NOVEL GENETIC DETERMINANTS RESPONSIBLE FOR DAPTOMYCIN RESISTANCE IN CLINICAL *E. faecium* ISOLATES

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ABSTRACT

Daptomycin (DAP) is used to treat Gram-positive bacterial infections and acts through cell membrane perturbation and depolarization. DAP resistance mechanisms are not completely understood, but often involve SNPs in *liaFSR* resulting in altered cell envelope homeostasis. Four DAP-resistant *E. faecium* clinical strains and the rare DAP hyper-susceptible *E. faecium* SJRP18 strain were isolated between September and November 2012 from infected patients in a hospital in Sao Jose do Rio Preto, Brazil, where DAP was not in use. In order to understand the DAP resistance mechanism, SJRP18 was submitted to *in vitro* selection with increasing DAP concentrations during 10 days in triplicate. Whole genome sequencing of the SJRP18, its resistant *in vitro*-selected strains and clinical DAP resistant strains were performed on an Illumina MiSeq and SNPs were identified by mapping reads from each resistant strain to the parent genome as a reference. The DAP-resistant strains selected *in vitro* contained no SNPs in *liaFSR* compared to the SJRP18 parent strain, and instead had SNPs in the same two genes in all 3 replicates: *lafB* and *dha*K. The first gene encodes the glycosyltransferase LafB, which is presumably responsible for adding the second glucose to glucosyl-diacylglycerol to help form the membrane anchor for lipoteichoic acid. The second gene codes for the dihydroxyacetone kinase DhaK, which is predicted to be involved in glycerol metabolism. Although SNPs in *liaFSR* were identified in the clinical resistant strains when compared to SJRP18, SNPs in *lafB* and *dhaK* genes were also detected. Overexpression of *lafB* with the C577T (R193W) mutation in the SJRP18 parent strain resulted in a 33-fold increase in DAP MIC (0.06 to 2 mg/L). Curiously, the same *lafB* mutation was found in many genomes already described including the DO strain, which suggests that SJRP18 is the one that mutated *lafB*, leading to its rare DAP hyper-susceptible phenotype. Overexpression of the wild-type, sensitive *dhaK* allele in a resistant *in vitro*-selected background strain led to a two-fold decrease in DAP MIC (from 8 to 4 mg/L). In conclusion, SNPs in *lafB* and *dhaK* form a new pathway to DAP resistance independent of *liaFSR* in *E. faecium* SJRP18.

Keywords: *Enterococcus faecium*, daptomycin resistance, genome analysis, *in vitro* selection.

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