Novel Rearrangements in the Staphylococcal Cassette Chromosome Mec Type V Elements of Indian ST772 and ST672 Methicillin Resistant *Staphylococcus aureus* Strains

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**Abstract**

*Staphylococcus aureus* is a commensal gram positive bacteria which causes severe and non severe infections in humans and livestock. In India, ST772 is a dominant and ST672 is an emerging clone of *Staphylococcus aureus*. Both cause serious human diseases, and carry type V SCCmec elements. The objective of this study was to characterize SCCmec type V elements of ST772 and ST672 because the usual PCR methods did not amplify all primers specific to the type. Whole genome sequencing analysis of seven ST772 and one ST672 *S. aureus* isolates revealed that the SCCmec elements of six of the ST772 isolates were the smallest of the extant type V elements and in addition have several other novel features. Only one ST772 isolate and the ST672 isolate carried bigger SCCmec cassettes which were composites carrying multiple ccr genes. These cassettes had some similarities to type V SCCmec element from M013 isolate (ST59) from Taiwan in certain aspects. SCCmec elements of all Indian isolates had an inversion of the mec complex, similar to the bovine SCCmec type X. This study reveals that six out of seven ST772 *S. aureus* isolates have a novel type V (SCCmec element while one each of ST772 and ST672 isolates have a composite SCCmec type V element (SC2&5) formed by the integration of type V SCCmec into a MSSA carrying a SCC mosaic element, in addition to the mec gene complex inversions and extensive recombinations.

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**Introduction**

*Staphylococcus aureus* is an important pathogen in hospitals and in communities causing a broad range of infections among both humans and animals. Treatment of severe infections is becoming a challenge due to development of multi-drug resistance. Methicillin resistance appeared soon after the introduction of this antibiotic in 1960. It is due to the presence of mec gene coding for the protein PB2A, which has a low affinity for β-lactam antibiotics [1–4]. The mecA gene is housed within a unique mobile genetic element known as Staphylococcal Cassette Chromosome mec (SCCmec) integrated in the staphylococcal genome. The SCCmec is comprised of (1) mec gene complex containing the mecA gene, its regulatory genes and associated insertion sequences, and (2) cassette chromosome recombinase (ccr) gene complex [5,6]. Six classes of mec gene complexes (A, B, C1, C2, D and E) and three ccr genes (ccrA, ccrB and ccrC) for integration and excision of the SCCmec element have been reported so far (www.sccmec.org). SCCmec elements are classified into types by the combination of the type of ccr gene complex and the class of mec gene complex. Eleven SCCmec elements are reported to date: SCCmec I to XI [5–8]. Among these, SCCmec types I–V are the most commonly reported. SCCmec types I–III are usually carried by hospital-associated methicillin resistant *S. aureus* (HA-MRSA) while types IV and V are usually carried by community-associated (CA) MRSA. However, now the distinction between HA and CA MRSA is getting blurred [9].

The first isolate to be characterized with SCCmec element V(5C2) containing ccrC was *S. aureus* strain WBG8318 (WIS) belonging to ST45 from Australia. The next was TSGH17 from Taiwan with ST59 genetic background and with two ccrC alleles [10–12]. Since then, many MRSA isolates such as M013 with SCCmec V elements containing two ccrCs, have been identified in various genetic backgrounds and with different ccrC alleles [13,14].

ST772, known as the Bengal Bay clone, prevalent in Bangladesh, India, and Malaysia, is a single locus variant of ST1 in *pvl* [15–18]. International travel has spread this clone to Japan and several European countries [19,20]. A recent German study has shown complex skin and soft tissue infections to be associated with Panton-Valentine Leukocidin (PVL) positive ST772 *S. aureus* among individuals returning from Asia [21]. The PVL-positive ST772 carrying SCCmec element V is one of the predominant clones present in Indian hospitals and the community, and is known to cause serious diseases [16,17]. A PVL-negative ST672 clone carrying SCCmec element V is also circulating among...
carriers and patients in India [22]. ST672 S. aureus, from Western Australia, carrying SCCmec elements IVa and V, is designated as a single locus variant of ST361 by Coombs et al. [23]. Very few ST672 isolates have been reported in the MLST database.

