Whole genome analysis of methicillin resistant *Staphylococcus aureus* from a tertiary care hospital in Southern India

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**Objectives**

Methicillin-resistant *Staphylococcus aureus* (MRSA) are one of the most important causes of hospital-acquired infections.1 Though the prevalence is reported to be high in India, the molecular epidemiology of MRSA in Indian hospitals remains largely unexplored. This study carried out a genome wide analysis to understand the epidemiologic patterns of MRSA causing infections at a tertiary care hospital in Southern India.

**Methods**

Nine MRSA isolates were obtained from patients admitted to surgical specialties at tertiary care hospital in Southern India. Susceptibility testing was performed by E-test. Multi-locus sequence typing (MLST) was also performed. The 9 isolates were genotyped for SCCmec types based on a ccr recombinase and mec PCR and Sanger sequencing2. Five isolates were chosen for whole genome sequencing via 2x150b paired end sequencing (Nextera XT sample preparation kit and Miseq, Illumina). Strain sequences were independently assembled using SPAdes v3.1.0 (de novo assembly), and scaffolds from each strain were ordered against published genome of *S. aureus* TW20 (ST239, accession number FN433596), and pseudo chromosomes were generated and compared using Mauve v2.3.1. Single Nucleotide polymorphisms (SNP) comparison among these pseudo chromosomes was done using GL C Genomics workbench (CLCbio, Denmark v7.5.1). Propaphage identification was done using http://phast.wishartlab.com.

**Results**

All isolates were resistant to oxacillin and showed susceptibility to vancomycin and linezolid by E-test. Of the 9 MRSA, SCCmec III was harboured by 5, SCCmec IV by 3, and SCCmec IV by 1 strain. The SCCmec III and IV harboring MRSA showed resistance (MIC ≥256 μg/ml) and intermediate (MIC 24 μg/ml) resistance to cefoxitin, respectively while SCCmec IV harbouring MRSA showed susceptibility (MIC 8 μg/ml) to cefoxitin. MLST types were ST22 (n=1), ST239 (n=2), ST772 (n=2), ST72 (n=1), ST368 (n=1), ST623 (n=1) and ST670 (n=1).

Comparative genome analysis of the Indian ST239 (IN-ST239-a) with the known predominant ST239 Asian clade strain, TW20 showed inter-clonal variation (Fig. 1). Comparative genome analysis of the Indian ST239 clones (IN-ST239-a and IN-ST239-b) revealed minimum number of SNPs (0.003%), which was higher with Asian clade (TW20) 0.05%, Turkish clade (T0131) 0.04% and was maximum with the European ST239 (EU-ST239) clone (1.3%). This comparison also enabled us to identify the major recombination region in these international ST239 clones using *Staphylococcus epidermidis* (accession number: NC_002976) (RP62A-SE) used as an outgroup (Fig. 2). An important marker of the Asian clade TW20 the pSPβ-like prophage was absent or disintegrated in the Indian ST239 isolates. Also Bacillus *SBP5* and *Staphylococcus* Twort prophages were identified as the major source of genomic variations in the Indian ST239 and other sequenced ST types (Table).

**Conclusions**

In this genome-wide analysis of Indian MRSA, we identified a recombination region that was present in ST239 MRSA from various continents. Even within the Indian ST239 MRSA that belonged to the same geographical region, genomic variation was observed.

**References**
