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COMMENTARY

Susceptibility Testing for the Polymyxins: Two Steps Back, Three Steps Forward?

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ABSTRACT Optimizing and standardizing susceptibility testing for the polymyxins have become pressing issues, given the rise in multidrug-resistant Gram-negative bacilli. Recently, both the CLSI and EUCAST have recommended broth microdilution (BMD) (without polysorbate) as the reference method for polymyxin susceptibility testing. In this issue, K. L. Chew et al. (J Clin Microbiol 55:2609–2616, 2017, https://doi.org/10.1128/JCM.00268-17) compare the performances of three commercial BMD panels and the Etest to the reference, BMD, for polymyxin B and colistin, using 76 *Enterobacteriaceae* isolates (21 of which were *mcr-1* positive). Although none of the commercial BMD panels strictly met FDA performance standards in this evaluation, possibly because of the small number isolates tested, the Sensititre panel achieved >90% categorical agreement for both polymyxin compounds. These results also reaffirm CLSI and EUCAST guidance that gradient diffusion testing, which had unacceptable error rates, should be abandoned. In a simulated analysis with lowered breakpoints (susceptible, ≤1 mg/liter; intermediate, 2 mg/liter; resistant, ≥4 mg/liter), error rates and agreement were improved across the various methods and the rate of detection of *mcr-1*-positive isolates improved. These observations, taken together with recent pharmacokinetic data on optimizing target attainment for the polymyxins, suggest that more-stringent (lower) breakpoints may be reasonable, although such an approach may be limited by the inherent reliability of current testing methodologies and a lack of robust clinical correlative data, which are sorely needed.

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Susceptibility testing for the polymyxins has been beleaguered over the years. The polymyxins were first isolated from *Paenibacillus polymyxa* in 1947 by two independent American groups (1, 2), and there has been a therapeutic renaissance in the use of both polymyxin B and colistin (polymyxin E) over the last decade, given the rise of multidrug-resistant (MDR) Gram-negative bacilli, such as MDR *Acinetobacter* and *Pseudomonas* and carbapenemase-producing *Enterobacteriaceae*.

Despite susceptibility testing being available for a long time, clarity has been lacking for appropriate methods of testing and optimal dosing for the polymyxins. Polymyxins are cationic polypeptides comprised of a heptapeptide ring, an exocyclic chain, and a fatty acid tail with positively charged residues which interact with and disrupt the Gram-negative lipopolysaccharide membrane; polymyxin B and colistin differ by only one amino acid in the heptapeptide ring (3, 4). Several reasons have accounted for difficulties with susceptibility testing for the polymyxins, including their cationic nature, their poor diffusion in agar due to their large molecular size, concerns over drug powder composition, and their heteroresistance. Further, the complex pharmacokinetics (PK) and pharmacodynamics (PD) of these compounds and the paucity of data correlating MIC data, drug concentration, and clinical outcomes have made setting clinical breakpoints challenging. The mechanisms underlying polymyxin resistance are

complex, and correlation between these has been comprehensively reviewed recently (4). Complicating this has been the discovery of plasmid-mediated resistance due to *mcr-1* and *-2* (5, 6).

Polymyxin susceptibility testing guidance by various professional bodies has varied over the years (e.g., there have been different breakpoints and disk antimicrobial contents for diffusion testing) (7), although efforts have recently been made to harmonize these. In 2016, the joint CLSI-EUCAST polymyxin breakpoint working group agreed that the ISO-20776 standard broth microdilution method (BMD) (which is the same method outlined in CLSI document M07-A010 [8]) should be used for colistin MIC determination and be performed with sulfate salts of colistin in plain polystyrene trays without additives like polysorbate-80 (P-80) (9) and that diffusion methods should be abandoned. P-80 had at one point been recommended by the CLSI as a supplement for colistin BMD quality control testing (10, 11) to mitigate the cationic properties of the polymyxins, which cause them to adhere to the negatively charged polystyrene surface. However, P-80 in itself has some antibacterial activity and may act synergistically with polymyxins to spuriously lower MICs, especially for organisms for which MICs are in the low range, i.e., near the breakpoints and/or epidemiologic cutoff values of 1 to 2 μg/ml (13, 14). This led to the removal of P-80 from BMD for the polymyxins in CLSI document M100-S27 (15, 16). Manual BMD, however, is laborious and not performed in many routine clinical microbiology laboratories, which often rely on diffusion or automated systems for susceptibility testing. Moreover, and somewhat paradoxically in view of the drugs' poor diffusion in agar, diffusion methods (both disk and gradient strips) have been found to have unacceptably high levels of false susceptibility (or very major errors [VMEs]) in multiple studies (12, 17–28) (Table 1). Both the CLSI and EUCAST have now advised against diffusion methods for the polymyxins, removing disk diffusion interpretive criteria for them from their guidance documents (9, 15, 22).

