

Testing the Mutant Selection Window Hypothesis in *Staphylococcus aureus* Resistance Studies with Linezolid Using a Mixture of Antibiotic-susceptible Cells and Resistant Mutants in an *In Vitro* Dynamic Model

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Background

- *In vitro* resistance studies with bacteria that exhibit low mutation frequencies often fail because of the lack of spontaneous resistant mutants (RMs) in the starting inoculum.
- To test the mutant selection window (MSW) hypothesis [1,2] as applied to linezolid-exposed *S. aureus*, a mixed inoculum of linezolid-susceptible and -resistant cells was used [3].

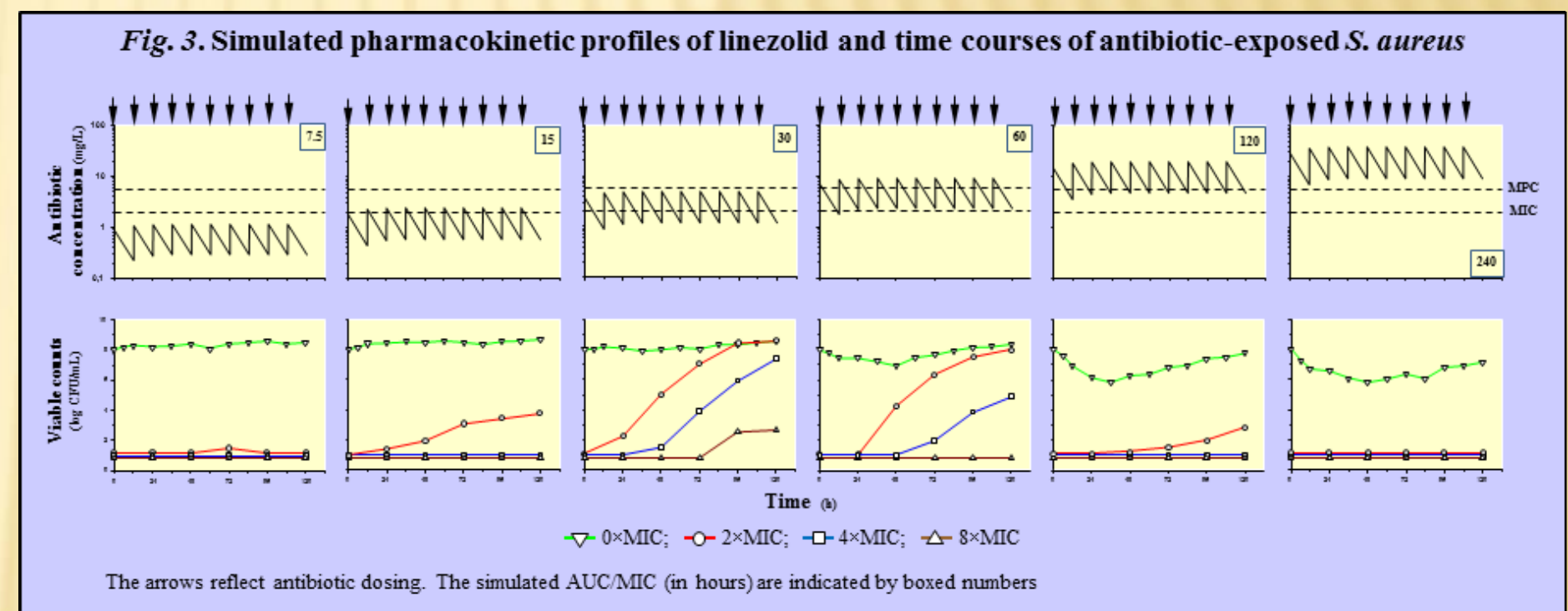
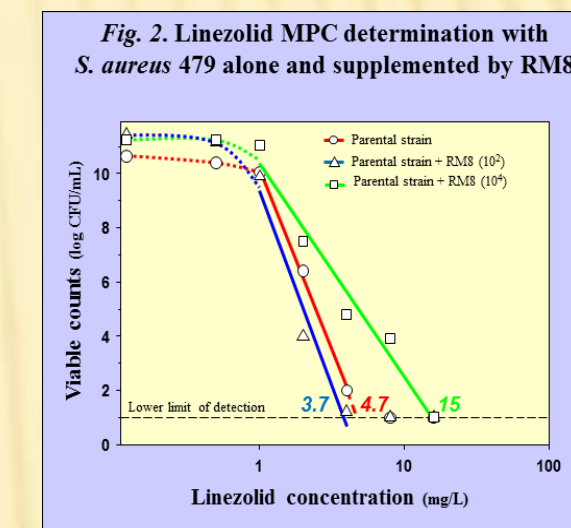
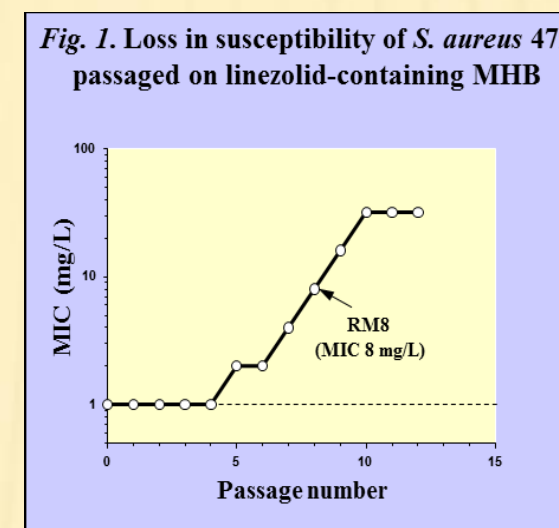
Materials/Methods

