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Genome Note

Draft genome sequence of a human-associated streptogramin-resistant *Staphylococcus aureus*☆

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ABSTRACT

Objectives: *Staphylococcus aureus* is one of the leading causes of nosocomial and community-acquired infections. Treatment of these infections with macrolide–lincosamide–streptogramin (MLS) antibiotics has led to resistance to these antibiotics via various mechanisms. *S. aureus* strain CIP108540, isolated from a human in France, has been previously shown to exhibit resistance to the streptogramins quinupristin and dalfopristin; the presence of streptogramin resistance genes was verified by PCR. However, the extent of MLS resistance genes in this strain is unknown. This study analysed the genome sequence of *S. aureus* CIP108540 to assess genes associated with antimicrobial resistance, including to streptogramins.
Methods: Genomic DNA of *S. aureus* CIP108540 was sequenced using Illumina MiSeq. The generated sequencing reads were de novo assembled using A5-miseq.
Results: The draft genome size was 3 014 273 bp with a GC content of 32.72%. There were 3063 predicted coding sequences with 59 tRNAs. Several antimicrobial resistance genes were identified conferring resistance to various antibiotics.
Conclusion: The draft genome sequence of *S. aureus* CIP108540 released here will provide valuable information for a better understanding of its genetic makeup and resistome.
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1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections are difficult to treat because they are not only resistant to clinically relevant β-lactam antibiotics but may also exhibit multidrug resistance [1]. As MRSA clones emerged with decreased susceptibility to glycopeptides, the streptogramin compound quinupristin/dalfopristin (QDA) came into use and has been used to treat MRSA infections in France since 1960; resistance of staphylococci to these drugs was reported in France in 1975 [1]. Resistance mechanisms to streptogramins include enzymatic modification of the antibiotics (*vgb* and *vat*), efflux/active transport (*msr* and *vga*) and alteration of the target site (*erm*) [1]. In the present report, the genome sequence of a human-associated streptogramin resistant MRSA was analysed to provide valuable insight into its resistance mechanisms and virulence factors.

2. Methods

S. aureus CIP108540 from the Collection of Institut Pasteur was isolated from a patient in Paris, France, in 1995 [2,3]. The streptogramin phenotype has been reported previously [2,3]. Additional antimicrobial susceptibility testing was performed by broth microdilution using a Sensititre™ semi-automated antimicrobial susceptibility system (Trek Diagnostic Systems, Inc., Cleveland, OH) and the Gram-positive plates GPN3F and CMV3AGPF. Antimicrobials and breakpoints were as follows: ampicillin, ≥0.5 μg/mL; ceftriaxone, ≥64 μg/mL; ciprofloxacin, ≥4 μg/mL; clindamycin, ≥4 μg/mL; daptomycin, ≥4 μg/mL; erythromycin, ≥8 μg/mL; gatifloxacin, ≥2 μg/mL; gentamicin, ≥16 μg/mL, ≥500 μg/mL; levofloxacin, ≥4 μg/mL; linezolid, ≥8 μg/mL; oxacillin, ≥0.5 μg/mL; kanamycin, ≥500 μg/mL; lincomycin,

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≥4 μg/mL; nitrofurantoin, ≥128 μg/mL; penicillin G, ≥0.25 μg/mL; QDA, ≥4 μg/mL; rifampicin, ≥4 μg/mL; streptomycin, ≥1024 μg/mL; tetracycline, ≥16 μg/mL; trimethoprim/sulfamethoxazole, ≥4/76 μg/mL; tigecycline, ≥0.5 μg/mL; tylosin, ≥32 μg/mL; and vancomycin, ≥16 μg/mL. Minimum inhibitory concentrations (MICs) were manually recorded by SensiTouch®, and Clinical and Laboratory Standards Institute (CLSI) standards were used to determine resistance [4], except for daptomycin, kanamycin, lincomycin, tigecycline, tylosin and streptomycin as resistance breakpoints have not been established by the CLSI; breakpoints for these antimicrobials were those defined by the National Antimicrobial Resistance Monitoring System (NARMS) (https://www.fda.gov/downloads/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/UCM581395.pdf). *S. aureus* ATCC 29213 was used as a quality control strain.

