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Short Communication

An invasive marker *Staphylococcus epidermidis* surface protein I (SesI) harboured by a ST239 methicillin-resistant *Staphylococcus aureus*

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ABSTRACT

Objectives: Recently, the virulence factor *sasX* was described on the mobile genetic element φSPβ prophage in a ST239-SCC*mec*III methicillin-resistant *Staphylococcus aureus* (MRSA) clone. The aim of this study was to identify *sesI*, an *sasX* homologue, in an MRSA ST239 strain.
Methods: MRSA strain VB1490 was isolated from a patient with MRSA bacteraemia in India. Staphylococcal cassette chromosome*mec* (SCC*mec*) typing and whole-genome shotgun sequencing were performed. Phylogenetic analysis of VB1490 and ST239 reference genomes from the NCBI database was performed. Amplification and sequencing of the *sasX* gene was performed to establish allele homology.
Results: The *sasX* gene identified in isolate VB1490 was more similar to the *sesI* gene of *Staphylococcus epidermidis* RP62A than the *sasX* gene of ST239 MRSA strain. Whole-genome analysis revealed the presence of the *sasX* gene on a φSPβ-like prophage that exhibited high sequence identity to that of *S. epidermidis* strain RP62A.
Conclusion: These finding suggests the dissemination of the invasion-determining virulence factor*sesI* from *S. epidermidis* to a ST239 MRSA strain.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) represents a great challenge to healthcare settings worldwide. The MRSA lineage ST239-SCC*mec*III has been previously reported to cause multiple epidemics and is one of the widely disseminated healthcare-acquired MRSA strains across the globe [1]. The highest incidence of healthcare-acquired MRSA infection is documented in Asia [2]. Furthermore, a study described ST239 strains as accounting for ≥90% of nosocomial MRSA infections in Asia [3].

Recently, a virulence factor *sasX* was described on a φSPβ prophage that promotes colonisation and immune evasion in MRSA ST239 [1]. The SasX surface protein exhibited high similarity to *Staphylococcus epidermidis* surface protein I (SesI), a marker of invasiveness in *S. epidermidis* [4]. Moreover, SasX is potentially involved in biofilm formation, cell aggregation and squamous cell adhesion of *S. aureus* [5]. Thus, the combination of multidrug resistance and *sasX* in ST239 MRSA lineage contributes to successful adaptation, survival and dissemination of this clone in hospital settings [6].

In India, reports on ST239 clone are limited. In addition, *sasX*-harbouring ST239-MRSA-III clone has ben previously reported in MRSA strains isolated from India. Here we report the draft genome sequence of strain VB1490 (ST239-MRSA-III) isolated from a 29-year-old patient with MRSA bacteraemia who recovered completely after treatment with vancomycin.

2. Methods

Strain VB1490 was isolated from a blood culture of a patient attending the outpatient department of Christian Medical College (Vellore, India) with complaint of weakness of the right upper limb, followed a month later by right humerus and tibial plating. Antimicrobial susceptibility testing was performed by the disk diffusion method for cefoxitin (30 μg), gentamicin (10 μg), erythromycin (15 μg), clindamycin (2 μg), ciprofloxacin (5 μg), tetracycline (30 μg), trimethoprim/sulfamethoxazole (SXT) (1.25/23.75 μg), rifampicin (5 μg) and linezolid (30 μg) according to Clinical and Laboratory Standards Institute (CLSI) guidelines [7]. The minimum inhibitory concentration (MIC) of vancomycin was determined by the broth microdilution method [8]. Etest was

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performed for determination of the teicoplanin and daptomycin MICs. Furthermore, isolate VB1490 was screened for a hetero-resistant vancomycin-intermediate *S. aureus* (hVISA) subpopulation by glycopeptide resistance detection (GRD) Etest and was confirmed by population analysis profile–area under the curve (PAP-AUC) method as previously described [9,10].

DNA was isolated from pure cultures using a QIAamp® DNA Mini Kit (QIAGEN, Hilden, Germany). Whole-genome shotgun sequencing was performed using an Ion Torrent PGM™ System (Life Technologies, Waltham, MA) with 400-bp chemistry. The raw data generated were assembled de novo using SPAdes Genome Assembler v.5.0.0.0 (http://cab.spbu.ru/software/spades/) embedded in Torrent Suite Software v.5.0.4. The genome sequence was annotated using PATRIC, the bacterial bioinformatics database and analysis resource (http://www.patricbrc.org) [11] and the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html). Downstream analysis was performed using the Centre for Genomic Epidemiology (CGE) server (http://www.genomicepidemiology.org/) and PATRIC. The following bioinformatics analyses were performed: staphylococcal cassette chromosome *mec* (SCC*mec*) type was identified using SCC*mec*Finder (https://cge.cbs.dtu.dk/services/SCCmecFinder); the resistance gene profile was analysed using ResFinder 2.1 (https://cge.cbs.dtu.dk//services/ResFinder/); virulence genes were determined using VirulenceFinder 1.5 (https://cge.cbs.dtu.dk/services/VirulenceFinder); the sequence type (ST) was determined using seven housekeeping genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi* and *yqiL*) with MLST 1.8 (Multi-Locus Sequence Typing) (https://cge.cbs.dtu.dk/services/MLST) and was further confirmed using the *S. aureus* MLST database (https://pubmlst.org/saureus/); the staphylococcal protein A (*spa*) type was identified with spaTyper 1.0 (https://cge.cbs.dtu.dk/services/spatyper); and PHASTER (PHAge Search Tool Enhanced Release) was used for annotation and identification of prophage sequences in the bacterial genome [12].

