Reply to “Reliability of Gradient Diffusion Methods for Detection of Acquired Colistin Resistance”

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We appreciate the interest of Nastro et al. in our recent article (1); their comments (2) offer an opportunity to discuss the work further. Our study was undertaken with a collection of Acinetobacter baumannii and Klebsiella pneumoniae clinical isolates for which colistin (Cst) MICs were elevated. It was observed that among the commercial methods for Cst susceptibility testing (ST), gradient diffusion methods (Etest and MTS) produced high rates of very major errors (VME) in comparison with broth microdilution (BMD). Error rates for K. pneumoniae were estimated using EUCAST breakpoints, as susceptibility breakpoints do not exist in CLSI documents; their applicability for this species may be limited in countries employing CLSI interpretive criteria. As the automated system Vitek2 yielded no VME, we concluded that such systems may be preferable for routine colistin ST over gradient diffusion methods. We should note herein that neither Vitek2 nor gradient diffusion methods are U.S. FDA approved for the testing of colistin for clinical purposes.

After reading thoroughly the letter of Nastro et al. (2), we still believe that our results are supported by the literature that they list. In response to the comments made by our colleagues, we point out the following. (i) Hindler and Humphries (3), applying the Etest to Cst-resistant Gram-negative bacteria, reported higher rates of VME (47 to 53%) than our study. (ii) Tan and Ng (4) recorded low rates of VME with Enterobacteriaceae, but their study included only 3 Cst-resistant Klebsiella spp. Of note, although detailed results per species were not presented, with the Etest, the MICs of 61% of Enterobacteriaceae were ~1 to more than ~2 log₂ dilutions lower than those determined with the standard method. (iii) Behera et al. (5) tested only 6 polymyxin B-resistant K. pneumoniae and A. baumannii isolates, and of those isolates, 2 (33.3%) produced VME with the Etest in comparison with the reference BMD method; of interest, 2 of 4 isolates for which polymyxin B MICs were 8 to 16 μg/ml gave VME by the Etest. (iv) Arroyo et al. (6) actually observed that the Etest misclassified as Cst susceptible a quite high proportion (9.1%) of the 22 A. baumannii isolates defined as resistant by BMD; the rate of 1.7% VME that Nastro et al. claim referred to the total of isolates (80.9% of those being Cst susceptible). (v) Lo-Ten-Foe et al. (7) did not report specific results, but they tested only 1 Cst-resistant A. baumannii isolate and 7 Cst-resistant K. pneumoniae isolates, and for all 8 isolates, the Cst MICs were very high (>64 μg/ml). VME are predictably limited for such MIC levels, and this was shown also in our original study (1), where gradient diffusion methods misidentified as susceptible few very isolates for which the Cst MIC was ≥32 μg/ml. (vi) Maelje et al. (8) included only 6 Cst-resistant K. pneumoniae isolates. (vii) Lee et al. (9) tested no Cst-resistant A. baumannii isolates. All the aforementioned data indicate that, as with our findings, important disagreements occur between the results of gradient diffusion and dilution methods for isolates resistant to Cst, particularly those for which the MICs are close to the resistance breakpoints.

Second, our collection actually included 35% of isolates for which Cst MIC values were at the resistance breakpoint; it is for such isolates that particular attention is required in clinical practice. The fact that other studies (3, 9), as claimed by Nastro et al., detected fewer isolates for which the MIC was 4 μg/ml cannot imply clonality for our isolates, which were temporally and geographically diverse and derived from four distinct hospitals. Pulsed-field gel electrophoresis (PFGE) analysis that was performed previously detected at least 3 major clonal types for the A. baumannii and 4 types for the K. pneumoniae isolates.

Third, evident heteroresistance was not detected in our study. It should be noted that all isolates in our study were incubated for 18 to 20 h, as is a common practice and was also the case in all studies that Nastro et al cited., which reported incubation times (3, 4, 6, 8). We should note that we compared the currently existing methods by following the available guidelines and aimed to draw conclusions applicable for clinical laboratories. It was not our intention to optimize the methodologies, and for that reason, we did not modify the recommended protocols. We agree that such an investigation would be important for the optimization of gradient diffusion methods.

In conclusion, resistance to Cst among A. baumannii and K. pneumoniae strains is an alarming and unfortunately increasingly observed evolution not only in Greece but also in several European regions (10). Its accurate detection in clinical laboratories is extremely important for the appropriate treatment of the respective infections. Our study was prompted by notifications from hospital laboratories that gradient diffusion methods were producing much lower Cst MICs than automated systems. Those preliminary observations were validated in our study when ST results were compared with those of the reference BMD method and were supported by most studies that tested isolates for which Cst MICs were elevated. Overall, it is clearly implied that gradient diffusion methods have to be used with caution for Cst ST of multidrug-resistant A. baumannii and K. pneumoniae isolates with potentially elevated Cst MICs, although their results may be of some...
clinical value for highly resistant strains. Further studies using these convenient and valuable methods should be conducted to optimize their calibration and improve their performance in accurately estimating Cst activity in daily practice.

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REFERENCES