



Genome Note

Genome sequence of a methicillin-resistant *Staphylococcus epidermidis* (MRSE) resistant to dalbavancin and teicoplanin isolated in Italy, 2023

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ABSTRACT

Objective: Methicillin-resistant *Staphylococcus epidermidis* (MRSE) is a commensal bacterium that colonizes the skin surface of humans and animals and represents an important opportunistic pathogen related to nosocomial and community-acquired infections. Here, we characterized the whole genome of an MRSE isolate, named SEPI150, resistant to dalbavancin and teicoplanin, collected from the blood of a patient with recurrent bacteraemic episodes.

Methods: DNA sequencing was performed using a hybrid approach with Illumina MiSeq and Minion Oxford Nanopore platforms. Hybrid de novo assembly was performed using Unicycler, and analysis of mutations or insertions was performed by mapping Illumina reads of an MRSE strain susceptible to dalbavancin and teicoplanin collected from the same patient against the assembled genome of the SEPI150 strain.

Results: The SEPI150 strain exhibited resistance to β -lactams, rifampicin, gentamicin, dalbavancin and teicoplanin, while remaining susceptible to vancomycin. The strain belonged to sequence type ST23 and carried different antimicrobial resistance determinants (*aant(9)-Ia*, *aadD*, *aac(6')-aph(2'')*, *mecA*, *blaZ*, *erm(A)* and *bleO*). Genome analysis in comparison with the dalbavancin-susceptible MRSE strain isolated from the same patient demonstrated that the SEPI150 strain carried wild-type *walkR*, *pbps* and *varR/S* genes, and specific mutations were observed within *rpoB* (F597L) and *varG* (D470G) genes.

Conclusions: Herein, we hypothesized that accumulation of different mutations within genes targeting different pathways (i.e., RNA transcription and cell-wall biosynthesis) may be involved in resistance to dalbavancin and cross-resistance with other glycopeptides.

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1. Introduction

Staphylococcus epidermidis is a common colonizer of the skin and mucous membranes of humans and animals that can become

an important opportunistic pathogen, especially among vulnerable patients. The antimicrobial resistance rate among *S. epidermidis* represents a major clinical concern due to reduced antimicrobial options available to treat infections due to this opportunistic pathogen [1]. Against MRSE, antimicrobial treatments are based on last resort molecules that retain antimicrobial activity. In this study, we characterized the genome of an MRSE strain, named SEPI150, resistant to dalbavancin and teicoplanin that emerged during dalbavancin-based treatment.

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Table 1
Phenotypic and genotypic characteristics of the glycopeptides-susceptible and -resistant methicillin-resistant *S. epidermidis* (MRSE) strains included in this study

Strain	Glycopeptide phenotype	Sequence type	β -Lactams resistance determinants	Other resistance genes	Predicted mutations					
					bpbs	walk/R	vraR/S	rpoB	vraG	VraG
SEPI148	Dalbavancin, teicoplanin and vancomycin susceptible	23	<i>mecA</i> , <i>blaZ</i>	<i>ant(9)-Ia</i> , <i>aac(6')-aph(2'')</i> , <i>erm(A)</i>	<i>pbp2</i> (T685I e S699L) ^a <i>pbp3</i> (G435D) ^a	WT ^a	WT ^a	E470D, I527M, F597L ^a	WT ^a	WT
SEPI150	Dalbavancin and teicoplanin resistant	23	<i>mecA</i> , <i>blaZ</i>	<i>ant(9)-Ia</i> , <i>aac(6')-aph(2'')</i> , <i>erm(A)</i>	<i>pbp2</i> (T685I, S699L) ^a <i>pbp3</i> (G435D) ^a WT ^b	WT ^a WT ^b	WT ^a WT ^b	E470D, I527M, F597L ^b	D470G ^a D470G ^b	D470G

^a Comparison with reference RP62A strain.
^b Comparison of the SEPI150 genome with SEPI148 reads.

2. Methods

The *S. epidermidis* strain object of this study was isolated from the Azienda Sanitaria Universitaria Friuli Centrale. Species identification was performed using the MALDI Biotyper (Bruker, Billerica, MA, USA) system. Antimicrobial susceptibility testing was performed using Vitek2 instrument (BioMérieux, Marcy-l'Étoile, France) and the MIC for dalbavancin and teicoplanin were confirmed by MIC test strips (Liofilchem, Roseto degli Abruzzi, Italy). MIC results were interpreted following the European Committee on Antimicrobial Susceptibility Testing guidelines (v16.0). Whole-genome DNA sequencing was performed as previously described [2]. Briefly, whole-genome sequencing was performed with a hybrid approach using Illumina paired-end and Oxford Nanopore long read technologies. Hybrid genome assembly was performed using Unicycler v0.5.1 (<https://github.com/ablab/spades>) and quality of the assembly was evaluated with QUAST v5.3.0 (<https://github.com/ablab/quast>). The multilocus sequence type was determined using MLST v2.23.0 (<https://github.com/tseemann/mlst>). Routine genome annotation was performed using Bakta v1.11.3 (<https://bakta.computational.bio>), while single nucleotide variations and/or insertions/deletions were investigated using Bre-Seq software by mapping reads of a clonally related MRSE strain susceptible to dalbavancin and teicoplanin (named SEPI148) isolated from the same patient to the assembled genome of the SEPI150 strain [2].