Two factors lead us to believe that the type V SCCmec elements of ST772 and ST672 could be different from each other and that of strain WIS. Firstly, primers identifying the ccrC and C2-mec gene complexes amplified while none of the joining (J) region primers amplified and secondly, during microarray analysis of SCCmec type V Indian isolates, ST772 and ST672 MRSA isolates tested positive for probe containing the ccr4MRSAZH47 region, while isolates belonging to ST8 were negative. This lead us to perform whole genome sequencing of 7 isolates of ST772 from different clinical backgrounds and one isolate of ST672 [16,17,22,24]. By identifying the SCCmec regions from whole genome sequences, we have shown that ST772 and ST672 SCCmec elements have novel rearrangements compared to extant type V elements.

Results and Discussion

We chose seven ST772 isolates and one ST672 S. aureus isolate from different clinical backgrounds for whole genome sequencing. Table 1 shows details of Indian isolates investigated in the present study and other reference strains used in comparison of genomes of SCCmec elements along with their accession numbers.

We identified the SCCmec elements from whole genome sequences by using previously published "Chromosome-SCCmec junction sequences at the SCC integration site. Six of the seven ST772 isolates with the exception of S. aureus 3957, carried SCCmec elements ranging in length from 24512 bp (LVP2) to 26528 bp (333). These are the smallest type V SCCmec elements so far reported with the number of open reading frames (orfs) varying between twenty six and twenty eight.

S. aureus 3957 contains the largest ST772 SCCmec element with 39 orfs. Table 2 presents data comparing the orfs present in the SCCmec element of 3957 to corresponding orthologs of one of the six similar ST772 isolates (118), ST672 isolate (GR1) and two reference strains WIS and M013 (representative of 5C2 and 5C2&5 SCCmec elements respectively). A similar Table S1 reports on the orfs present in the SCCmec element of S. aureus VH60 (highest number of orfs) in comparison with all the other ST772, ST672 and reference strains. While there is 100% identity in the sequence of RNA methyl transferase (orfX), the hypothetical proteins (HPs) coded by orfs 2 – 8 and orfs 10 – 13 present in 3957 are not identified in 118. Two HPs coded by orfs 7 and 15 in VH60 are not identified in most of the isolates (Table S1). The sequenced isolates came from different clinical backgrounds and it is apparent that there are differences in HPs, insertional sequences and transposases.

Small-sized SCCmec elements carried by six ST772 strains

OrfX insertion site and the characteristic terminal inverted and direct repeats, generated upon insertion of SCCmec, were almost similar to extant SCCmec elements. Genomic maps and comparison of SCCmec elements of strains WIS, 118 (representing six similar ST772 SCCmec elements), 3957, GR1 and M013 are illustrated in Figure 1a drawn using Easy Fig software [25]. The differences and similarities between the genomic structures of various SCCmec elements are highlighted below.

Sequence analysis of this region from Figure 1a and Table 2 reveals large differences in HPs, IS431 transposases and the C2-mec gene complex between WIS and 118, between 118 and 3957, and between 3957 and GR1 and M013. All sequenced isolates contain the class C2 mec gene complex; the arrangement of genes downstream of orfX between the two IS431 transposases however, are significantly different. In the SCCmec elements of ST772 and ST672 isolates, there are inversions in the mec gene complex with the absence of mecR1/ mecR. This inversion is similar to the inversion reported in isolate JCS6945 (ST398) collected from a Canadian participant in an International Pig Veterinary Conference. JCS6945 contains SCCmec element X with C1 mec gene complex, while ST772 and 672 isolates carry SCCmec element V with a C2 mec gene complex [7]. Figure 1b depicts similar mec gene complex inversions in strain 118 and JCS6945 but with a different orientation of IS431 which might have occurred during a horizontal transfer. S. aureus ST398 is associated with livestock and human infections and is an important pathogen [26,27]. SCCmec type V element has been characterized recently in UMCG-M4, a ST398 human isolate containing PVL but does not harbour mec gene complex inversion [28].

Downstream of mecA, next to the second IS431, our isolates and M013, unlike WIS, contain a PhnB-like protein, which adopts structural folds similar to bleomycin resistance protein. The Hps downstream of the PhnB-like protein are similar among ST772, and ST672 isolates.

