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 SCC*mec* types based on a *ccr* recombinase and *mec* PCR and Sanger sequencing[2]. Five isolates were chosen for whole genome sequencing via 2×150b 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 CLC Genomics workbench (CLCbio, Denmark v7.5.1). Prophage 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, SCC*mec* III was harboured by 5, SCC*mec*V by 3, and SCC*mec* IV by 1 strain. The SCC*mec* III and IV harboring MRSA showed resistance (MIC ≥256 µg/ml) and intermediate (MIC 24 µg/ml) resistance to cefoxitin, respectively while SCC*mec*V 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).

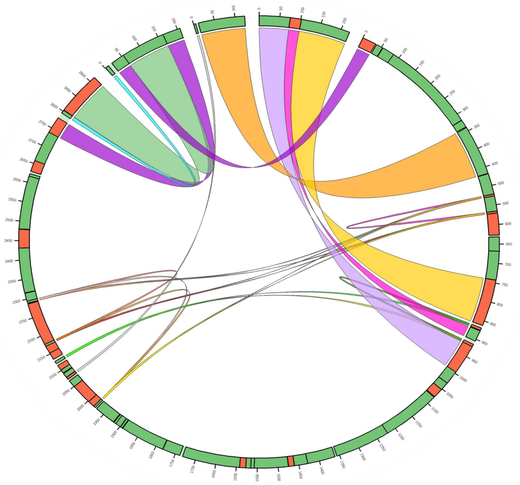

Fig. 1: Comparison of TW20 with IN-ST239-b using Circos. Only contigs (IN-ST239-b) with multiple synteny blocks rearranged differently in the genome are shown. Green and red bars depict the direction of synteny blocks on the positive and negative strands, respectively.

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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-ST2339-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 φSPβ-like prophage was absent or disintegrated in the Indian ST239 isolates. Also *Bacillus SPBc2* and *Staphylococcus* Twort prophages were identified as the major source of genomic variations in the Indian ST239 and other sequenced ST types (**Table**).

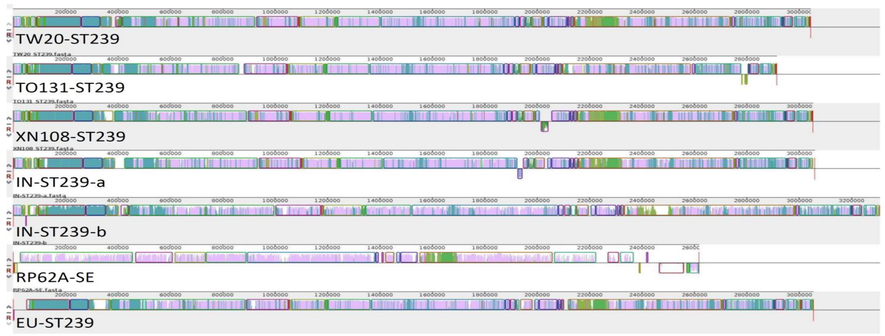

Fig. 2: Comparison of Indian ST239 clones (IN-239a, IN239b) with published genomes of the predominant Asian clone TW20, XN108 (accession number. CP007447.1), Turkish clade (TO131, accession number NC_017347) European ST239 (EU-ST239, unpublished data), and *S. epidermidis* RP62A as an outgroup. Recombination regions were highlighted in green.

Sample ID	Scc mec type	Source	MLST (ST) type	Genome Size (Mb)	Prophages Identified	Virulence factors		
						Adherence	Toxins	Exoenzyme
IN-ST239a	III	Wound Swab	ST239	~2.94	7	*clfA*	*tsst*	*splD* & *splF*
IN-ST368	III	Tissue	ST368	~2.98	7	-	-	*splD* & *splF*
IN-ST623	III	Tissue	ST623	~2.95	8	-	-	*splD* & *splF*
IN-ST239b	III	Wound Swab	ST239	~3.02	8	-	-	*splD* & *splF*
IN-ST670	III	Pus	ST670	~3.10	7	-	-	*splD* & *splF*
TW-20	III	Blood	ST239	~3.01	7	*clfA*	*tsst*	*splD* & *splF*
T0131	III	Blood	ST239	~2.91	6	*clfA*	*tsst*	*splD* & *splF*
EU-ST239	III	Blood	ST239	~2.92	10	-	-	-

Table: Strain characteristics. Comparison of sequenced strains from this study with published genomes (bold).

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

1. Wielders CLC, et. al ., *mecA* Gene Is widely disseminated in *Staphylococcus aureus* population. *J.Clin. Micro.*. 2002.
2. Kondo Y, et. al., Combination of multiplex PCRs for Staphylococcal cassette chromosome *mec* type assignment: rapid identification system for *mec*, *ccr*, and major differences in junkyard regions. Antimicrob Agents Chemother 2007.

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