A phylogenetic tree comprising ST239 reference genomes ($n = 16$) retrieved from the NCBI database (http://www.ncbi.nlm.nih.gov) and the genome sequence of strain VB1490 was constructed using single nucleotide polymorphism calling and CSI Phylogeny [13]. Annotation and BLAST comparison of the φSPβ-like prophage between VB1490 and reference genomes (GenBank accession nos. **CP000029**, **FN433596**, **CP006838** and **LC036194**)

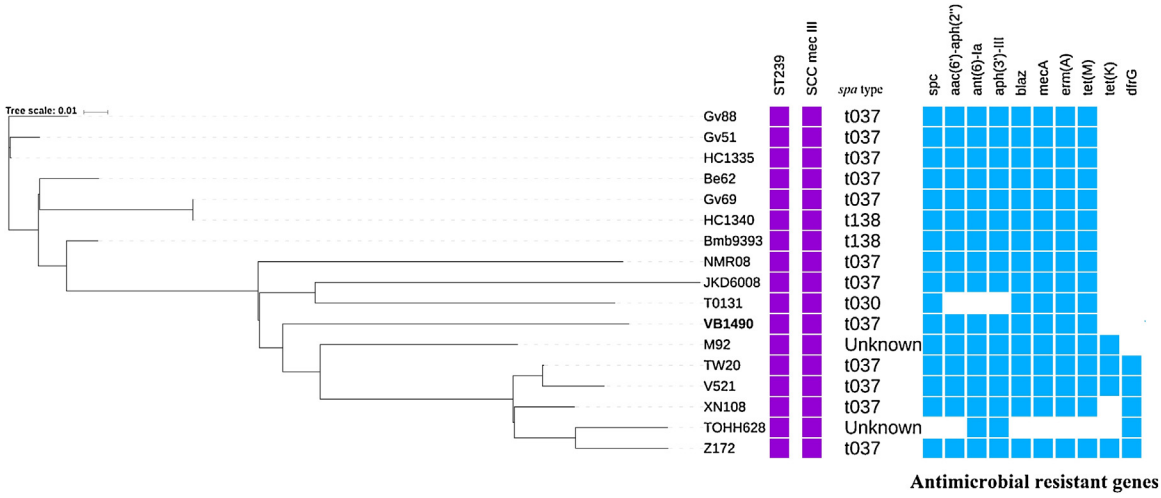

Fig. 1. Phylogenetic tree inferred by CSI Phylogeny. The tree was constructed using ST239 reference genomes ($n = 16$) and the genome sequence of methicillin-resistant *Staphylococcus aureus* (MRSA) strain VB1490 (in bold) and was labelled with the metadata using iTOL v.4. The scale bar indicates the number of nucleotide substitution per 100 nucleotides. Columns represent, respectively, the isolate ID, sequence type (ST), staphylococcal cassette chromosome *mec* (SCC*mec*) type (purple) and staphylococcal protein A (*spa*) type. The presence of various antimicrobial resistance genes in the genomes is indicated as a blue box. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