All the six isolates have similar arrangements with minor variations in HPs, IS431 transposases and non coding regions. IS431 transposases of all six isolates located at both upstream and downstream of the mecA gene are truncated to different extents as shown in Table S2. IS431 transposases (represented as Atnp in figure 1) located at the downstream of mecA in strains VH60, 118, 333, 3989 and LVP2 are truncated to the same size (48 aa), while 120 has a larger sized Atnp (157 aa). Similarly, IS431 transposases (represented as Atnp in figure 1) located at the upstream of mecA in two isolates from eye infections, 333 and LVP2, have larger Atnps (133, 155 aa respectively) while the Atnps of 60, 118, 120 and 3989 are of same size (92 aa) and smaller. S. aureus 118 and all the other 5 isolates have a ccrC1 (allele 2) downstream of the mec gene complex while WIS has ccrC1 (allele 1). All six ST772 isolates and WIS carry SCCmec elements type V (5C2) although with many differences. It is evident that WIS and these 772 isolates have evolved independently. To our knowledge, no other type V (5C2 or 5C2&3) SCCmec element has been reported with inversion of the mec complex.

SCCmec elements of S. aureus 3957, and GR1

While GR1 has two Atnps of equal size, 3957 has an IS431 transposase at the upstream of mecA and an IS similar to IS1181 having a transposase of 440 amino acids long. Similar partial IS431 transposases have been found in S. aureus TSGH17 and ZH47. S. aureus 3957 and GR1 contain the same mec gene complex inversion as in other ST772 isolates and, in addition, type 5 ccr gene complex comprising of ccrC1 (allele 8) in the region between orfX and mec gene complex. S. aureus 3957 and GR1 carry two other truncated (split) ccrCs, downstream of mec gene complex, split into two due to a frame shift mutation. Orfs 28 and 29 of 3957 and orfs 29 and 30 of GR1 have 98, 97, 100 and 95% identity respectively with ccrC1 (allele 5) from S. haemolyticus JCS1435 (YP_251971.1) which is an intact protein of 558 amino acids carrying serine recombinase, zinc finger, and flmA domains. In GR1 split ccrCs are encoded by orf 29 (311 amino acids) and 30 (247 amino acids) carrying two recombinase domains (one serine recombinase and zinc finger domain, respectively) while in 3957, they are encoded on orf 28 (83 amino acids as initial part of serine recombinase) and orf 29 (478 amino acids) with latter part of serine recombinase, resolvase, zinc finger and flmA domains respectively. ClustalW alignments of the split ccrCs of S. aureus 3957 and GR1 with ccrC1 (allele 5) sequences are shown in Figure 1c.
| Isolate/Strain | Place/Source | Clinical History | Year of isolation | ST/CC | PVL | Agr type | Gen Bank Accession No<sup>a</sup> | DDBJ Accession No<sup>b</sup> | SCC<sub> mec </sub> size (bp) | No of ORFs |
|---------------|-------------|-----------------|-------------------|-------|-----|---------|-----------------------------|-----------------------------|-------------------|-----------|
| 118           | Bangalore / blood | Pyomyositis | 2008 | 772/1 | +   | II      | AJGE0000000000              | AB777516                    | 25,389            | 26        |
| VH60          | Bangalore/nasal swab | Carrier | 2007 | 772/1 | +   | II      | ALWG0000000000              | AB781450                    | 25,396            | 28        |
| 3989          | Hyderabad/sputa | Pneumonitis | 2007 | 772/1 | +   | II      | ALWH0000000000              | AB781447                    | 24,870            | 27        |
| 120           | Bangalore/pus | Cerebral Acsess | 2009 | 772/1 | +   | II      | ALWE0000000000              | AB781444                    | 25,288            | 26        |
| 333           | Madura/corneal ulcer | Endophthalmitis | 2010 | 772/1 | +   | II      | ALMF0000000000              | AB781445                    | 26,528            | 26        |
| LVP2          | Bhubaneshwar/keratitis | Microbial keratitis | 2010 | 772/1 | +   | II      | AOFV0000000000              | AB781449                    | 24,512            | 26        |
| 3957          | Hyderabad/pus | Breast abscess | 2007 | 772/1 | +   | II      | AOFU0000000000              | AB781446                    | 36,199            | 39        |
| GR1           | Delhi/blood | Septicemia | 2007 | 672/361 | -  | I       | AJLX0000000000              | AB781448                    | 34,776            | 40        |
| M013<sup>c</sup> | Taiwan/ | Wound infection | 2002 | 59 | +   | I       | CP003166                     | -                          | 41,265            | 39        |
| W5<sup>d</sup> | Australia | Skin carriage | 1995 | 45/45 | -  | -       | AB121219                     | -                          | 28,612            | 25        |
| JCSC6945<sup>e</sup> | Denmark | Carrier | 2006 | 398 | -   | -       | AB505653.1                   | 51,483                     | 54        |
| 85/2018<sup>f</sup> | Newzealand | - | 1985 | 239/8 | -  | I       | AB037670.1                   | 68,256                     | 80        |

