Evaluation of L6 Ribosomal Protein Alterations in Fusidic Acid-Resistant *Staphylococcus aureus*: Fitness Cost and Time Kill Analysis

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Objectives: To evaluate fitness cost and activity by time kill experiments on three *S. aureus* (SA) clinical strains displaying elevated fusidic acid (FA) MIC values (4-8 mg/L) and L6 ribosomal protein (RP) alterations. L6 RP (encoded by *fusE*) has been recognized as a FA secondary action site and L6 mutations have been described in FA-resistant (R) SA small colony variant laboratory mutants.

Methods: Three SA clinical strains showing FA-R MIC values were identified in 2008 and 2009. Two strains were detected from one hospital during a surveillance study and one strain was isolated following FA therapy. MIC values were determined by CLSI broth microdilution method (M07-A8, 2009). Isolates were screened for known FA-R mechanisms by PCR and sequencing. Clonality was assessed by PFGE and *spa* typing. Growth rate studies were performed Q30 min for 10h. Time kill analyses were performed over 48h and tested FA at 8X MIC and at the clinical trough plasma level (80 mg/L).

Results: SA clinical strains showing FA-R (MIC, 4-8 mg/L) were screened for the presence of *fusB*, *fusC,* and *fusD,* as well as mutations in *fusA*, yielding negative results. All three SA showed *fusE* alterations. Two SA clinical strains were recovered from different patients in Michigan and possessed a 22 amino acid deletion in *fusE* starting at position 78 (compared to SA Mu50). These strains were shown to be identical by two typing methods, and belonged to USA300 clone. A third strain (FA MIC, 8 mg/L) recovered after FA therapy had a stop codon in L6 at position 77. This strain had identical PFGE and *spa* typing results as the pre-treatment isolate (FA MIC, 0.12 mg/L). SA displaying L6 RP alterations did not reach log phase in 10h (very slow generation times), while controls [NSR384 (USA300) and pre-treatment strain] demonstrated a mass doubling time of 90 min. Time kill studies showed rapid killing at the clinical trough plasma level for all FA-R clinical strains and controls.

Conclusions: L6 alterations have not been described in SA strains from patient infections. In this study, three SA clinical strains displaying L6 RP alterations were associated with modest elevation of FA MICs, but possess a remarkable difference in fitness cost. Furthermore, under experimental conditions, simulated FA plasma trough levels attained in vivo killed these R mutant strains.