RESEARCH ARTICLE

# Comparison of Multilocus Sequence Typing and *Spa* Typing of *Staphylococcus aureus* Isolated from Bovine and Bubaline Mastitis

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## Abstract

*Staphylococcus aureus* (*S. aureus*) is the leading causative agent of mastitis in dairy cows, causing reduced milk quality and production. The present study describes the genotypes and clonal distributions of 35 *S. aureus* strains isolated from cases of mastitis from southern India by multi-locus sequence typing (MLST). The resultant *S. aureus* sequence types (STs) were compared with their *Staphylococcus* protein A (*spa*) types and correlated to methicillin resistance. A total of 27 methicillin-sensitive *Staphylococcus aureus* (MSSA) and eight methicillin-resistant *Staphylococcus aureus (*MRSA) strains were identified. Twenty different STs with nine new alleles and nine new STs were obtained. The STs were grouped into four clonal complexes (CCs) CC1, CC5, CC8 and CC97. The predominant STs 2454 and 2459 belonged to CC8 and CC97, respectively. Eight STs were not clustered under any of the CCs. Comparison of genotypes of MLST with that of *spa* types revealed that some of the isolates with same ST yielded different *spa* types that were closely related to each other. On the contrary, some of the isolates with same *spa* types gave different STs in which one ST was the single locus variant of the other in all the cases. Overall, high diversity of *S. aureus* isolates was observed, excepting for the isolates from a particular geographical region (Bidar) in Karnataka.

**Key words:** Bovine milk, Clonal complexes (CCs), MRSA, Multi-Locus Sequence Typing (MLST), Sequence types (STs), *Staphylococcus protein A* (*spa*) typing.

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## Introduction

*Staphylococcus* species are important etiological agents of mastitis in cattle and buffaloes resulting in huge economic losses in dairy industry worldwide responsible for up to 74% of bovine mastitis cases causing per-acute, acute, sub-acute, chronic, gangrenous and subclinical types of mastitis (Sharma *et al.,* 2012). There are recent reports emphasizing the need to characterize the isolates distributed worldwide to improve our understanding of pathogenesis in the natural populations (Klein *et al*., 2007). Molecular epidemiological studies contribute considerably to our understanding of sources, transmission routes and prognosis for many bovine mastitis pathogens, and to our understanding of mechanisms of host-adaptation and disease causation (Zadoks *et al*., 2011). Newer DNA sequencing methods including multi-locus sequence typing (MLST), *spa* typing, SCC*mec* typing and toxin gene profiles (TGP) typing are more practical methods for detecting evolutionary changes and transmission events. They possibly aid in implementing control measures in an outbreak situation, have high discriminatory power and the data can be compared across laboratories by making them accessible through the Internet (Mehndiratta and Bhalla, 2012).

For monitoring short-term, local outbreaks, *spa* typing is most appropriate (Hallin *et al*., 2007), whereas MLST based on sequencing of more stable house-keeping genes has been proposed for long-term global studies and for the assessment of evolutionary relationships among *S. aureus* strains (Cooper and Feil, 2004). By employing *spa* typing and MLST, studies have been carried out in various parts of the

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world to characterise the *S. aureus* strains of human origin. But the data on virulence potential and clonal diversity of *S. aureus* originating from bovine mastitis is still limited in many countries and necessitate investigation. The aim of this study was to determine the genotypes and clonal distributions of the *S. aureus* associated with bovine and bubaline mastitis in Southern India by MLST and compare them with *spa*-typing and correlate them with methicillin resistance (phenotypic).

## Materials and Methods

### Bacterial Isolates

Bacterial strains of bovine (n=28) and bubaline (n=7) mastitis origin maintained in the Microbiology Departments of Veterinary College, Bengaluru and Shivamogga (Karnataka, India) were used in the present study. The samples were collected from both organised and unorganised farms located in southern India, mainly Karnataka and Maharashtra (Table 1).