<sup>a</sup>: Whole genome sequenced contigs;
<sup>b</sup>: Annotations for the SCC<sub> mec </sub> region;
<sup>c</sup>Huang TW, Chen F, Miu WC, Liao TL, Lin AC, et al. (2012) J. Bacteriol 194:1256-1257;
<sup>d</sup>O’Brien FG, Coombs GW, Pearson JC, Christiansen KJ, Grubb WB. (2005) Antimicrob. Agents Chemother 49: 5129-5132;
<sup>e</sup>Li S, Skov RL, Han X, Larsen AR, Larsen J, et al. (2011) Antimicrob. Agents Chemother 55: 3046-3050;
<sup>f</sup>Ito, T., Y. Katayama, K. Asada, N. Morin, K. Tsutsumimoto, C. Tienasitorn, and K. Hiramatsu. 2001. Antimicrob. Agents Chemother. 45: 1323-1336.
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| Orf   | Position | Predicted Function                                      | 118 (ST772) | GR1 (ST672) | WIS (ST45) | M013 (ST59) | Others                      |
|-------|----------|--------------------------------------------------------|-------------|-------------|------------|-------------|-----------------------------|
|       |          |                                                        | % Identity  | % Identity  | % Identity | % Identity  | % Identity                  |
|       |          |                                                        | C O         | C O         | C O        | C O         | C O                         |
| orf 1 | 369_848.160 aa | rRNA methyltransferase                               | 100         | 118.1       | 100        | GR1.1       | 100                        | BAD24821.1 | 100 | YP_005296500.1 |
| orf 2 | 1094.1399, 102 aa | HP<sup>+</sup>                                        | NI          | 100         | GR1.2      | NI          | 100                        | BAD24832.1 | 100 | YP_005296501.1 |
| orf 3 | 1598.246, 288 aa | HP                                                      | NI          | 100         | GR1.3      | NI          | 100                        | BAD24833.1 | 100 | YP_005296502.1 |
| orf 4 | 2569.404, 492 aa | HP                                                      | NI          | 100         | GR1.4      | NI          | 100                        | BAD24834.1 | 100 | YP_005296504.1 |
| orf 5 | 4270.5370, 367 aa | HP (repair, recombination and replication)             | NI          | 100         | GR1.5      | 77.13       | 100                        | BAD24831.1 | 100 | YP_005296505.1 |
| orf 6 | 5363.5734, 124 aa | HP                                                      | NI          | 100         | GR1.6      | 81.15       | 100                        | BAD24832.1 | 100 | YP_005296506.1 |
| orf 7 | 7371.7374, 548 aa | Putative Primase                                       | NI          | 100         | GR1.7      | 81.38       | 100                        | BAD24833.1 | 100 | YP_005296507.1 |
| orf 8 | 7367.7444, 26 aa | HP                                                      | NI          | 100         | GR1.8      | NI          | 100                        | BAD24834.1 | 100 | YP_005296508.1 |
| orf 9 | 7600.9276, 559 aa | CcC1 (allele 8)                                       | 95.2        | 118.17      | 99.82      | 100         | 100                        | BAD24831.1 | 100 | YP_005296509.1 |
| orf 10| 9382.9720, 113 aa | HP                                                      | NI          | 100         | GR1.10     | 49.55       | 100                        | BAD24835.1 | 100 |                       |
| orf 11| 9722.9823, 34 aa | HP                                                      | NI          | 100         | GR1.11     | NI          | 100                        |                       | 97 (S. epidermis) | CAL22884.1 |
| orf 12| 9816.10127, 104 aa | HP                                                      | 100         | GR1.12     | 50.94      | 100         | 100                        | BAD24837.1 | 100 | YP_005296510.1 |