- To obtain RMs, a clinical isolate of *S. aureus* 479 (MIC of linezolid 2 mg/L) was serially passaged in linezolid-containing Mueller-Hinton broth.
- The mutant prevention concentrations (MPCs) were determined for the mixtures of linezolid-susceptible cells (0.9 mL of 10¹⁰ CFU/mL suspension) with RM isolated after the eighth passage (RM8; 0.1 mL of 10³ and 10⁵ CFU/mL suspension resulting in 10² and 10⁴ CFU/mL in the mixture, respectively) and without RM8.
- To provide linezolid concentrations within and out of the MSW, five-day treatments with twice daily linezolid (half-life of 6 h, in accordance with values reported in humans [4]) were simulated in an *in vitro* dynamic model [3] over a 32-fold range of the ratio of area under the concentration – time curve (AUC) to the MIC.
- The amplification of resistant mutants was monitored by plating on agar plates containing 2×, 4×, 8×MIC of linezolid. Time courses of resistant mutants were characterized by the area under the bacterial mutant concentration – time curve (AUBC_M) [5] calculated from time zero to 144 h and corrected for the area under the lower limit of detection.

- To relate AUBC_M with simulated AUC/MICs, a modified Gaussian type function was used.

Results

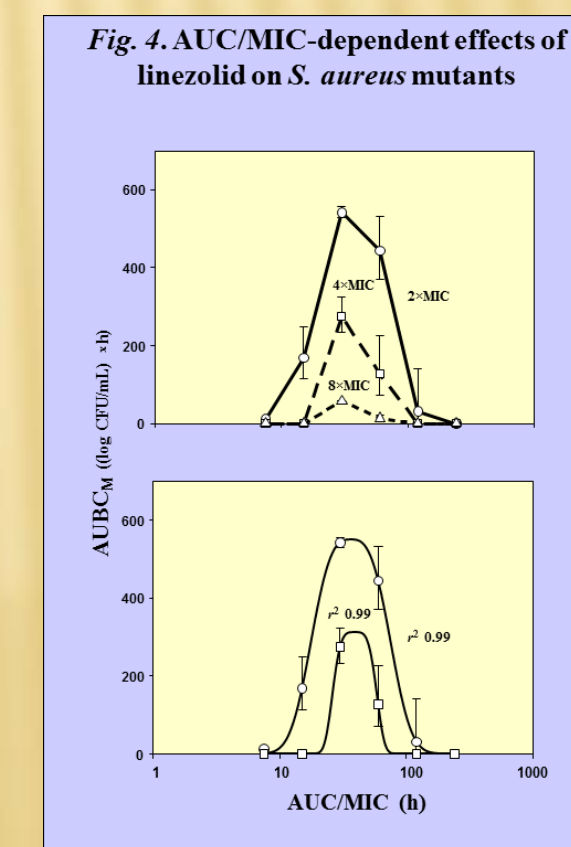
- RMs were enriched starting at the 5th passage, with continued loss in susceptibility up to the 10th passage. MICs of linezolid determined for the isolated RMs were 2-, 4-, 8-, 16 and 32-fold higher than for the parental strain (Fig. 1). The MICs for RMs remained constant after 20 passages on antibiotic-free medium.
- MPCs determined for the parental strain of *S. aureus* 479 with RM8 (10² but not 10⁴ CFU/mL) and without RM8 were similar (4-5 mg/L, Fig. 2).
- Therefore, an inoculum containing 10¹⁰ CFUs of susceptible cells (10⁸ CFU/mL per a 100 mL central unit) and 10² CFUs of the RM8 bacterial suspension (10² CFU/mL per 1 mL), i.e., one linezolid-resistant cell per 10⁸ CFU of susceptible cells, was exposed to linezolid in *in vitro* model studies.
- As seen in Fig. 3, *S. aureus* mutants resistant to 2×MIC of linezolid were enriched in a concentration-dependent pattern: little or no enrichment at the low (7.5 and 15 h) and high (120 and 240 h) AUC/MIC ratios in contrast to pronounced enrichment at the intermediate AUC/MICs (30 and 60 h). The enrichment of mutants resistant to 4× and 8×MIC of linezolid was less pronounced and occurred only at AUC/MICs 30 h.
- Pronounced enrichment of mutants at AUC/MICs 30 and 60 h is concordant with the time inside the MSW (T_{MSW}) of 59% and 52% of the dosing interval respectively.



- Regardless of the level of resistance, AUC/MIC relationships with the AUBC_M were bell-shaped (Fig. 4) in concordance with the MSW hypothesis. Gaussian functions fit well the data obtained with mutants, resistant to 2× and 4×MIC of linezolid.

Conclusion

These findings support the MSW hypothesis as applied to linezolid-exposed *S. aureus*.



References

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