### Phenotype and PCR-based confirmation of *S. aureus*

The pure cultures obtained from the revival of the maintained stock cultures were subjected to routine cultural, morphological and biochemical analyses for confirmation of the species by phenotypic properties as described earlier (Sundareshan, 2012).

Genomic DNA of the isolates was extracted using a bacterial DNA extraction kit (UniFlex™ DNA isolation kit, GeNei™, Bengaluru), following the manufacturer's instructions. Molecular confirmation of *S. aureus* was performed by PCR amplification of the species-specific staphylococcal nuclease (*nuc*) gene as described previously (Hegde, 2011).

### Detection of MRSA by Phenotypic Method

The isolates were tested for phenotypic methicillin resistance by employing the Kirby-Bauer disk diffusion method. Cefoxitin discs (30 µg/disc, HiMedia) were used to test methicillin resistance. The isolates were categorized as either methicillin-resistant *Staphylococcus aureus* (MRSA) or methicillin-sensitive *Staphylococcus aureus* (MSSA) according to the guidelines recommended by the Clinical and Laboratory Standards Institute (CLSI, 2018).

### Multi-Locus Sequence Typing of *S. aureus*

A total of 35 representative isolates (28 from bovines and 7 from buffaloes) from different geographical areas under study having different *spa* types (Table 1) were subjected to MLST analysis, based on the sequencing of 7 house-keeping genes described previously (Enright *et al*., 2000). The sequence of each locus was compared to allele sequences in the MLST database (http://saureus.mlst.net). The combination of alleles at the seven loci was used to define the allelic profile for each isolate and assigned to a sequence type (ST). The allele and/or ST number of putative novel alleles and the allelic profiles of novel STs were obtained from the database curator after submitting them. Subsequently, the isolates were grouped in clonal complexes (CCs) and analysed in conjunction with the entire *S. aureus* MLST database (http://saureus.mlst.net). The clonal complex relationship of the isolates was determined using goeBURST [enhanced version of BURST (Based Upon Related Sequence Types)] (http://goeBURST.pyloviz.net/).

### Analysis of Diversity of *S. aureus* MLST STs

The diversity of MLST STs determined for the isolates was studied and was compared with the *spa* types obtained in another study (Sheela *et al*., 2019) using the same isolates by amplification of the *spa* repeat region as per the procedure described previously (Aires-de-Sousa *et al*., 2006) using the database (http://spatyper.fortinbras.us/) available online. *Spa* types with similar repeat profiles were grouped into a *spa* complex as described earlier (Ruppitsch *et al*., 2006).

## Results and Discussion

### Multi-Locus Sequence Typing of *S. aureus*

The MLST analysis of 35 *S. aureus* isolates revealed 20 different sequence types (STs) which were further grouped into four clonal complexes (CCs) CC1, CC5, CC8 and CC97 according to *S. aureus* MLST database (http://pubmlst.org/saureus) (Table 1). Eight STs were not clustered under any of the clonal complexes. A total of nine new alleles (583, 588 and 598 for *arc*C, 735 and 737 for *aro*E, 685 for *glp*, 556 for *tpi* and 658 and 659 for *yqi*L) and nine new STs (ST4967, ST4968, ST4975, ST4976, ST4992, ST5098, ST 5113, ST5273 and ST5360) were obtained (Table 1). Discovery of new alleles and sequence types (STs) suggests *S. aureus* strains causing mastitis in this region are evolving locally and may have unique evolutionary lineages not seen elsewhere. In addition, it also indicates that the local bacterial population is genetically diverse, possibly due to varied animal husbandry practices, antibiotic usage, or environmental pressures. High diversity also complicates vaccine or treatment strategies, as a one-size-fits-all approach may not be effective. Of the 35 *S. aureus* isolates subjected to MLST, eight were MRSA and the remaining 27 were found to be MSSA.