| orf 13| 10143.10649, 169 aa | DUF1643 superfamily protein                            | NI          | 100         | GR1.13     | 65.87       | 100                        | BAD24838.1 | 100 | YP_005296511.1 |
| orf 14| 11277.10609,223 aa | IS431 mec                                              | 95.6        | 118.3       | 98.9       | 100         | 100                        | BAD24823.1 | 100 | YP_005296512.1 |
| orf 15| 11589.13595,669 aa | Penicillin binding protein PBPZ                        | 100         | 118.4       | 100        | GR1.15     | 99.04                      | 100         | 100 | YP_005296516.1 |
| orf 16| 14069.13641, 143 aa | HP                                                      | 100         | 118.5       | 100        | GR1.16     | 99.21                      | 100         | 100 | YP_005296515.1 |
| orf 17| 14909.14166, 248 aa | ugpQ<sup>+</sup>                                       | 100         | 118.6       | 100        | GR1.17     | 100                        | 100         | 100 | YP_005296514.1 |
| orf 18| 15993.15826, 56 aa | HMG-CoA                                                | 100         | 118.7       | 100        | GR1.19     | 100                        | 100         | 100 | YP_005296513.1 |
| orf 19| 16251.16234, 28 aa | HP                                                      | NI          | NI          | NI         | NI          | 88 (USA300-TCH959)          | ZP_04865955.1 |
| orf 20| 16377.17696, 440 aa | Transposase for IS1181                                | NI          | NI          | NI         | NI          | 99 (S. aureus A9781)        | ZP_05642705.1 |
| orf 21| 18368.17940, 143 aa | PhnB like proteins<sup>+</sup>                        | 100         | 118.9       | 99.3       | GR1.21     | 99.3                      | 100         | 100 | YP_005296517.1 |
| orf 22| 18449.19378, 310 aa | HP (transcriptional regulation response to unknown ligand) | 100         | 118.10      | 100        | GR1.22     | 99.3                      | 100         | 100 | YP_005296518.1 |
| orf 23| 19540.21528, 663 aa | HP                                                      | 100         | 118.11      | 100        | GR1.23     | 99.85                      | 100         | 100 | YP_005296522.1 |
| orf 24| 21723.22832, 370 aa | HP (Family A polymerase functions in DNA repair)      | 100         | 118.12      | 100        | GR1.24     | 96.75                      | 100         | 100 | YP_005296523.1 |
| orf 25| 22825.23193, 123 aa | HP                                                      | 100         | 118.14      | 100        | GR1.26     | 65.57                      | 100         | 100 | YP_005296524.1 |
| orf 26| 23193.24809, 539 aa | HP (distant relative to ccr)                          | 100         | 118.15      | 100        | GR1.27     | 77.09                      | 100         | 100 | YP_005296525.1 |
| orf 27| 24802.24879, 26 aa | HP (IS-125)                                            | 100         | 118.16      | 100        | GR1.28     | 99.11                      | 100         | 100 | YP_005296508.1 |
| orf 28| 25034.25285, 84 aa | Truncated ccrC (serine recombinase domain)            | NI          | 97.59       | 100        | GR1.29     | 89.16                      | 100         | 100 | YP_005296526.1 |
| orf 29| 25282.26718, 479 aa | ccrC                                                   | 97.07       | 118.17      | 100        | GR1.29     | 94.97                      | 100         | 100 | YP_005296526.1 |
| orf 30| 26807.27145, 113 aa | HP                                                      | 98.21       | 118.18      | 100        | GR1.31     | 91.96                      | 100         | 100 | YP_005296527.1 |