Genomic DNA (gDNA) of *S. aureus* CIP108540 was extracted using a DNeasy Blood and Tissue Kit (QIAGEN, Germantown, MD). The concentration of extracted gDNA was calculated using a Qubit® double-stranded DNA (dsDNA) high-sensitivity (HS) Assay Kit (Life Technologies, Inc., Carlsbad, CA), and the integrity of the gDNA was determined using a NanoDrop™ spectrophotometer. Illumina sequencing libraries were prepared using a Nextera™ XT DNA Sample Preparation Kit and Nextera™ XT Index Kit (Illumina Inc., San Diego, CA). The Illumina library was quantified using Qubit® DNA HS Assay Kit in a Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA), and the size of the library was checked using an Agilent 2100 Bioanalyzer System with an Agilent HS DNA Kit (Agilent Technologies, Santa Clara, CA). The library was then sequenced on an Illumina MiSeq platform (Illumina Inc.) using a MiSeq v.3 reagent kit with 600 cycles and a paired-end read length of 2 × 300 bp. Resulting paired-end reads were de novo assembled into contigs using A5-miseq assembler [5]. The contigs were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) [6]. Antimicrobial resistance genes were predicted using ARG-ANNOT [7]; virulence genes and in silico plasmid replicon types were predicted using VirulenceFinder (https://cge.cbs.dtu.dk/services/VirulenceFinder/) and PlasmidFinder (https://cge.cbs.dtu.dk/services/PlasmidFinder/), respectively; and multilocus sequence typing (MLST) and *spa* typing were determined using MLST 1.8 (https://cge.cbs.dtu.dk/services/MLST/) and the web-based *spa* server (https://www.spaserver.ridom.de/), respectively.

3. Results

The assembly yielded 64 contigs and a genome size of 3 014 273 bp with a GC content of 32.72%. The N_{50} value obtained was 140 427 bp with a median coverage of 206×. The total number of genes was 3272, including 3063 coding sequences and 90 RNA genes. *S. aureus* CIP108540 was resistant to ampicillin, ceftriaxone, gentamicin, kanamycin, oxacillin, penicillin, QDA and tetracycline. In silico analysis using ARG-ANNOT predicted several genes encoding resistance to β-lactams (*mecA* and *blaZ*), aminoglycosides (*spc*, *aph*, *aadD*, *ant6-Ia*, *aph3-III*, *sat4A* and *aac6-aph2*), macrolides (*ermA*), streptogramins (*vgaA*, *vgaB* and *vatB*) and tetracycline (*tetM* and *tet38*), explaining the resistance phenotypes. The fosfomycin resistance gene *fosB* was found but was not tested phenotypically. Plasmid replicon types predicted using PlasmidFinder were rep7, rep20, rep21 and repUS12. Several virulence factors were detected, including exoenzyme *aur*, *splA*, *splE* (with 100% identity) and *splB* (99.45% identity), as well as the toxin genes *sek*, *lukD*, *seb*, *hlgA*, *hlgB*, *seq* (100% identity) and *lukE* and *hlgC* (99.89% identity). *S. aureus* CIP108540 was identified as ST112 and *spa* type t200.

4. Discussion

Genome sequencing of the historic streptogramin-resistant *S. aureus* isolate revealed a pool of resistance and virulence genes. As the strain was isolated from a human, antibiotic use in the patient may have played a role in the streptogramin and multidrug resistance observed. The origin of the plasmids carrying one or more streptogramin resistance determinants was unclear; however, the presence of the isolate is itself significant and poses a serious problem as it could easily disseminate in the nosocomial setting. Streptogramin resistance in clinical MRSA is reported rarely and it is possible that the isolate could be of animal origin as virginiamycin was used as growth promoter both in the USA and Europe and was only restricted in Europe in 1999 [1]. The draft genome sequence presented here will be helpful in understanding the resistance and virulence mechanisms.

5. Nucleotide sequence accession no.

This draft genome sequence of *S. aureus* CIP108540 has been deposited at DDBJ/ENA/GenBank under accession no. **QVEL00000000**. The version described in this paper is version **QVEL01000000**.

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Competing interests

None declared.

Ethical approval

Not required.

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