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Is Colistin Susceptibility Testing Finally on the Right Track?

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Colistin susceptibility testing has changed over the years, and it is still a subject of ongoing debate. Such changes include modifications in both the methodology for testing and breakpoints. Currently, both the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommend the use of broth microdilution (BMD) as the only valid method, even though colistin binds to plastic materials because of its amphipathic nature (1). For some time and in order to overcome this drawback, the CLSI recommendation was to add polysorbate-80 to perform BMD (2), yet this recommendation has changed again. At this time, none of the committees recommend its use, as it appears to act synergistically with colistin (1). Regarding colistin breakpoints, EUCAST lowered them for *Pseudomonas* spp. in 2017, and currently they are the same as those of CLSI (3, 4). EUCAST has breakpoints for *Enterobacteriaceae*, whereas CLSI has only established epidemiological cutoff values for some species (3, 4). Although diffusion methods are not accepted now by any of the two committees, CLSI had disk diffusion breakpoints for *Pseudomonas aeruginosa* until 2016 (3–6). The gold standard method used in comparative studies on colistin susceptibility testing methods in the literature is not always the same (7), which has been causing researchers to sail into troubled waters. Therefore, the reported susceptibility results are contradictory and misleading. In this context, the report by Karvanen et al. (8) has enlightened us on the issue of colistin susceptibility testing, and perhaps it will finally lead to a reliable and reproducible method.

This report leads us to question the accepted recommendation by the joint CLSI/EUCAST polymyxin breakpoint working group about the BMD reference method using polystyrene trays not treated before use (1). Karvanen et al. showed that colistin was considerably adsorbed on both plain polystyrene and polypropylene in a concentration-dependent manner. As a result of the loss of colistin, they found nonlinearity in the MIC tests determined by BMD (8). Given these findings, we wonder about the results of the previous comparative studies on colistin susceptibility testing methods, in which BMD was considered the reference method. Accordingly, this may cast doubts on the recent EUCAST warning specifically advising against the use of diffusion gradient techniques (6). If colistin binds to the plastic of the BMD trays, a higher colistin MIC by the reference method would be expected. This fact would also explain the previous reported paradoxical effect of high false-susceptibility rates (very major errors) using the gradient diffusion technique even though colistin is a big molecule that does not diffuse well into the agar (7). In agreement with the results of Karvanen et al. (8), we consider that some reported data need to be reevaluated. More studies taking into consideration the use of low-protein-binding materials should be performed, and some other important issues that may contribute to the variability of the MIC results, such as

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heteroresistance, should be addressed. This comprehensive report reasserts the need to continue with the revision of the issue of colistin susceptibility testing.

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