**Table 2.** Comparison of orfs from SCCmec elements of *S. aureus* 3957 and corresponding orthologs of other ST772, ST672 and reference strains.
### Table 2. Cont.

| Orfs   | Position       | Predicted Function                                         | 118 (ST772) | GR1 (ST672) | WIS (ST45) | M013 (ST59) | Others          |
|--------|----------------|------------------------------------------------------------|-------------|-------------|-------------|-------------|----------------|
|        |                | % Identity C O                                                 | % Identity C O | % Identity C O | % Identity C O | % Identity C O | % Identity C O |
| orf 31 | 31499..28380, 104 aa | hsdR (type I restriction enzyme R protein)               | 100         | 118.22      | 100         | 96.54       | 100 (HP)       |
| orf 32 | 27568..28071, 168 aa | orf 33 | 27568..28071, 168 aa | HP          | 100         | 118.20      | 100         | GR1.34 92.81    | BAD24838.1 95.21 | YP_005296529.1 |
| orf 34 | 36285..28306, 386 aa | hsdS (type I restriction modification DNA specificity domain) | 100         | 118.23      | 100         | 36.56       | 100         | GR1.37 36.56    | BAD24841.1 34.72 | YP_005296532.1 |
| orf 35 | 34144..32630, 505 aa | hsdM (type I restriction modification DNA methyltransferase subunit M) | 100         | 118.24      | 100         | 96.23       | 100         | GR1.38 96.23    | BAD24842.1 98.21 | YP_005296533.1 |
| orf 36 | 34329..35204, 292 aa | HP (nucleotidyltransferase domain of 2'5'-oligoadenylate synthetase) | 100         | 118.25      | 100         | 98 (S.epidermidis W23144) ZP_04797658.1 |
| orf 37 | 35428..35976, 183 aa | HP (nucleotidyltransferase domain of 2'5'-oligoadenylate synthetase) | 100         | 118.26      | 100         | 99 (S.epidermidis W23144) ZP_04797657.1 |

*aIdentity of the amino acid sequence to each ortholog (orf);
*bCorresponding Ortholog in the reference strain,
*cHypothetical Protein;
*dugpQ: glycerophosphoryl diester phosphodiesterase;
*ePhnB-like proteins adopting structural fold similar to bleomycin resistance proteins;
*fNo Identity.

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The designations for the truncated ccrCs have to await the decision from the International Working Group on SCCmec elements. The type V SCCmec elements of S. aureus 3957 and GR1 are composite cassettes (5C2&5) formed by the integration of type V SCCmec element into a SCC element of a MSSA isolate and have in all probability evolved independently from the more common 5C2 ST772 isolates.

SCCmec element in S. aureus 3957 is an exception to other ST772 isolates as it carries the region similar to M013 in GR1.

**Figure 1. Schematic representation of genetic structures of type V SCCmec elements.** a) Schematic representation and comparison of genetic structures of type V (SC2) SCCmec element of 118 (ST772), and type V (SC2&5) SCCmec elements of 3957 (ST772) and GR1(ST672) and the reference strains WIS (ST45) and M013 (ST59). Structures of these elements are illustrated based on nucleotide sequences deposited in DDBJ/EMBL/GenBank database under the accession numbers AB777516, AB781446, AB781448, AB121219 and CP003166. Coding sequences are marked in the direction of transcription as arrows. Transposases for IS431 are indicated in black arrows. Intact transposases for IS431 have been labeled as tnp, truncated transposases for IS431 have been labeled as Δtnp. Transposase for IS1181 and transposase for M013 are indicated in red arrows and have been labeled as tnp* and Δtnp, respectively. Color coding for the genes not labeled are shown in the legend. Conserved region with more than 95% homology are indicated in light brown and mec gene complex inversions are shown in purple as determined by BLASTN. Genomic picture was generated using Easy Fig software. 1b: Comparison of mec gene complexes in S. aureus 118 and JCSC6945.

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S1. The designations for the truncated ccrCs have to await the decision from the International Working Group on SCCmec elements.

The type V SCCmec elements of S. aureus 3957 and GR1 are composite cassettes (5C2&5) formed by the integration of type V SCCmec element into a SCC element of a MSSA isolate and have in all probability evolved independently from the more common 5C2 ST772 isolates.

SCCmec element in S. aureus 3957 is an exception to other ST772 isolates as it carries the region similar to M013 in GR1.
(ST672). To check the frequency of appearance of SCCmec elements similar to 3957, we screened 45 ST 772 isolates from our collection with primers specific for 3957 and did not find any other ST772 isolate with a unique SCCmec element found in 3957. Hence it appears that the recombination event is not frequent although it is stable in this isolate. ST672 may not be highly prevalent for the same reason.