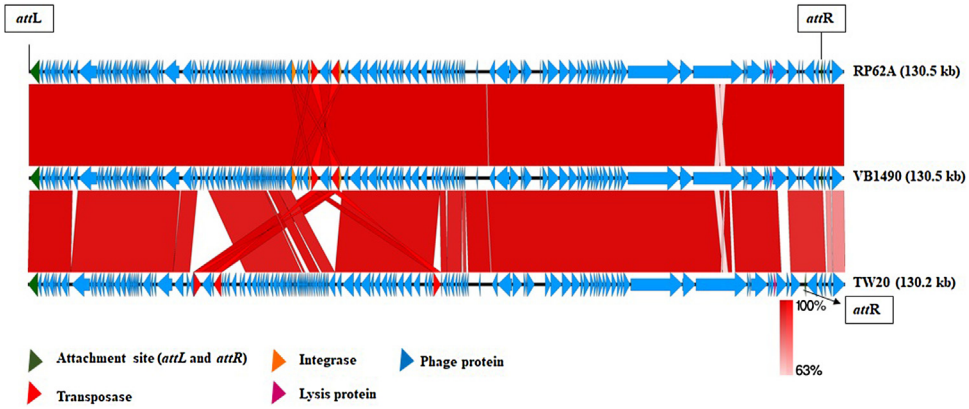

Fig. 2. Comparison between the *sesI*-carrying φSPβ-like prophage of methicillin-resistant *Staphylococcus aureus* (MRSA) strain VB1490 with *Staphylococcus epidermidis* RP62A (GenBank accession no. **CP000029**) and *S. aureus* TW20 (**FN433596**). The red shading between the prophage genomes denotes regions of identity. Overall identity was >95%. Prophage genome alignment was constructed using BLASTn and was depicted using Easyfig v.2.2.2. Genes encoding structural proteins are colour-coded. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

was performed using Glimmer 2.1 and the genome comparison visualiser Easyfig v.2.2.2. The *sasX* gene was amplified and sequenced as previously described [4]. Multiple sequence alignment (MSA) of *sasX* was performed using ClustalW as applier in BioEdit software v.7.0.9.1. For homology analysis, the *sasX* genes of *S. aureus* TW20 (GenBank accession no. **FN433596**), *S. aureus* TOHH628 (**LC036194**) and *S. epidermidis* RP62A (**CP000029**) were used as references. From this point onwards, *sasX* will refer to *S. aureus* isolates, whilst *sesI* will be specified for *S. epidermidis*.

3. Results

MRSA isolate VB1490 was resistant to cefoxitin, gentamicin, erythromycin, clindamycin, ciprofloxacin, SXT and tetracycline but remained susceptible to rifampicin, linezolid, teicoplanin (MIC = 0.5 μg/mL) and daptomycin (MIC = 0.5 μg/mL). The isolate was found to have a vancomycin MIC of 1 μg/mL and was screened and confirmed as hVISA with a PAP-AUC ratio of 1.0.

The complete genome of MRSA VB1490 contains a 2.9-Mbp chromosome with coverage of 57.7×. The number of DNA coding sequences (CDS) is 3011 with a GC content of 33%. Genome annotation by PATRIC predicted a total of 2298 proteins with assigned function, 58 tRNAs and 8 rRNAs in the sequenced genome. VB1490 belongs to ST239 lineage and harbours SCC*mec* III and *spa* type t037. Genome analysis of VB1490 revealed a multidrug-resistant profile that is similar to ST239 reference genomes (Fig. 1). The draft genome was deposited in NCBI GenBank under accession no. **MLQB00000000**.

In the genome of VB1490, the *sasX* gene (position 1 659 382 to 1 659 987) encoding a cell surface protein was identified within a 72.6-kb (position 1 650 692 to 1 723 299) region of a φSPβ-like prophage. The prophage was integrated into the chromosome at *attL* (TAAAATAATAATA) and *attR* (TAAAATAATAATA) attachment sites. Comparative analysis of the φSPβ-like prophage showed high sequence identity to the φSPβ prophage of *S. epidermidis* strain RP62A (Fig. 2). Furthermore, MSA of *sasX* identified in the study

