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CORRESPONDING AUTHOR

Marta Bottagisio marta.bottagisio@unimi.it

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UNIVERSITÀ DEGLI STUDI DI MILANO DIPARTIMENTO DI SCIENZE VETERINARIE PER LA SALUTE, LA PRODUZIONE ANIMALE E LA SICUREZZA ALIMENTARE

Phenotypic and genomic identification of Staphylococcus epidermidis GOI1153754-03-14 isolated from an infected orthopedic prosthesis

Marta Bottagisio^{1,2*}, Alessio Soggiu¹, Arianna B. Lovati², Marco Toscano³, Cristian Piras¹, Carlo L. Romanò⁴, Luigi Bonizzi¹, Paola Roncada⁵, Lorenzo Drago^{3,6}

¹University of Milan, Department of Veterinary Medicine, Italy ²IRCCS Galeazzi Orthopedic Institute Cell and Tissue Engineering Laboratory, Milan, Italy ³University of Milan, Department of Biomedical Science for Health, Italy ⁴IRCCS Galeazzi Orthopedic Institute Center for Reconstructive Surgery of Osteoarticular Infections Milan, Italy ⁵Istituto Sperimentale Italiano Lazzaro Spallanzani, Rivolta D'Adda, Cremona, Italy ⁶IRCCS Galeazzi Orthopedic Institute Laboratory of Clinical Chemistry and Microbiology, Milan, Italy

Abstract

Staphylococcus epidermidis GOI1153754-03-14 is able to colonize orthopedic implants and to cause septic non-unions, as validated in a recent in vivo study (Lovati *et al.*, 2016). To the mechanisms leading to the biofilm formation on metallic implants, we carried out the phenotypic and genotypic characterization of the clinical isolate *S. epidermidis* GOI1153754-03-14.

The antimicrobial susceptibility and minimum inhibitory concentration (MIC) of the strain were evaluated through the Vitek2 System (Biomerieux), as well as its ability to form biofilm in vitro through a spectrophotometric assay (Stepanovich *et al.*, 2000).

The genomic DNA was extracted by Bacterial Genomic DNA Isolation Kit (Norgen Biotek Corp.). Libraries were prepared with the ThruPLEX DNA-seq (Rubicon Genomics) and then sequenced on the Illumina MiSeq platform through the MiSeq Reagent Kit v3 (600-cycles) to produce 300 bp paired-end reads (Illumina Inc.). Reads were quality-trimmed and gene annotated thanks to the RAST software (Aziz *et al.*, 2008).

The antimicrobial susceptibility along with the MIC values are reported in Table 1. The outputs resulted in 51 contigs (Average = 50,720.6 Mb) with 396X fold average coverage. The total genome is 2,586,753 bp long with a GC content of 31.84% and an N50 value of 7 bp. The whole genome is composed by 2,467 protein-encoding genes and 64 RNAs (55 tRNAs and 9 rRNAs). The entire genome sequence has been deposited in the European Nucleotide Archive (ENA) under the accession no. FWCG01000000 (Bottagisio *et al.*, 2017).

The genotypic and phenotypic characterization of the *S. epidermidis* GOI1153754-03-14 will enable a better comprehension of the mechanisms involved in the biofilm formation on orthopedic implants, paving the way for innovative preventative and therapeutic strategies. Moreover, the sequence of this clinical strain is mandatory to develop dedicated proteomics analysis in order to highlight functional mechanism of biofilm formation.

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Antibiotic	Antimicrobial susceptibility	MIC
Benzylpenicillin	Resistant	≥0.5
Oxacillin	Resistant	≥ 4
Levofloxacin	Resistant	4
Rifampicin	Resistant	\geq 4
Gentamicin	Susceptible	≤ 0.5
Erythromycin	Susceptible	≤ 0.25
Clindamycin	Susceptible	≤ 0.12
Linezolid	Susceptible	1
Daptomycin	Susceptible	0.25
Teicoplanin	Susceptible	4
Vancomycin	Susceptible	≤ 0.5
Tetracycline	Susceptible	≤ 1
Tigecycline	Susceptible	≤ 0.12
Fusidic acid	Susceptible	≤ 0.5
Trimethoprim/ Sulfamethoxazole	Susceptible	≤ 10

Table 1: Antimicrobial susceptibility and MIC values

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