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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 to the dalbavancin-susceptible MRSE strain isolated from the same patient demonstrated that the SEPI150 strain carried wild-type *walK/R, pbps,* and *varR/S* genes, while specific mutations were observed within *rpoB* (F597L) and *vraG* (D470G) genes.

Conclusion: 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.

Keywords: Dalbavancin-based treatment, Whole-Genome Sequencing, mutations *walK/walR, rpoB, varG*

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 characterize the genome of an MRSE strain, named SEPI150, resistant to dalbavancin and teicoplanin that emerged during dalbavancin-based treatment.

2. Material and 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, Massachusetts, US) system. Antimicrobial susceptibility testing (AST) was performed using Vitek2 instrument (Biomerieux, France) and MIC for dalbavancin and teicoplanin were confirmed by MIC test strips (Liofilchem, Italy). MIC results were interpreted following EUCAST guidelines v16.0.

Whole-genome DNA sequencing was performed as previously described (2). Briefly, whole-genome sequencing was performed with 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 v.5.3.0 (https://github.com/ablab/quast). Multi-locus 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 SNVs and/or indels were investigated using BreSeq 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 the 12th 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 in order to obtain an optimal exposure in terms of pharmacokinetic/pharmacodynamic (PK/PD) 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 Figure S1 in the Supplementary Material. 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), while maintained susceptibility to vancomycin (MIC 2 mg/L) and linezolid (MIC ≤ 0.5 mg/L) (Table S1 in the Supplementary material). 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 tRNA, 2 rRNAs, 46 ncRNAs, 29 ncRNAs regions, 1 CRISPR arrays and 3 oriCs (Figure S2 in the Supplementary material). Genome-based typing revealed that the strain belonged to the ST23 and carried different genes associated to the 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 pro-phage regions showed that the MRSE SEPI150 strain harbored 2 pro-phage regions within its genome (Figure S3 in the Supplementary material). Genetic analysis demonstrated that no virulence factors and antimicrobial resistance genes were found within the pro-phage regions of the MRSE SEPI150 strain.

Also, we evaluated the genetic variations within the genome of SEPI150 strain in comparison to *S. epidermidis* RP62A strain used as reference. Analysis of the genetic determinants related to the resistance to dalbavancin showed that no differences were observed among w*alK/R, pbp1,* and *VarR/S*

genes, while mutations were observed within *pbp2,* (T685I, S699L), *pbp3* (G435D) and *rpoB* (E470D and I527M) genes in comparison to RP62A strain.

In order to characterize the genetic determinants associated to 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 a MRSE SEPI148 strain susceptible to dalbavancin and teicoplanin collected from blood of the same patient two-month early. The two MRSE strains collected exhibited a high genomic homology with an Average Nucleotide Identity based on BLAST (ANIb) 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 to SEPI148 strain (Table S2 in the Supplementary material). 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, a 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 agreement with previous studies showing that mutations within the *rpoB* gene correlated with a reduced susceptibility to glycopeptides [6-9]. Modifications in *rpoB* gene were associated to 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* (VISA) phenotype [10]. Also, mutations within *vraG* gene were associated to 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 ATP-binding cassette (ABC) 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 accumulation of mutations, although not exclusive, within genes associated with different regulatory pathways (i.e. transcription of DNA into RNA and cell-wall biosynthesis 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 SNPs among these genes, given their relevance in the context of the clinical impact of MRSE infections.

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Competing interests

None declared

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AI statement

N/A

Ethical approval

Written informed consent was not needed for demographic, physiological and hospital-outcome data analyses based of the retrospective nature of the study that not modify existing diagnostic or therapeutic strategies.

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**.