In the present study, CC8 (n=11) was the most predominant CC represented by STs 2454 (n=9, the predominant ST), ST4968 (n=1), ST4975 (n=1) and ST4975 being a single locus variant (SLV) of ST2454 (Fig. 1). ST4968 though differed from ST2454, both were placed in the same CC *i.e.* CC8. However, based on the *spa* typing they were placed in different *spa* complexes (Table 3). Isolates with different *spa* types t7867, t7286, t18547, t4522, t18314 along with three strains (P144, P145 and P171) with non-typeable *spa* types were associated with ST2454 (Fig. 2). Nevertheless, all of these isolates, except one (t18314), exhibited a similar repeat profile in their *spa* repeats (Table 3) suggesting microevolution within strains of the same ST. On the contrary, isolates (P144 and P169) belonging to the same s*pa* type (t4522) belonged

**Table 1:** MLST allelic profile/ sequence types of *S. aureus* isolates obtained in the study

| Isolate ID | Location | Species | *Spa* Type | MRSA/MSSA | MLST-ST | CC | *Arc* allele | *aroE* allele | *glpF* allele | *Gmk* allele | *Pta* allele | *Tpi* allele | *yqilL* allele |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BP2 | Dharwad | Bubaline | t442 | MSSA | 5 | CC5 | 1 | 4 | 1 | 4 | 12 | 1 | 10 |
| BP5 | Dharwad | Bubaline | t359 | MRSA | 4967# | CC97 | 583* | 1 | 280 | 168 | 1 | 5 | 3 |
| BP7 | Dharwad | Bubaline | t359 | MRSA | 4967# | CC97 | 583* | 1 | 280 | 168 | 1 | 5 | 3 |
| BP8 | Dharwad | Bubaline | t4570 | MRSA | 2459 | CC97 | 3 | 240 | 280 | 168 | 1 | 5 | 3 |
| BP14 | Bengaluru | Bubaline | t7696 | MRSA | 1687 | CC97 | 3 | 1 | 208 | 1 | 1 | 5 | 3 |
| BP15 | Bengaluru | Bubaline | t7867 | MSSA | 2454 | CC8 | 3 | 3 | 1 | 1 | 264 | 1 | 10 |
| BP19 | Bengaluru | Bubaline | t18547 | MSSA | 2454 | CC8 | 3 | 3 | 1 | 1 | 264 | 1 | 10 |
| P10 | Bengaluru | Bovine | t3380 | MRSA | 2459 | CC97 | 3 | 240 | 280 | 168 | 1 | 5 | 3 |
| P12 | Bengaluru | Bovine | t4793 | MRSA | 6 | CC5 | 12 | 4 | 1 | 4 | 12 | 1 | 3 |
| P22 | Bengaluru | Bovine | t7287 | MSSA | 5360# | CC1 | 1 | 13 | 1 | 1 | 1 | 10 | 658* |
| P26 | Bengaluru | Bovine | t267 | MSSA | 4992# | - | 3 | 737* | 280 | 168 | 1 | 5 | 475 |
