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Evolutionary Genetic Reversion of Daptomycin Resistance in a Ca-mrsa St5-iv Clinical Isolate

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Abstract: Background: Daptomycin (DAP; approved for treatment of MRSA) disrupts the cytoplasmic membrane (CM), leading to depolarization and cell death, and the cell wall (CW), postulated to be an additional target. DAP resistance in *S. aureus* includes mutations in *mprF* (CM surface charge; most clinically relevant gene), *walkR* (CW), *dltA*, and *rpoB*. Mutations are accompanied by fitness cost with two evolutionary known responses: elimination of the cost by reverting to antibiotic sensitivity or adapt by compensatory mutations without compromising antibiotic tolerance. Rifampicin resistance (*rpoB*) imparts a fitness cost in the absence of antibiotics through compensatory mutations at a second site of *rpoB*. Methods consecutive CA-MRSA isolates (ST5-SCCmec type IV-PVL+) were recovered from blood cultures (patient with chronic kidney failure, dialysis): SA6819 (VSSA-DAP-S), SA6820 (VISA-DAP-R) and SA6850 (VSSA-DAP-S). Susceptibility testing were performed by E-test, agar dilution (CLSI), Phoenix and Vitek2c. DAP/ β -lactams synergy was done in SA6820-infected *G. mellonella* larvae, and WGS by Illumina MiSeq and *mprF* functional studies by allelic replacement/complementation (wild type or L826F mutated). Results: DAP resistance in SA6820 (DAP-R) was acquired by mutations in *mprF* (L826F) and *vraS* (cell wall sensor; frame shift mutation T3H), and *rpoB* demonstrating mutual CM/CW modifications. Inactivation of SA6820 mutated *mprF* increased sensitivity to DAP (resistance levels were restored by complementation with mutated L826F *mprF*). Genomic evolution to DAP sensitivity (SA6850) was characterized by: presence of a different *rpoB* mutation identical to the DAP-S parent but distinct of DAP-R strain, absence of SNPS in *mprF* and new *lySR* SNPs (main regulator of decreased antibiotic tolerance and cell death leading to DAP-S phenotype). DAP/ β -lactam combination significantly enhanced both in-vitro and in-vivo efficacy against DAP-R MRSA infections. Conclusions: This study provides novel findings on the mechanisms of reversion of DAP-R subsequent to treatment with VAN and /or DAP in dialyzed patients.