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Mechanism of Daptomycin Resistance Reversion in a CA-MRSA ST5-IV Clinical Isolate

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Objectives. To investigate the mechanism of reversion of daptomycin (DAP) resistance from a DAP resistant (DAP-R) to a DAP-susceptible (DAP-S) phenotype in a VISA (vancomycin intermediate *S. aureus*) CA-MRSA ST5-IV strain isolated from a patient in Argentina, and to establish the mechanistic role of *mpr*F mutations during this process.

Methods. PFGE and whole genome sequencing (WGS) were performed in both the clinical and the *in-vitro* derivative strains. SA6820 (VISA, DAP-R) was subcultured during 35 days in drug-free BHI to reproduce *in-vitro* the DAP-R/-S reversion. Competitive cultures were performed by mixing the two strains in equal proportion followed by 10 days of subculture in drug-free BHI to compare their fitness. Additionally, SA6820ΔmprF mutant strain was obtained from SA6820 by 80α transduction, and compared with the other strains.

Results. Following vancomycin (VAN) and DAP treatment, a VISA DAP-R strain (SA6820) with a DAP MIC= 2 mg/L and VAN MIC= 4 mg/L was isolated from a diabetic patient. Three months after treatment, the patient developed a recurrent infection produced by a VAN-S DAP-S strain (SA6850) with DAP MIC= 0.25 mg/L and VAN MIC=1 mg/L. PFGE and WGS confirmed the highly homology between the clinical isolates. SA6820 strain harbored the *mprF* L826F mutation, previously shown to be a major determinant of DAP resistance. The competitive culture between SA6820 and SA6850 showed a rapid decrease of the resistant strain (SA6820) displaying lower fitness compared to its revertant counterpart (SA6850). Experiments directed to obtain the *in-vitro* revertant phenotype showed that after 35 days of passage in drug-free BHI, SA6820 was able to revert to a DAP-S phenotype (SA6820-DAP-S). Whole genome sequencing of the *in-vitro* revertant SA6820-DAP-S showed a mutation Y116. leading to an early stop codon in MprF. Competitive cultures performed to assess the importance of *mpr*F during the reversion process showed the increased fitness of SA6820Δ*mprF* over SA6820, while no difference was observed between SA6820Δ*mprF* and SA6850.

Conclusion. The present study provides the first full description of phenotypic and genotypic events leading to reversion from a DAP-R to a DAP-S phenotype in a clinical MRSA strain and its *in vitro*-obtained derivative. The difference of fitness found between the DAP-R and DAP-S strains may explain the clearance of the resistant sub-population when the antibiotic pressure was removed following the patient treatment. Moreover, the *in-vitro* experiments highlight the key mechanistic role of *mprF* during this process.