Endpoints in Bacterial Resistance Studies Using *In Vitro* Dynamic Models: an Integral Parameter *versus* a Point Parameter

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Background

- Post-exposure number of mutants (N_M) is a conventional endpoint in bacterial resistance studies using *in vitro* dynamic models that simulate antibiotic pharmacokinetics.
- To compare N_M with a recently introduced integral parameter $AUBC_M$ (the area under time course of resistance mutants) [1], the enrichment of resistant *Staphylococcus aureus* was studied *in vitro* by simulating single [daptomycin (DAP), doxycycline (DOX)] and combination treatments [DAP + rifampicin (RIF), linezolid (LZD) + RIF].

Materials/Methods

- Two strains of *S. aureus*, ATCC 43300 (MICs of DOX, DAP and RIF 0.1, 0.39 and 0.012 mg/L, respectively) and a clinical isolate *S. aureus* 10 (MICs of LZD and RIF 2.0 and 0.016 mg/L, respectively) were exposed to 5-day treatments with the antibiotics in an *in vitro* dynamic model [2].
- Pharmacokinetic profiles that mimic time courses of once-daily DAP, DOX and RIF and twice-daily LZD in humans were simulated with their respective half-lives (9, 15, 3 and 6 h [3-6]).
- The designed ratios of 24-hour area under the concentration – time curve (AUC) to the MIC were 64 (DAP), 90 (DOX), 100-1850 (RIF) and 60, 120 h (LZD).
- The enrichment of mutants resistant to 2×MIC of DAP and 2×, 4×, 8× and 16×MIC of RIF and DOX was monitored by plating on agar plates containing the respective antibiotic.
- Time courses of resistant mutants were characterized by N_M at the end of each experiment (120 h) and $AUBC_M$ calculated from time zero to 120 h and corrected for the area under the lower limit of detection.

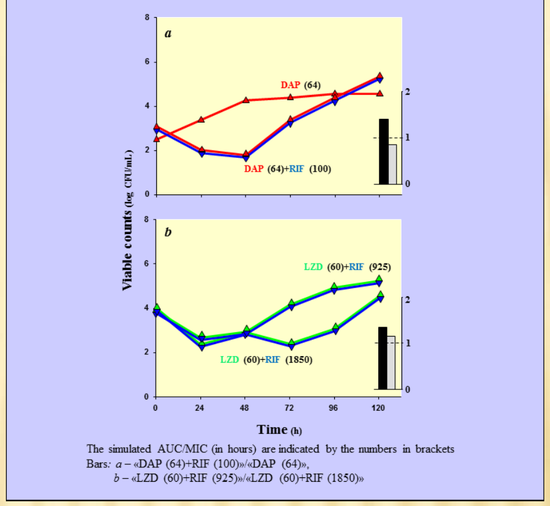
Results

- With DAP given alone, DAP-resistant mutants were enriched soon after the start of single treatment of *S. aureus* ATCC 43300, whereas with DAP + RIF the amplification of mutants occurred only on the third day of treatment. The enrichment of RIF-resistant mutants of *S. aureus* 10 was observed later at higher RIF AUC/MICs than at lower ratios when given in combination with LZD (Fig. 1).
- Time courses of *S. aureus* 10 mutants resistant to 2×, 4×, 8× and 16×MIC of RIF combined with LZD as well as time courses of *S. aureus* ATCC 43300 mutants resistant to 2×, 4×, 8× and 16×MIC of DOX were distinctly stratified: the higher the resistance level, the lower the numbers of mutants (Fig. 2).
- These differences were clearly reflected by $AUBC_M$ but not N_M, because in most cases post-exposure numbers of resistant mutants were similar, masking the true effects of the AUC/MIC ratio and different levels of resistance (diagrams in right lower corners of both figures).

Conclusion

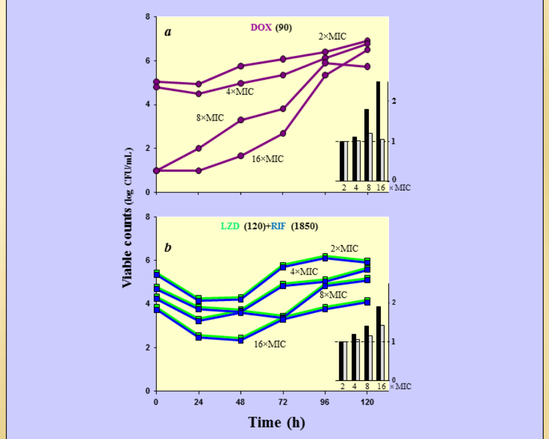
These findings suggest that $AUBC_M$ is a more reliable endpoint of the amplification of resistant mutants than N_M.

Fig. 1. **Time courses of subpopulations of *S. aureus* (*a* - *S. aureus* ATCC 43300, *b* - *S. aureus* 10), resistant to 2×MIC of DAP (*a*) and 4×MIC of RIF (*b*). In right lower corner - $AUBC_M$ (black bars) and relative N_M (gray bars) relations**



The simulated AUC/MIC (in hours) are indicated by the numbers in brackets
Bars: *a* – «DAP (64)+RIF (100)»/«DAP (64)»,
b – «LZD (60)+RIF (925)»/«LZD (60)+RIF (1850)»

Fig. 2. **Time courses of subpopulations of *S. aureus* (*a* - *S. aureus* ATCC 43300 and *b* - *S. aureus* 10) mutants with different susceptibility to DOX and RIF, relatively. In right lower corner - $AUBC_M$ (black bars) and relative N_M (gray bars) relations.**



The simulated AUC/MIC (in hours) are indicated by the numbers in brackets
Bars: relation of $AUBC_M$s and N_Ms calculated at 2×, 4×, 8 × and 16×MIC to those at 2×MIC

References:

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