| P41 | Bengaluru | Bovine | t7286 | MSSA | 2454 | CC8 | 3 | 3 | 1 | 1 | 264 | 1 | 10 |
| P51 | Bengaluru | Bovine | t213 | MSSA | 12 | - | 1 | 3 | 1 | 8 | 11 | 5 | 11 |
| P103 | Bidar | Bovine | t4522 | MSSA | 2454 | CC8 | 3 | 3 | 1 | 1 | 264 | 1 | 10 |
| P107 | Bidar | Bovine | t10760 | MSSA | 4968# | CC8 | 3 | 735* | 1 | 1 | 443 | 4 | 3 |
| P123 | Bidar | Bovine | t2297 | MSSA | 2453 | CC97 | 3 | 1 | 280 | 168 | 1 | 5 | 178 |
| P144 | Bidar | Bovine | NT | MSSA | 2454 | CC8 | 3 | 3 | 1 | 1 | 264 | 1 | 10 |
| P145 | Bidar | Bovine | NT | MSSA | 2454 | CC8 | 3 | 3 | 1 | 1 | 264 | 1 | 10 |
| P169 | Bidar | Bovine | t4522 | MSSA | 4975# | CC8 | 3 | 3 | 1 | 1 | 264 | 1 | 658* |
| P171 | Bidar | Bovine | NT | MSSA | 2454 | CC8 | 3 | 3 | 1 | 1 | 264 | 1 | 10 |
| P172 | Bidar | Bovine | t3841 | MSSA | 672 | - | 4 | 3 | 1 | 1 | 11 | 72 | 11 |
| P181 | Bidar | Bovine | t519 | MSSA | 4976# | - | 588* | 1 | 1 | 1 | 12 | 556* | 659* |
| UP9 | Uppinangadi | Bovine | t 668 | MSSA | 5 | CC5 | 1 | 4 | 1 | 4 | 12 | 1 | 10 |
| UP11 | Uppinangadi | Bovine | t002 | MRSA | 5 | CC5 | 1 | 4 | 1 | 4 | 12 | 1 | 10 |
| UP12 | Uppinangadi | Bovine | t1236 | MSSA | 97 | CC97 | 3 | 1 | 1 | 1 | 1 | 5 | 3 |
| UP13 | Uppinangadi | Bovine | t1236 | MSSA | 97 | CC97 | 3 | 1 | 1 | 1 | 1 | 5 | 3 |
| UP15 | Uppinangadi | Bovine | t7287 | MSSA | 5098# | - | 598* | 13 | 1 | 1 | 1 | 10 | 658 |
| UP16 | Uppinangadi | Bovine | t7656 | MSSA | 88 | - | 22 | 1 | 14 | 23 | 12 | 4 | 31 |
| UP17 | Uppinangadi | Bovine | t657 | MRSA | 772 | CC1 | 1 | 1 | 1 | 1 | 22 | 1 | 1 |
| SMG2 | Shimoga | Bovine | t3992 | MSSA | 2459 | CC97 | 3 | 240 | 280 | 168 | 1 | 5 | 3 |
| SMG24 | Shimoga | Bovine | t18314 | MSSA | 2454 | CC8 | 3 | 3 | 1 | 1 | 264 | 1 | 10 |
| SMG46 | Shimoga | Bovine | t5019 | MSSA | 2459 | CC97 | 3 | 240 | 280 | 168 | 1 | 5 | 3 |
| M4 | Mumbai | Bovine | t18320 | MSSA | 5273# | - | 3 | 3 | 149 | 1 | 264 | 1 | 10 |
| M11 | Mumbai | Bovine | t18320 | MSSA | 2454 | CC8 | 3 | 3 | 1 | 1 | 264 | 1 | 10 |
| M35 | Mumbai | Bovine | NT | MSSA | 5113# | - | 3 | 3 | 685* | 1 | 264 | 1 | 10 |

