Concentration-resistance Relationships with *Staphylococcus aureus* Exposed to Linezolid in an *in Vitro* Dynamic Model

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Background

- Antibiotic concentration relationships with the selection of resistant mutants (RMs) of Gram-positive and Gram-negative pathogens have been shown useful in predicting “anti-mutant” fluoroquinolone dosing [1-6].
- To delineate relationships between the enrichment of linezolid-resistant *S. aureus* and the ratio of 24-hour area under the concentration-time curve (AUC) to the MIC or the mutant prevention concentration (MPC), linezolid pharmacokinetics were simulated in an *in vitro* dynamic model.
- Given the low mutation frequency exhibited by *S. aureus* and, therefore, the possible lack of spontaneous RMs in the starting inoculum, a mixed inoculum of linezolid-susceptible cells and previously selected RMs was used [7].

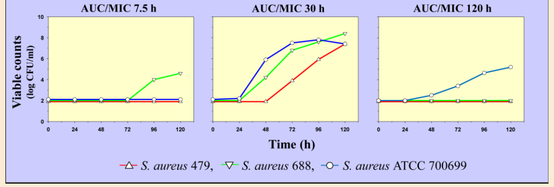
Materials/Methods

- Three methicillin-resistant *S. aureus* strains susceptible to linezolid (MIC 2 μg/ml) were used in the study: two clinical isolates 479, 688 and a well-characterized strain Mu50 (ATCC 700699).
- Their RMs, selected respectively after the 7th (RM7), 23rd (RM23) and 28th (RM28) passages, had a stable MIC of 8 μg/ml.
- The presence of RM7, RM23 and RM28 in the mixture with the parent strains (10^2:10^{10}) did not shift the MPCs of linezolid against *S. aureus* 479 (5 μg/ml), *S. aureus* 688 (6 μg/ml) or *S. aureus* ATCC 700699 (10 μg/ml).
- Mixed inocula at the same ratio of RMs to the parent strain were used in simulated five-day treatments with twice daily linezolid over a 32-fold range of the AUC/MIC ratio that provided antibiotic concentrations below the MIC, between the MIC and MPC and above the MPC.
- The amplification of RMs was monitored by plating on media with 2×, 4×, 8×MIC of linezolid. Time courses of resistant mutants were characterized by the area under the RM concentration – time curve ($AUBC_M$ [8]) corrected for the area under the lower limit of detection.
- To relate $AUBC_M$ with simulated AUC/MICs and AUC/MPCs, a modified Gaussian type function was used.

Results

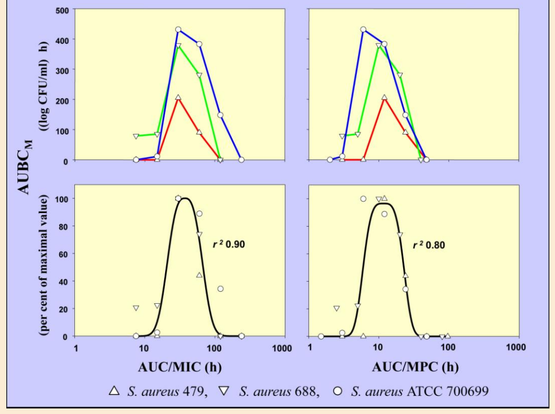
- With each *S. aureus* strain, mutants resistant to 2×, 4× and 8×MIC of linezolid were enriched at the AUC/MIC ratios of 30 and 60 h. The selection of mutants resistant to 4× and 8×MIC of linezolid was less pronounced than to 2×MIC. Minor if any enrichment of RMs occurred at the lower (7.5 and 15 h) and higher (120 and 240 h) AUC/MIC ratios.
- Time courses of mutants resistant to 4×MIC (as an example) at three characteristic AUC/MICs are shown in Fig. 1. As seen in the figure, at AUC/MIC 30 h RMs of all studied strains were enriched, while at 7.5 h – only RMs of *S. aureus* 688 and at 120 h – only RMs of *S. aureus* ATCC 700699.

Fig. 1. **Time courses of *S. aureus* subpopulations resistant to 4×MIC of linezolid at three characteristic AUC/MIC ratios**



- With each *S. aureus* RM, AUC/MIC and AUC/MPC curves of the $AUBC_M$ were bell-shaped. Although the maximal $AUBC_M$s were strain-specific, descending portions of the curves were not stratified (see for example curves plotted for mutants resistant to 4×MIC of linezolid – Fig. 2, upper panel).
- As seen in the figure, clinically attainable linezolid exposure (120 h [9]) may be sufficient to restrict the emergence of resistant *S. aureus* 479 and *S. aureus* 688 but not *S. aureus* ATCC 700699.
- To combine data obtained with individual *S. aureus* RMs, $AUBC_M$s were normalized to the maximal value for each strain. As seen in the bottom panel of Fig. 2, a Gaussian function fits AUC/MIC and AUC/MPC relationships with resistance with high r^2s (0.9 and 0.8, respectively).

Fig. 2. **AUC/MIC and AUC/MPC relationships with $AUBC_M$ (*S. aureus* mutants resistant to 4 MIC of linezolid)**



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Conclusions

- The amplification of *S. aureus* RMs is AUC/MIC- and AUC/MPC-dependent.
- Bell-shaped patterns of the $AUBC_M$-AUC/MIC or -AUC/MPC relationships support the mutant selection window hypothesis as applied to linezolid-exposed *S. aureus*.

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