3. Results

The MRSE SEPI150 strain was isolated on 12 May 2023 from a blood sample collected from a 78-year-old male patient admitted to the Azienda Sanitaria Universitaria Friuli Centrale for a chronic infection involving an ascending aortic vascular graft, complicated by recurrent episodes of MRSE bacteraemia. Before isolation of the SEPI-150 strain, the patient was treated with dalbavancin at a dose of 1500 mg at variable intervals to obtain an optimal exposure in terms of pharmacokinetic/pharmacodynamic target attainment and maintained over time [3]. Medical history of infection, bacterial isolates and antimicrobial treatments of the clinical case presented in this study are shown in Supplementary Fig. S1. The SEPI150 strain was susceptible to erythromycin (MIC >4 mg/L), oxacillin (MIC >2 mg/L), rifampicin (MIC >1 mg/L), teicoplanin (MIC >4 mg/L) and dalbavancin (MIC 0.5 16 mg/L), although it maintained susceptibility to vancomycin (MIC 2 mg/L) and linezolid (MIC ≤ 0.5 mg/L) (Supplementary Table S1). Hybrid genome assembly produced a draft with a total size of 2 455 66 bp with a 31.97% G+C content, composed of 21 contigs (ranging from 745 730 to 1 158 bp in length) with a total of 2259 CDS, 47 transfer RNA, 2 ribosomal RNAs, 46 noncoding RNAs, 29 noncoding RNAs regions, 1 CRISPR arrays and 3 oriCs (Supplementary Fig.

S2). Genome-based typing revealed that the strain belonged to the ST23 and carried different genes associated with resistance to different antimicrobials, including aminoglycosides [*ant(9)-Ia*,*aac(6')-aph(2'')*], β -lactams (*mecA*, *blaZ*) and macrolides [*erm(A)*]. Also, analysis of the prophage regions showed that the MRSE SEPI150 strain harboured two prophage regions within its genome (Supplementary Fig. S3). Genetic analysis demonstrated that no virulence factors or antimicrobial resistance genes were found within the prophage regions of the MRSE SEPI150 strain. Also, we evaluated the genetic variations within the genome of SEPI150 strain in comparison with the *S. epidermidis* RP62A strain used as a reference. Analysis of the genetic determinants related to the resistance to dalbavancin showed that no differences were observed among *walk/R*, *pbp1* and *VarR/S* genes, although mutations were observed within *pbp2*, (T685I, S699L), *pbp3* (G435D) and *rpoB* (E470D and I527M) genes in comparison with the RP62A strain. To characterize the genetic determinants associated with the in vivo evolution of the reduced susceptibility to dalbavancin, we performed a genomic analysis of the MRSE SEPI150 strain resistant to dalbavancin and teicoplanin in comparison with the whole-genome reads obtained from an MRSE SEPI148 strain susceptible to dalbavancin and teicoplanin collected from blood of the same patient 2 months earlier. The two MRSE strains collected exhibited a high genomic homology with an Average Nucleotide Identity based on BLAST equal to 99.98%, thus indicating longitudinal evolution of the same strain. Genome comparison showed that SEPI150 exhibited a total of 34 predicted mutations in comparison with the SEPI148 strain (Supplementary Table S2). In particular, the MRSE SEPI150 strain exhibited predicted mutations within *rpoB* (phenylalanine to leucine at position 597) and *vraG* (aspartic acid with glycine at position 470) genes (Table 1).

4. Discussion

Here, we described the genome of SEPI150, an MRSE strain exhibiting resistance to dalbavancin and teicoplanin. The SEPI150 strain exhibited mutations within the *rpoB* and *vraG* genes, previously described genes associated with decreased vancomycin susceptibility [4,5]. Our data are in agreement with previous studies showing that mutations within the *rpoB* gene correlated with a reduced susceptibility to glycopeptides [6–9]. Modifications in the *rpoB* gene were associated with a thickened cell wall and reduction of the cell-surface negative charge, thus resulting in a reduced susceptibility to daptomycin and heterogeneous vancomycin-intermediate *S. aureus* phenotype [10]. Also, mutations within the *vraG* gene were associated with the regulation of genes involved in peptidoglycan synthesis and remodelling observed in glycopeptide-intermediate staphylococci [11,12]. These results are in agreement with recent studies demonstrating a close correlation between the *vraG* gene, encoding an adenosine triphosphate–

binding cassette transporter and the reduced susceptibility to glycopeptides by modulating the *VraSR* two-component regulatory system, which upregulates genes involved in cell wall biosynthesis and repair [13,14].

In conclusion, these data hypothesize that the accumulation of mutations, although not exclusive, within genes associated with different regulatory pathways (i.e., transcription of DNA into RNA and cell-wall biosynthesis) may be involved in resistance to dalbavancin and cross-resistance with other glycopeptides. Further research will be performed to define the effect of different single nucleotide polymorphisms among these genes, given their relevance in the context of the clinical impact of MRSE infections.

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Data availability: The draft genome assembly of the SEPI150 strain has been deposited in the NCBI BioSample database under accession number SAMN54743267 and BioProject ID: PRJNA1405501.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.jgar.2026.05.006](https://doi.org/10.1016/j.jgar.2026.05.006).

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