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Phenotypic and genotypic correlates of reduced vancomycin susceptibility in vancomycin-intermediate methicillin-resistant *Staphylococcus aureus*

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Background: Although vancomycin (VAN) is one of the alternative for the treatment of methicillin-resistant *S. aureus* (MRSA) infections, the usage of VAN has resulted in the emergence of VAN-intermediate (VISA) or -resistant *S. aureus* (VRSA). Using an isogenic pair of sequence type (ST) 72 CA-MRSA isolates from the bloodstream of a VAN-treated patient who failed VAN therapy, we investigated potential relationships between the reduced vancomycin susceptibility and with: i) cross-resistance to daptomycin (DAP) and host defense cationic antimicrobial peptide (HD-CAP); ii) alterations in cell envelope phenotypes (i.e autolysis, membrane potential, surface positive charge); and iii) transcriptional profiles of genes associated with HD-CAP and DAP resistance.

Methods: Strains: Previously well-characterized isogenic pair of VAN-susceptible & VAN-intermediate ST72- MRSA strains, VSSA303 and VISA072 (VAN MICs of 1 and 4 μg/ml, respectively).

Autolysis assays: TritonX-100 induced autolysis assays.

Daptomycin challenged Population analysis profiles

Membrane potential: Daptomycin challenged flow cytometric analysis using the lipophilic dye DiOC5

HD-CAP susceptibility assays: 2h killing assays using 5 ×103 CFU *S. aureus* against polymyxin B (PMB), LL-37(human cathelicidin found in neutrophil and skin), BMAP(Bovine serum), K9CATH().

Surface charge: cytochrome c binding assays.

Transcriptional expression: standard qRT-PCR.

Results: The VISA strain, VISA072, showed enhanced surface positive charge and, in turn, exhibited significantly increased susceptibilities to DAP, PMB, and LL-37. Reductions in both autolysis rate and membrane potential were observed in VISA072 strain. Further, the VISA strain displayed altered expression profiles of *mprF, dltA, and graRS* genes compared to those of the VSSA strain.

Conclusion: These results demonstrate that alterations in cell wall synthesis physiology, cell membrane potential, cell surface positive charge are associated with reduced VAN susceptibility in the ST72-VISA strain.

Key words : Vancomycin-intermediate resistant *S. aureus* (VISA); daptomycin(DAP); host defense cationic antimicrobial peptides (HD-CAPs)

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