References

1. Siciliano V, Passerotto RA, Chiuchiarelli M, Leanza GM, Ojetti V. Difficult- to-Treat Pathogens: A Review on the Management of Multidrug-Resistant *Staphylococcus epidermidi*s. Life (Basel) 2023;13(5):1126.
2. Gaibani P, Bovo F, Bussini L, et al. Dynamic evolution of imipenem/relebactam resistance in a KPC- producing *Klebsiella pneumoniae* from a single patient during ceftazidime/avibactam-based treatments. J Antimicrob Chemother 2022;77(6):1570-7.
3. Cojutti PG, Gatti M, Tedeschi S, et al. Usefulness of a hub and spoke TDM-guided expert clinical pharmacological advice program of dalbavancin for optimizing very long-term curative or suppressive treatment of chronic staphylococcal infections. Antimicrob Agents Chemother 2025;69(4):e0183024.
4. Gardete S, Kim C, Hartmann BM, et al. Genetic pathway in acquisition and loss of vancomycin resistance in a methicillin resistant *Staphylococcus aureus* (MRSA) strain of clonal type USA300. PLoS Pathog 2012;8(2):e1002505.
5. Watanabe Y, Cui L, Katayama Y, Kozue K, Hiramatsu K. Impact of *rpoB* mutations on reduced vancomycin susceptibility in *Staphylococcus aureus*. J Clin Microbiol 2011;49(7):2680-4.
6. Rishishwar L, Kraft CS, Jordan IK. Population Genomics of Reduced Vancomycin Susceptibility in *Staphylococcus aureus*. MSphere. 2016;1 (4):e00094-16.
7. Mwangi MM, Wu SW, Zhou Y, et al. Tracking the in vivo evolution of multidrug resistance in *Staphylococcus aureus* by whole-genome sequencing. Proc Natl Acad Sci U S A. 2007;104(22):9451-9456.

8. Bongiorno D, Lazzaro LM, Stefani S, Campanile F. In Vitro Activity of Dalbavancin against Refractory Multidrug-Resistant (MDR) *Staphylococcus aureus* Isolates. Antibiotics (Basel). 2020 Dec 3;9(12):865.
9. Hotz JF, Staudacher M, Schefberger K, et al. Unraveling novel mutation patterns and morphological variations in two dalbavancin-resistant MRSA strains in Austria using whole genome sequencing and transmission electron microscopy. BMC Infect Dis. 2024;24(1):899.
10. Cui L, Isii T, Fukuda M, et al. An RpoB mutation confers dual heteroresistance to daptomycin and vancomycin in *Staphylococcus aureus*. Antimicrob Agents Chemother. 2010;54(12):5222-33.
11. Meehl M, Herbert S, Götz F, Cheung A. Interaction of the GraRS two-component system with the VraFG ABC transporter to support vancomycin-intermediate resistance in Staphylococcus aureus. Antimicrob Agents Chemother 2007;51(8):2679-89.
12. Yang SJ, Bayer AS, Mishra NN, et al. The Staphylococcus aureus two-component regulatory system, GraRS, senses and confers resistance to selected cationic antimicrobial peptides. Infect Immun. 2012;80(1):74-81.
13. Werth BJ, Jain R, Hahn A, Cummings L, Weaver T, et al. Emergence of dalbavancin non-susceptible, vancomycin-intermediate Staphylococcus aureus (VISA) after treatment of MRSA central line-associated bloodstream infection with a dalbavancin- and vancomycin-containing regimen. Clin Microbiol Infect 2018; 24:429.e1-429.e5.
14. Werth BJ, Ashford NK, Penewit K, Waalkes A, Holmes EA, et al. Dalbavancin exposure in vitro selects for dalbavancin- non-susceptible and vancomycin-intermediate strains of methicillin-resistant Staphylococcus aureus. Clin Microbiol Infect 2021;27:910.e1-910.e8.

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

Strain	Glyocpeptide phentoype	Sequence type	B-lactams resistance determinants	Other resistance genes	Predicted Mutations					
					bpbs	***walK/R***	***vraR/S***	***rpoB***	***vraG***	**VraG**
SEPI148	Dalbavancin, Teicoplanin and Vancomicin Susceptible	23	*mecA, blaZ*	*ant(9)-Ia, aac(6')-aph(2''), erm(A)*	pbp2 (T685I e S699L)[a] pbp3 (G435D)[a]	WT[a]	WT[a]	E470D, I527M, F597L[a]	WT[a]	WT
SEPI150	Dalbavancin and Teicoplanin resistant	23	*mecA, blaZ*	*ant(9)-Ia, aac(6')-aph(2''), erm(A)*	pbp2 (T685I, S699L)[a] pbp3 (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

Competing of interest

All the authors declare no competing of interest