**Note:** MRSA: methicillin-resistant *Staphylococcus aureus,* MSSA: methicillin-susceptible *Staphylococcus aureus,* MLST-ST: multi-locus sequence typing-sequence type, CC: clonal complex, *arcC*: gene encoding carbamate kinase, *aroE*: gene encoding shikimate dehydrogenase, *glpF*: gene encoding glycerol kinase, *gmk*: gene encoding guanylate kinase, *pta*: gene encoding phosphate acetyl transferase, *tpi*: gene encoding triosephosphate isomerise, *yqilL*: gene encoding acetyl coenzyme A acetyl transferase, #: new MLST profile or sequence type (ST), *: new allele.

to two different sequence types, *viz*., ST2454 and ST4975, respectively, in which ST4975 is a SLV of ST2454. Similarly isolates with same *spa* type t=18320 belonged to different STs, *viz.,* 5273 and 2454 (5273 SLV of 2454) and two more isolates (P22 and UP15) with same *spa* type t=7287 belonged to different STs 5098 and 5360, where 5098 is a SLV of 5360 (Fig. 2) indicating recent evolutionary divergence, possibly from a common ancestor. The conservation of the *spa* type despite ST divergence may imply stabilizing selection on the *spa* gene or slower mutation rate.

It was found in the present study that some STs were associated with certain *spa* types. A set of two isolates with same *spa* type yielded same sequence type (t359 and ST4967) and two more isolates with *spa* type t1236 yielded same sequence type *i.e.* ST97.

All the isolates of CC8 (n=11) were MSSA which comprised 41 % of the total MSSA (n=27) strains. Among the nine bovine isolates from Bidar, six of them belonged to CC8, exhibiting least diversity, whereas the isolates from another geographical region (Bengaluru) exhibited greatest genetic variability (Table 2). The CC8-MSSA was reported as a frequent clone from bovine mastitis in Western Switzerland (Sakwinska *et al*., 2011). The CC8 was reported as the predominant CC in bovine *S. aureus* isolates in Italy, mostly associated with MSSA strains (Luini *et al*., 2015). In a study, a close genetic relationship was demonstrated between MSSA isolates from dairy cow mastitis and the prominent human CC8, suggesting human-to-bovine jump associated with the combined loss of β-haemolysin converting prophages and gain of a new SCC (without *mecA/mecR1*) probably acquired in the animal environment (Resch *et al*., 2013). Notably, CC8-MRSA is seldom recovered from livestock; one case of bovine mastitis by ST8 was described in Belgium (Bardiau *et al*., 2013) and one out of 95 *S. aureus* strains collected from bulk tank milk in Minnesota was ST8 with *spa* type t121 (Haran *et al*., 2012).

In the current study, CC97 strains (n=10) were recovered from different regions, as well as from both cattle (n=6) and buffaloes (n=4) comprising of both MRSA (n=5) and MSSA (n=5) strains. CC97 (n=10) was represented by STs 2459 (n=4, the second predominant ST) along with 2453 (n=1), 4967(n=2) and 97(n=2) which are double locus and triple locus variants (DLV, TLV) of ST 2459, respectively. Another ST of CC97, *viz.,* ST1687 (n=1) is a SLV of ST97 (Fig. 1). Although the isolates of CC97 were of different *spa* types, most of them exhibited similarity in their *spa* repeat profiles (Table 3).

The CC97 identified as the second most prevalent CC in the present study has been reported as a leading cause of bovine mastitis worldwide and occasionally in small ruminants, pigs and humans (Butaye *et al*., 2016). Bovine *S. aureus* isolates of CC97 were the most frequently detected in USA (Smith *et al*., 2005), Japan (Hata *et al.,* 2010), Netherlands (Ikawaty *et al*., 2009) and in UK. Contrary to this, CC97 has been rarely detected among isolates from human beings suggesting that ruminants are reservoirs for this CC (Smith *et al*., 2005). Human strains of CC97, a widely disseminated human CA-MRSA type descended from bovine MSSA, has been hypothesized to acquire SCC*mec* during a bovine to human host jump approximately 40 years ago. This is in contrast to the livestock clade of CC398 that acquired SCC*mec* after jumping from humans to pigs (Butaye *et al*., 2016).

The *S. aureus* clones under CC5 in this study were ST5-t002, ST5-t442, ST5-t668 and ST6-t4793 (DLV of ST5), comprising of both MRSA and MSSA strains (Fig. 2). A striking observation regarding CC5 is that strains of this lineage especially ST5, have had host jumps to poultry, in which it is frequently implicated in disease (Hasman *et al*., 2010). The *S. aureus* clone t002-ST5-MRSA identified in the present study was also reported from bovine mastitic isolates from Japan and Korea (Hata *et al*., 2010; Hwang *et al.,* 2010), in isolates

