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P2841 Whole-genome analysis of daptomicin-resistant *Staphylococcus aureus* isolates

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Background: Daptomycin (DAP) is a cyclic lipopeptide with in vitro activity against a variety of Gram-positive pathogens, including multidrug-resistant organisms. Although DAP-resistance is uncommon in clinical practice, development of this phenomenon during therapy has been widely described in clinically important organisms such as *Staphylococcus aureus*. Here, we performed whole-genome sequencing (WGS) to identify potential determinants of DAP-resistance in 3 *S. aureus* clinical strains.

Materials/methods: Antibiotic susceptibility were determined by both disk diffusion and the microdilution technique. Interpretation of results was done according to the EUCAST clinical breakpoints. Genomic DNA was extracted using the MagNA Pure96 System (Roche), and quantified using Qubit 1.0 Fluorometer (Invitrogen). The Nextera XT DNA Sample Preparation Kit (Illumina) was used to prepare sequencing libraries from 1ng of genomic DNA according to the manufacturer's instructions. WGS was performed using 150 bp paired-end reads on a MiSeq (Illumina). Sequence reads were trimmed and filtered according to quality criteria, and de novo assembled into contigs by means of CLC Genomics Workbench 10.0 (Qiagen). *In silico* phenotyping and molecular typing (*agr*-typing, *spa*-typing and MLST) was performed using web-based bioinformatics tools.

Results: One *S. aureus* was susceptible to all antibiotics tested, except to DAP, and two presented also resistance to ciprofloxacin and cefoxitin being methicillin-resistant *S. aureus* (MRSA). We identified heterogeneity of lineages, such as ST22 (t2357 and t3914) in MRSA isolates, and a new ST (single locus variant of ST20) with a *spa* typing t22 in Sa850. Regarding DAP-resistance, we detected SNPs and deletions in the multipeptide resistance factor gene (*mprF*) and the *yycFG* components. Both loci are involved in key cell membrane events, with *mprF* being responsible for the synthesis and outer cell membrane translocation of the positively charged phospholipid, lysyl-phosphotidylglycerol, while the *yyc* operon is involved in the generalized response to stressors such as antimicrobials.

Conclusions: In summary, we confirm that point mutations in genes coding for membrane phospholipids are associated with the development of reduced susceptibility to DAP in *S. aureus*. Furthermore, we attested that WGS has the potential of providing a better tool for the detection of the mechanisms involved in DAP-resistance.