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AUTHOR REPLY

Reply to Prim et al., "Is Colistin Susceptibility Testing Finally on the Right Track?"

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We thank Prim et al. (1) for their comment on our recent paper (2). Prim et al. raise the interesting question of method comparisons and the discrepancies shown in many instances of previous work.

As a general note, colistin diffuses poorly in agar, raising reasonable concerns with regard to disk diffusion and other methods that rely on diffusion of the drug through the agarose matrix. Worth noting is that gradient strip methods, such as Etest, do not rely on diffusion of the drug according to the manufacturer (AB Biodisk, personal communication). Rather, a drug gradient is merely transferred to the surface of the agar, where it is available for uptake by the bacteria and possible subsequent diffusion. This is foundational for understanding how Etests work.

With regard to the discrepancies between the "gold standard" (GS) and other methods, all we can currently do is to enter the realms of hypothesis and conjecture. For the sake of argument, let us assume (i) that the concentration data presented in our paper (2) are, in colloquial terms, true, (ii) that the GS method exposes the bacteria to colistin according to the concentrations presented for large polystyrene tubes at 24 h (2), and (iii) that a developer of a new MIC method would devise an "ideal" test in which the bacteria are in fact exposed to the nominal concentrations of colistin. Under these premises, a reference strain that would give an MIC of 1 mg/liter with the GS would give an MIC of 0.5 mg/liter with the ideal test (Table 1). As such a discrepancy from the results of the GS is observed, the developer might justifiably think that the new method has a built-in skewing factor and make a linear adjustment to the measuring scale of the method (i.e., shift the scale one dilution step) so that the MICs for the reference strains are accurate against those determined by the GS. However, a linear adjustment of the scale will not be sufficient, as the adsorption of colistin is nonlinear (Table 1). Thus, one expects that the ideal assay would yield an MIC that is 3 dilutions lower than those of the GS at 0.125 mg/liter (two steps in the scale-adjusted assay). In an ideal assay based on materials that eliminate the adsorption of colistin, MICs over 8 mg/liter are not expected to be affected. However, with a gradient strip test that is based on a

TABLE 1 Expected MICs resulting from the gold standard assay, a corresponding ideal assay, and a scale-adjusted ideal assay

MIC (μg/liter) determined by the:		
Gold standard	**Ideal assay**	**Scale-adjusted ideal assay**
8	8	
4	2	8
2	1	2
1	0.5	1
0.5	0.12	0.5
0.25	0.03	0.12
0.125	0.015	0.03

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transferred gradient relying on a reference strain with an MIC of around 1 mg/liter (according to the GS), there may be discrepancies even at higher concentrations.

In conclusion, the ideal assay would yield lower MICs than the GS, with increasing errors as the MICs decrease. A liquid broth-based ideal assay would not be much different from the GS at high MICs, whereas a transferred gradient test could justifiably be expected to yield increasing errors as MICs increase. Interestingly, Etests performed according to manufacturer recommendations (on BBL Mueller-Hinton agar) appear to yield MIC values that are lower than those of the GS, although the large variability and very low MICs yielded by resistant strains appear to render the test useless (3).

REFERENCES

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