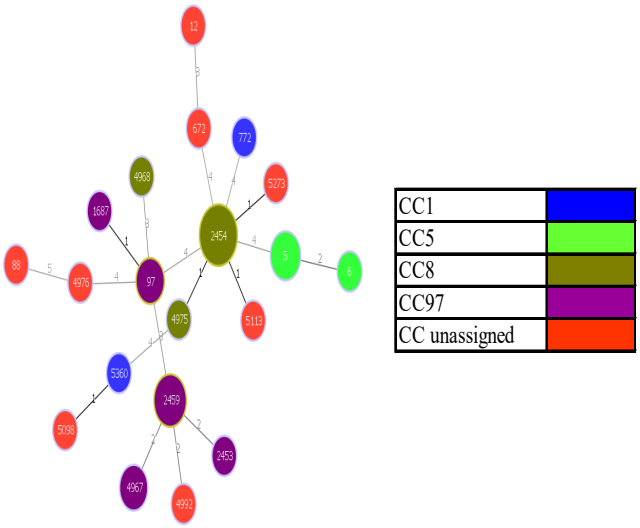


**Fig. 1:** Analysis and visualization of MLST clonal relationships of *S. aureus* isolates integrating clonal complex data using goeBURST full minimum spanning tree (level 5)

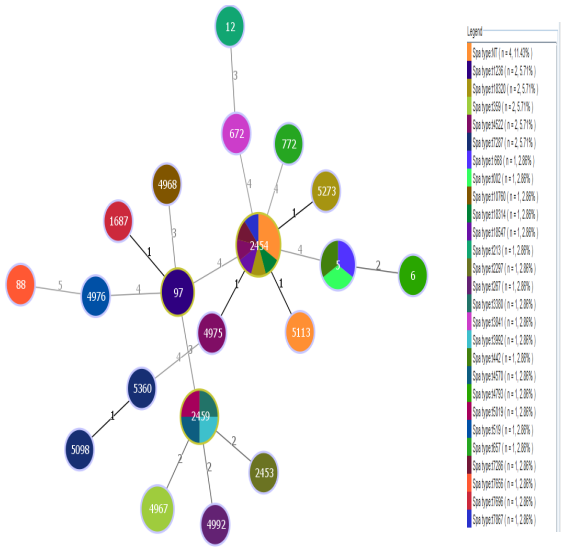

**Fig. 2:** Analysis and visualization of MLST clonal relationships of *S. aureus* isolates integrating *spa* type data using goeBURST full minimum spanning tree (level 5)

