TITLE: *Staphylococcus haemolyticus* MD43: A COMPLETE ARSENAL OF RESISTANCE AND VIRULENCE READY TO BE DISSEMINATED

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ABSTRACT:

Staphylococcus haemolyticus is one of the most frequent coagulase-negative staphylococci isolated from nosocomial infection. However, its pathogenic potential and its virulence factors are still underexplored. In 2012, it was described a new virulence gene in a strain of methicillin resistant *Staphylococcus aureus* (MRSA) named *sasX* and an orthologous gene has been described in *Staphylococcus epidermidis* (*sesI*). In both cases the encoded proteins demonstrated to be involved in pathogenesis, besides that SasX has been identified as a major factor in the rapid spread of MRSA in Asia, and may be the responsible for the emergence of new resistant and virulent clones. In a previous work we extend the research to *S. haemolyticus*. We detected the *sasX-like* gene in 33/62 strains isolated from a Rio de Janeiro hospital. A deep characterization was done in the strain MD43. Then we obtained the complete sequence of the new orthologous *sasX*-like (now named by us *shsA*), which showed 92 and 96% of identity with *sasX* and *sesI* respectively, and evidence that in the *S.haemolyticus* MD43 it would be in the same genetic environment as the *sasX* and *sesI*, inserted into Φ SPβ -like prophage. This fact and others features related to *S.haemolyticus* MD43 regarding the resistance profile and evidence of horizontal gene transfer (HGT), motivated us to sequence this genome. Whole-genome sequencing was performed (Illumina MiSeq), the sequences were assembled (CLC) and annotated (RAST). After annotation and manual curation, we confirmed the previous results on the genetic environment of *shsA* and, unlike some studies that found *sasX* and variants in prophages with loss of up to 60Kb, there is the maintenance of all phage CDS coding for packaging and structural proteins. Evidencing the possibility of acting as a prophage and a probable selective pressure that kept the prophage intact in the genome. Resistance traits were identified with ResFinder tool and we found the genes *mecA, blaZ, erm(C), aac(6')-aph(2")*. By disk diffusion test the strain showed resistance to Penicillin, Ampicillin, Oxacillin, Cefoxitin, Gentamicin, Ciprofloxacin, Erythromycin, Trimethoprim-sulfamethoxazole, Clindamycin, Rifampin. The results of some phenotypic tests suggested that this strain could have the *aac(6')-aph(2")* gene located in plasmid rather than in chromosomal DNA, as described until now for *S. haemolyticus*, other genes related to resistance also may be associated with plasmids. In a preliminary search for the sequence of the conjugative plasmid PGO1, which carries the *aac (6')-aph (2")* gene in some strains of *S. aureus*, this seems to be a probable context for the gene mentioned, now we are conducting a manual curation in order to confirm this finding. In this study has been described a new virulence gene in *S. haemolyticus*. The strain analyzed presents at the same time a virulent potential and resistance to several antimicrobials. In addition, it has the ability to transmit these features by HGT besides the lack of CRISPR array, becoming a complete arsenal for the reception and dissemination of virulence and resistance genes in the *Staphylococcus* genus.

Keywords: *Staphylococcus haemolyticus* MD43, *sasX/sesI/ shsA*, resistance/virulence, CRISPR, HGT

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