Acinetobacter spp. and time–kill studies

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Sir,

Multidrug-resistant isolates of Acinetobacter spp. have become a worldwide threat for hospitalized patients in recent years. Two papers recently published in JAC referred to in vitro tests of combinations destined to kill carbapenem-resistant Acinetobacter isolates.1,2 The respective authors found effective combinations that may be useful for the treatment of severe infections due to such isolates. However, in my view there are technical considerations regarding the time–kill studies that put in doubt the validity of the results obtained.

Tan et al.1 wrote that they used minocycline and colistin combinations at 1 × MIC for each antibiotic for isolates susceptible to both drugs, but turbidity after 24 h of incubation can be inferred from the results of Table 1 in their article (the average concentrations at 24 h were between 10⁶ and 10⁷ cfu/mL). The authors attempted to explain this anomaly by supposing the presence of a heteroresistant population in colistin-susceptible Acinetobacter baumannii instead of realizing that they were not working with colistin at 1 × MIC. They determined the MICs by macrodilution, so lack of turbidity should be visualized at an equivalent antimicrobial concentration at the end of both the time–killing studies and the dilution tests, if they really were working at the MIC.1

Curves of imipenem and colistin in the work of Song et al.2 in a similar way, were performed at concentrations below the MIC, and not at the MIC as they stated, but in this case, regrowth in the tubes of the time–kill assay may be a more likely explanation accounting for the differences between volumes used in microdilution and those used in time–kill studies. This problem is frequently found when one wants to work at 1 × MIC, because the MIC is a parameter that has an uncertainty of ±1 dilution, and from one experiment (i.e. broth dilution) to another (time–kill study) it is possible that differences occur. To evaluate synergy in a static time–kill assay, they should have worked with different antibiotic concentrations and one of the antibiotics should have been tested at a concentration that did not inhibit the growth of the organism.

However, their results seem to be better than the authors thought, because combinations were bactericidal with both antibiotic concentrations below the respective MICs (Figure 1 in Tan et al.1). Another issue regarding the work of Song et al.2 is their use of colistin methanesulphonate rather than colistin sulphate, which is normally recommended for in vitro testing of colistin.

However, what I find particularly difficult to accept is that in both studies, the isolates apparently grew to titres of >10¹¹ cfu/mL (antibiotic-free curve in Figure 1 in Tan et al.1 and Figures 1 and 2 in Song et al.2). Normally Acinetobacter isolates reach in the stationary phase at least 10⁸, 5 × 10⁸ or, rarely, 10⁹ cfu/mL. Taking into account that the volume of one bacterial cell is >1 μm³, a bacterial suspension containing 10¹² cfu/mL would have the consistency of a near solid paste rather than fluid. Still more impressive are the results of Song et al.2 where bacterial concentrations in the presence of colistin grew to concentrations of 10¹³–10¹⁵ cfu/mL. To my mind, this raises the possibility that the apparently high counts reflect organism carry-over while undertaking 10-fold dilutions. If that was so, unfortunately their whole work would lose its value.

Finally, Song et al.2 failed to provide the time–killing curves of rifampicin and sulbactam for the eight selected isolates. This omission precludes discussion of synergy, as the activity of the drugs in combination cannot be interpreted in the absence of knowledge of the activity of the individual drugs.2 On the other hand, I did not understand why Tan et al.1 said that one isolate did not fulfil the criteria for synergy when at 24 h the colony count in the combined antibiotic test fell below the detectable threshold (20 cfu/mL). I think that if they started with a concentration of 5 × 10⁸ cfu/mL and this result was reproducible, the synergy should be warranted.

Transparency declarations
None to declare.

References


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Comment on: Acinetobacter spp. and time–kill studies

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