**Table 2:** Frequency of *spa* types and clustering based on their repeat succession

| Sl. No. | *Spa* types | *Spa* repeats | | | | | | | | | | | | *Spa* complexes | No of repeats | Susceptibilityto methicillin | | Occurrence of this clone/ *spa* type (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | | | MSSA | MRSA | |
| 1 | t3992* | r26 | r23 | r12 | r21 | r17 | r34 | r34 | | r33 | r34 | | | | 9 | 3 | 2 | 5 |
| 2 | t1236 | r26 | r23 | r12 | r21 | r17 | r34 | r34 | r34 | r33 | r34 | | | I | 10 | 2 | | 2 |
| 3 | t657 | r26 | r23 | r13 | r21 | r17 | r34 | r26 | | r33 | r34 | | | | 8 | | 1 | 1 |
| 4 | t002* | r26 | r23 | r17 | r34 | r17 | r20 | r17 | r12 | r17 | r16 | | | | 10 | | 1 | 1 |
| 5 | t668 | r26 | r23 | r17 | r34 | r17 | r20 | | | r17 | r16 | | | II | 8 | 1 | | 1 |
| 6 | t3841 | r26 | r22 | r17 | | | r20 | r17 | r12 | r17 | r16 | r16 | | | 9 | 6 | | 6 |
| 7 | t442 | r35 | | r17 | r34 | r17 | r20 | r17 | r12 | r17 | r16 | | | | 9 | 1 | | 1 |
| 8 | t359* | r07 | r23 | r12 | r21 | r17 | r34 | r34 | r33 | r34 | | | | | 9 | 16 | 15 | 31 |
| 9 | t3380 | r07 | r23 | r12 | r21 | r17 | r34 | r34 | | | | | | III a | 7 | | 1 | 1 |
| 10 | t4570 | r07 | r23 | r12 | r21 | | r34 | | | | | | | | 5 | | 2 | 2 |
| 11 | t2297* | r07 | r23 | r12 | r12 | r21 | r17 | r34 | r34 | r34 | r33 | r34 | | | 11 | 2 | | 2 |
| 12 | t267 | r07 | r23 | r12 | | r21 | r17 | r34 | r34 | r34 | r33 | r34 | | III b | 10 | 2 | | 2 |
| 13 | t7696 | r07 | r23 | r12 | | r437 | r17 | r34 | r34 | r34 | r33 | r34 | | | 10 | | 3 | 3 |
| 14 | t5019 | r07 | r23 | | | | r17 | r34 | r34 | r34 | r33 | r34 | | | 8 | 1 | | 1 |
| 15 | t213 | r07 | r23 | r12 | | r21 | r24 | r33 | r22 | r17 | | | | | 8 | 2 | | 2 |
| 16 | t7867* | r07 | r16 | r12 | r23 | r02 | r02 | r34 | r34 | | | | | | 8 | 9 | 4 | 13 |
| 17 | t7286 | r07 | r16 | r12 | r23 | r02 | | r34 | r34 | | | | | IV a | 7 | 1 | 1 | 2 |
| 18 | t18547 | r07 | r16 | r21 | | r02 | r02 | r34 | r34 | | | | | | | 1 | | 1 |
| 19 | t18320* | r26 | r16 | r12 | r23 | r02 | r12 | r23 | r02 | r02 | r02 | r02 | r34 | IVb | 12 | 3 | | 3 |
| 20 | t4522 | r07 | r16 | r12 | r23 | r02 | r12 | r23 | r02 | r02 | r02 | | r34 | | 11 | 5 | | 5 |
| 21 | t4793* | r11 | r10 | r21 | r17 | r34 | | r22 | r25 | r25 | | | | V | 8 | 1 | 1 | 2 |
| 22 | t10760 | r11 | r19 | r21 | r12 | r34 | r24 | r22 | r25 | | | | | | 8 | 1 | | 1 |
| 23 | t519 | r04 | r20 | r17 | r25 | | | | | | | | | | 4 | 1 | | 1 |
| 24 | t7287 | r125 | r21 | r17 | r34 | r12 | r23 | r02 | r12 | r23 | | | | Singletons | 9 | 3 | 2 | 5 |
| 25 | t7656 | r07 | r12 | r21 | r17 | r13 | r13 | r13 | r13 | r34 | | | | | 11 | 1 | | 1 |
| 26 | t18314 | r07 | r16 | r17 | r16 | r34 | r34 | r16 | r34 | | | | | | 8 | 1 | | 1 |
| 27 | NT (P103) | r07 | r16 | r12 | r23 | ? | r12 | r23 | r02 | r02 | r02 | r34 | | | | 1 | | 1 |
| 28 | NT (P145) | r07 | r16 | r12 | r23 | ? | r12 | r23 | r02 | r02 | r02 | r34 | | | | | 1 | 1 |
| 29 | NT (P171) | r07 | r16 | r12 | r23 | ? | r12 | r23 | r02 | r02 | | r34 | | | | 1 | | 1 |
| 30 | NT (M35) | | | | | ? | r12 | r23 | r02 | r02 | r34 | r34 | | | | 1 | | 1 |
| | | | | | | | **Total** | | | | | | | | | **66** | **34** | **100** |

**Note:**
MSSA : methicillin-sensitive *Staphylococcus aureus*
MRSA : methicillin-resistant *Staphylococcus aureus*
* : prototype of the complex
**?** : repeat not assigned
Cluster I : r26-r23-r12/13-r21-r17-(r34)n-(r26)-r33-r34
Cluster II : r26/r35-r23/22-r17-(r34-r17)-r20-(r17-r12)-r17-(r16)n
Cluster IIIa : r07-r23-r12-r21-(r17)-(r34)n-(r33)-r34
Cluster IIIb : r07-r23-(r12)n-r21/437-(r24-r33-r22)-r17-(r34)n-(r33)-(r34)
Cluster IVa : r07-r16-r12/21-(r23)-(r02)n-(r34)2
Cluster IVb : r7/26-r16-r12-r23-r02-r12-r23-(r02)n-r34
Cluster V : r11-r10/19-r21-r17/12-r34-(r24)-r22-(r25)n
NT : Non-typeable (No. in parenthesis indicates the isolate ID)

