadhyay *et al.* ,2010; Khichar *et al.*,2014 ; Yadav *et al.*,2015; Nathawat *et al.*,2015 and Sharma *et al.* (2016) reported *cap5K* prevalence rates ranging from 46.5% to 92.86%, further supporting a regional trend favouring this genotype. Studies, by Camussone *et al.* (2012) in Argentina and Salimena *et al.* (2016) in Brazil, also documented higher frequencies of *cap5K* over *cap8K*. Similarly, Kumar *et al.* (2011) from India observed a similar trend in bovine isolates. In contrast, Gharaibeh and Abu-Qatouseh (2022) and Singhal *et al.* (2020) reported a higher prevalence of *cap8K* in their MRSA collections from the same region, suggesting the existence of niche or host-specific variations. This variability was further emphasized by Diwakar *et al.* (2023), who found a predominance of *cap8K* (94.11%) over *cap5K* (35.29%) in *S. aureus* isolates from caprine clinical mastitis cases in India.

Interestingly, isolates co-expressing both *cap5K* and *cap8K* genes were more frequently found in human sources (50.00%) than animal sources (e.g., 8.00% in animal pus), hinting at possible host-specific adaptation or selective pressures influencing gene expression. On the other hand, *cap5K*-only genotypes were significantly more common in animal isolates, particularly in unprocessed meat and pus samples, suggesting potential roles in virulence or environmental persistence in animal reservoirs. Reinoso *et al.* (2008) also observed a higher frequency of *cap5K* in human isolates than in bovine strains, which supports our findings. However, their report of complete absence of *cap8K* in bovine isolates differs from our study, where this genotype was detected in 32.00% of animal pus and 23.53% of mastitis milk isolates, possibly reflecting differences in local strain dynamics or animal husbandry practices. The detection of non-typeable strains exclusively from animal pus samples is consistent with previous reports by Upadhyay *et al.* (2010), Yadav *et al.* (2015), Sharma *et al.* (2016), Bhati and Kataria (2019), and Singhal (2020). Such NT strains may represent novel capsular types not targeted by conventional *cap5*/*cap8* primers or strains that lack a capsule altogether. Additionally, the absence of *cap8K* (Proietti *et al.,* 2010) or *cap5K* (Soares *et al.,* 2017) in certain isolate collections elsewhere further demonstrates the genetic diversity among *S. aureus* strains.

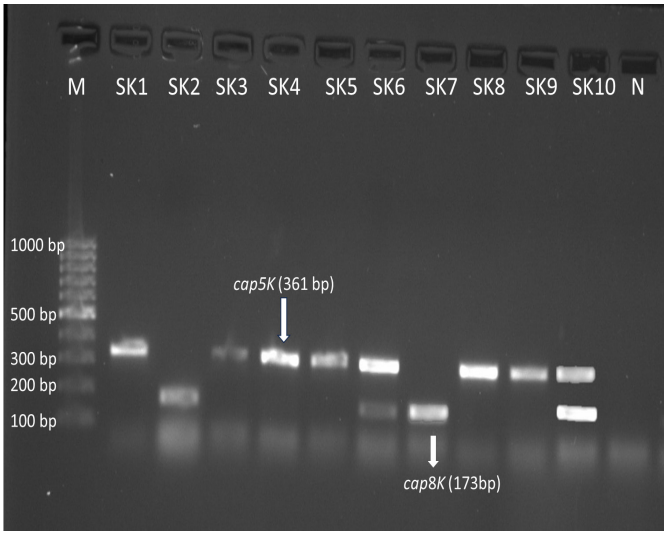

Fig. 1: Agarose gel electrophotogram showing duplex PCR amplicons of *cap5K* and *cap8K* genes of MRSA isolates. Lane 1: 100bp ladder; Lane 2-10: Duplex PCR amplicons of different MRSA isolates

Conclusion

This study highlights the predominance of the *cap5K* gene among MRSA isolates from both human and animal sources in Western India, with notable host-specific differences in capsular gene distribution. The higher occurrence of *cap5K* in animal isolates and dual gene presence (*cap5K* and *cap8K*) in human isolates suggests potential host adaptation. The presence of non-typeable strains indicates possible genetic diversity beyond the commonly targeted capsular types. These findings emphasize the need for ongoing molecular surveillance to better understand the epidemiology and virulence of MRSA in diverse hosts.

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