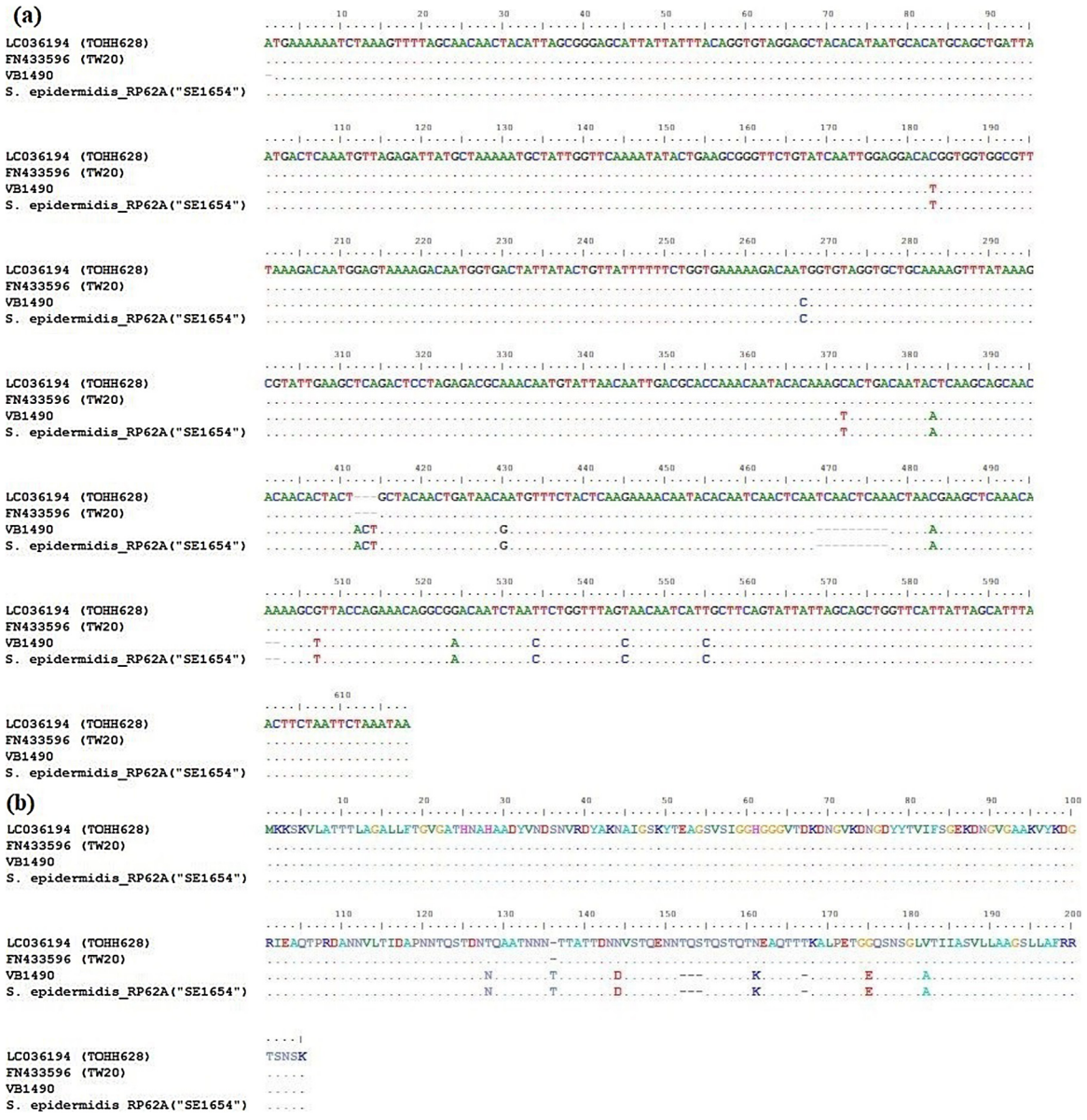

Fig. 3. BioEdit conservation plot analysis obtained with multiple sequence alignment (MSA) of the *sasX* gene of methicillin-resistant *Staphylococcus aureus* (MRSA) strain VB1490 and ST239 reference strains (*S. aureus* TOHH628, *S. aureus* TW20 and *Staphylococcus epidermidis* RP62A): (a) MSA of nucleotides encoding *sasX*; and (b) MSA of amino acids encoding *sasX*.

isolate showed 100% homology to the *sesI* gene of *S. epidermidis* RP62A strain (Fig. 3).

4. Discussion

ST239 is a frequently observed lineage in many Asian countries, but less from India [14]. In this study, an *sasX*-positive ST239-MRSA-III strain was found. Any virulence factor that promotes colonisation of *S. aureus* can be considered as a risk factor for infection. Several virulence factors are widely reported in *S. aureus* [15]. However, none of them have been epidemiologically linked to an MRSA outbreak. *sasX* was first described in an MRSA outbreak strain of ST239 in London (UK) using whole-genome sequencing [16]. Furthermore, Li et al. described the vital role of SasX in nasal colonisation and its contribution to MRSA outbreaks [1]. A multicentre study from China reported an increased frequency of *sasX*-positive isolates from 21% in 2003 to 39% in 2011 [1]. Although exclusive to ST239, few studies have reported *sasX* in other MRSA lineages including ST5, ST59 and ST45 [1,2]. ST239-III carrying *sasX* was reported as the predominant strain causing invasive infections [17].

MSA of *sasX* of strain VB1490 exhibited higher similarity to *S. epidermidis* RP62A than to other ST239 *sas*X genes. Likewise, a previous study reported an *sesI*-harbouring Indian ST239 MRSA strain [18]. Similar to the previous report, *sasX* was also identified on a φSPβ-like prophage in the study isolate [4]. *sasX* was identified on a φSPβ-like prophage, which could potentially spread this invasive gene to *S. aureus* and other lineages [16,18,19].

Collectively, these data show the horizontal acquisition of *sesI* in ST239 MRSA from *S. epidermidis*. The presence of the native *sasX* homologue *sesI* on a φSPβ-like prophage promotes dissemination to *S. aureus*.

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Competing interests

None declared.

Ethical approval

Not required.

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