**Table 3:** Distribution of STs, *spa* types and MRSA in different CCs

| Clonal Complexes (CC) | No. of isolates | Associated STs | Associated *spa* types* | MSSA | MRSA |
|---|---|---|---|---|---|
| CC1 | 2 | ST772, ST5360 | t657 (1), t7287 (1) | 1 | 1 |
| CC5 | 4 | ST5, ST6 | t442 (1), t4793 (1), t 668 (1), t002 (1) | 2 | 2 |
| CC8 | 11 | ST2454, ST4968, ST4975 | t4522 (2), t7286 (1), t7867 (1), t10760 (1), t18314 (1), t18320 (1), t18547 (1), NT (3) | 11 | 0 |
| CC97 | 10 | ST97, ST1687, ST2453, ST2459, ST4967 | t359 (2), t1236 (2), t2297 (1), t3380 (1), t3992 (1), t4570 (1), t5019 (1), t7696 (1) | 5 | 5 |
| CC unassigned | 8 | ST12, ST88, ST672, ST4976, ST4992, ST5098, ST5113, ST5273 | t213 (1), t267 (1), t519 (1), t3841 (1), t7287 (1), t7656 (1), t18320 (1), NT (1) | 8 | 0 |

*Numbers in the parenthesis indicate no. of isolates belonging to the *spa* type.

from poultry in Denmark (Hasman *et al*., 2010) and in human MRSA isolates (Aires-de-Sousa *et al*., 2006).

In the present study, the *S. aureus* clones under CC1 were ST772-t657 and ST5360-t7287 from MRSA and MSSA strains, respectively. The CC1/ST1-t127 clone reported in MSSA isolates in dairy cows from Brazil (Silva *et al*., 2013) is one of the major clones circulating in Italy, where it was reported for the first time and also has been implicated in mastitis in cows in Italy (Luini *et al*., 2015) and also in China (Li *et al*., 2017).

Nine different *S. aureus* clones were identified with CC8, while eight *S. aureus* clones were identified with CC97, and eight *S. aureus* clones were not identified with any clonal complexes (Table 1). The CCs 1 and 5 detected in the present study along with CC30 have been reported in bovine mastitic cases and also implicated with human infections worldwide. The genetic background of these CCs is more suited for spread among human beings (Gomes *et al.,* 2006). In this study also the source of infection in the farms might be from humans. The analysis of samples from animal handlers also might help in understanding the source.

### Analysis using goeBURST Full Minimum Spanning Tree

The goeBURST full MST algorithm analysis of 35 *S. aureus* isolates comprising 20 STs allowed the visual exploration of the clonal relationship of the STs. At level of 5, all the STs of the present study were clustered in a single group with links along with numbers of allelic differences. The links are colour-coded for the number of differences; darker links represent less allelic differences in the profile than lighter links. The number of differences in the profiles it is connecting is displayed on the link. The MST was divided in groups by keeping links up to a determined level when the tree was constructed at the level of 1 to 5. The node size varied linearly (in logarithmic scale) with the number of isolates of a given ST. No ST under the study differed from the other in six alleles.

## Conclusion

The study highlights local evolution, strain diversity, and resistance gene dissemination in bovine mastitis-associated *S. aureus* in Southern India. It underscores the need for region-specific surveillance and typing efforts. All the isolates of the predominant CC8 were MSSA, whereas both methicillin susceptible and resistance isolates were equally distributed in CC97, CC5 and CC1 indicating that resistance is not just clonal, but also dispersed across multiple lineages, likely due to horizontal gene transfer (HGT) of resistance elements like *SCCmec*. From a One Health perspective, the presence of MRSA and MSSA in livestock has implications for zoonotic transmission to humans.

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