**Restriction-Modification systems**

Like other type V SCCmec elements, ST772 and ST672 elements code for a complete type I restriction modification system proteins hsdR, hsdS and hsdM. HsdR and hsdM are conserved among all ST772s and ST672 with respect to WIS and M013, while hsdS domain is different from that of WIS and M013 and has 80% similarity to bovine S. aureus LGA251 carrying SCCmec XI.

**Hypothetical Proteins**

Several HPs from S. aureus 3957 and GR1 are not present in the other six ST772 isolates but have 100% identity with M013 proteins. To our knowledge, ST 59 S. aureus isolates have not been detected in India but are present in Taiwan, China and Hong Kong. A common HA-MRSA present in India, China and Taiwan is ST239 which is the first bacterial hybrid to be found in nature [29]. We compared the nucleotide sequences (blastn) of SCCmec element of S. aureus 85/2082 (AB037671.1) with VH60, 3957 and GR1, and found 54%, 64%, and 67% query coverage respectively, with >97% identity. Blastp results indicate that 22/40, 21/39 and 10/28 proteins in GR1, 3957 and VH60 have more than 70% identity to 85/2082 SCCmec element proteins (Table S3). More specifically, 9 proteins between orfs 2-13 in GR1, 3957 and VH60 have more than 90% identity with 85/2082 and M013 proteins. It is likely that some of these proteins have originated from ST239 through horizontal transfer to generate SCCmec elements of ST772 and ST672. M013 SCCmec element has perhaps been generated through similar independent recombinations. HPs coded by orf 10 and orf 11 are homologous with SCCmec elements of ZH47 and S. epidermidis (CAL22884.1 and EJE32225.1). The last two HPs present in ST772 and ST672 SCCmec elements originate from S. epidermidis (ZP_04797658.1 and ZP_04797657.1) and are not present in WIS or M013.

SCCmec V element present in most Indian ST772 isolates is the smallest perhaps rendering the organism fitter to survive. The generation of Indian ST772 and ST672 type V SCCmec elements point to novel rearrangements due to recombination events (deletion/addition) involving other S. aureus including ST239 isolates, bovine SCCmec elements and elements from other Staphylococci.

**Materials and Methods**

**Ethical Statement**

The sequenced S. aureus and other ST772 isolates were obtained from clinicians from different hospitals in India. These hospitals have their own ethical boards which give them permission to collect these samples. M013 and WIS were obtained through the courtesy of Prof. Etienne, University of Lyon, France. The eight sequenced and 45 ST772 S. aureus isolates used to check the frequency of appearance of SCCmec elements similar to S. aureus 3957, were part of this collection.

The collection and molecular characterization of S. aureus isolates were carried out as described in earlier publications [16,17]. Whole genome sequencing of two S. aureus isolates, one ST772 and one ST672 respectively, have been described earlier [22,24]. Whole genome sequencing was performed using Illumina HiSeq-1000 sequencer. The raw reads obtained were assembled into contigs using VELVET [30], and gene predictions were made using GLIMMER 3.02 [31]. The relative arrangement of the SCCmec structural elements in different contigs was determined by BLASTP and TBLASTN [32]. The arrangement of contigs corresponding to SCCmec elements was determined by Mummer [33]. The sequences were validated and joined by performing overlapping PCRs (Figure S2) and Sanger sequencing (Figure S3). We used previously published chromosome- SCCmec junction sequences to identify the sequences of SCCmec elements present in ST772 and ST672 [10,22,24]. The SCCmec element annotations for all eight isolates have been deposited in the DNA database of Japan (DDBJ).

**Supporting Information**

**Figure S1** ClustalW alignment of ccrC1 (allele 5) of JGC1435 with 3957(Orf28 and 29) and GR1 (Orf 29 and 30).

**Figure S2** Verification of SCCmec contig sequences by overlapping PCRs.

**Figure S3** Example of Sanger sequencing.

**Table S1** Comparison of orfs from SCCmec elements of S. aureus VH60 and corresponding orthologs of other ST772, ST672 and reference strains.

**Table S2** Length of IS431 transposases among sequenced ST772 and ST672 isolates.

**Table S3** Comparison of SCCmec element orfs of 85/2082 with corresponding orthologs of VH60, 3957 and GR1.

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**Author Contributions**

Conceived and designed the experiments: GA. Performed the experiments: JB SP. Analyzed the data: JB SP GA. Wrote the paper: GA.

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