



A Novel Mechanism of Antibiotic Resistance : Thickened Cell Wall as the Major Phenotypic Determinant of Low-Level Vancomycin Resistance in *S. aureus*

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Since the first isolation of low-level vancomycin-resistant *S. aureus* (L-VRSA, or VISA) strain Mu50, there have been several L-VRSA strains with a similar level of vancomycin resistance reported from USA, South Korea, France, UK, Greece, Italy, South Africa and Brazil. The subsequent worldwide isolation of L-VRSA has confirmed that emergence of vancomycin resistance in *S. aureus* is a global issue. The morphology study on sixteen L-VRSA isolates from six countries shows significant thickening of cell wall when compared to vancomycin-susceptible strains. Vancomycin-susceptible derivatives (VRSA-P strains: P stands for “passage”) were prepared from sixteen VRSA clinical strains by drug-free passage, and vancomycin-resistant revertants were obtained from VRSA-P strains by one-step selection with vancomycin. Morphologic observation by transmission electron microscopy shows that the thickening of cell wall of L-VRSA strains is lost together with loss of vancomycin resistance (as judged by MIC values) during drug-free passage, but is regained in the vancomycin-resistant revertants. A pair-wised comparison demonstrates a strong correlation between cell-wall thickness and the degree of vancomycin resistance. Subsequently, direct evidences for the thickened cell wall as the determinant of vancomycin resistance were obtained using experimentally prepared isogenic cells of Mu50 with different cell-wall thickness. A study of biosynthesis, vancomycin consumption and quantitative measurement of membrane-bound vancomycin using these preparations demonstrated that *S.aureus* resists to vancomycin through the novel strategy; i.e., by thickening its cell wall. Thick cell wall significantly retards the timing that vancomycin molecules reach the cytoplasmic membrane to shut down the peptidoglycan synthesis by affinity trapping vancomycin molecules within thickened cell wall layers. In an effort to understand the genetic determinants of vancomycin resistance, DNA microarray technique was employed to fish out several candidate genes, and their functions were evaluated by its selective overexpression in vancomycin-susceptible *S. aureus* cells. The results indicated that vancomycin resistance in *S.aureus* could be influenced by multiple genetic determinants.