Parsing the literature for the comparative performances of the various susceptibility testing methods for the polymyxins may be somewhat confusing, with studies seemingly giving contradictory results; Table 1 attempts to summarize data published from 2001 to date. Several important reasons accounting for these disparities should be borne in mind when evaluating studies. These include different susceptibility breakpoints used and various proportions of isolates studied for which the MICs are near the breakpoint. With regard to the number of isolates near susceptibility breakpoints, studies often lack a sizeable number of resistant isolates (especially resistant isolates for which the polymyxin MICs are low [4 to 8 mg/liter]), which may obscure an accurate estimation of VME rates. These MICs are also of clinical relevance given what we currently know about the pharmacokinetics of polymyxin B and colistin, dose optimization, and target attainment (29, 30). Indeed, VMEs or major errors (MEs) may be underreported if the MICs for the isolates studied fall into extremes, with either very high MICs (high-level resistance) or very low MICs (very susceptible isolates). VME rates should also be calculated with the number of resistant isolates as the denominator and ME rates with susceptible isolates as the denominator, rather than expressed as a percentage of the total number of isolates (12). Unfortunately, the literature has not been consistent, and VMEs and MEs have often been expressed as percentages of the number of isolates tested (resistant and susceptible) (Table 1, footnote *c*). Studies may also vary depending on the relative proportions of *Enterobacteriaceae* and/or nonfermenting Gram-negative bacilli, and heteroresistance has been described in some species, such as *Enterobacter*, *Pseudomonas aeruginosa*, and *Acinetobacter* spp. The most important reason for the variation of reported results in the literature for polymyxin antimicrobial susceptibility testing (AST), however, has been the lack of consensus in what constitutes a "gold standard" comparator. Until further data are available, per CLSI-EUCAST guidance, BMD with no supplementation should be the standard comparator.

In this issue, Chew et al. (31) pragmatically evaluate four commercial polymyxin B and colistin AST methods commonly used and/or more easily implemented in routine clinical microbiology laboratories than BMD performed by the reference ISO-20776

TABLE 1 Summary of published comparative susceptibility studies for polymyxin B and colistin from 2001 to 2017[a]

First author/yr (reference)	Antimicrobial(s) studied	Organisms studied	Methods compared (manufacturer, potency)	Reference standard	Breakpoint(s) used, resistance (mg/liter)	No. (%) of isolates resistant to polymyxin B or colistin	No. of isolates near the following resistance breakpoint (mg/liter)[b]: 1	2	4	8	VME rate (%)	Notes
Gales/2001 (17)	Polymyxin B	200 Gram-negative bacilli	BMD (PML Microbiologics), DD (BD/Difco, 300 U polymyxin)	BMD	≥4	30 (15)	NR	31	1	7	DD (with S indicated by a ≥8-mm diam) 12/30 (40)[c]	Disk diffusion is unreliable. Only 14 resistant isolates were studied for AD (1 isolate each was near the resistance breakpoints of 4 and 8 mg/liter).
	Colistin		BMD (PML Microbiologics), DD (BD, 10 μg colistin), AD (performed only on a subset of 35 isolates)		≥4	30 (15)	NR	8	2	2	AD (0); DD (with S indicated by a >8-mm diam) 12/30 (40)[c]; DD (wth S indicated by a ≥14-mm diam) 7/30 (23.3)[c]	
Hogardt/2004 (38)	Polymyxin B and colistin	401 *P. aeruginosa*, 50 *Achromobacter xylosoxidans*, and 50 *Stenotrophomonas maltophilia* isolates from CF patients and 100 *P. aeruginosa* isolates from non-CF patients	AD for polymyxin B, BMD (Merlin Diagnostika) for colistin	Unclear, AD (polymyxin B) was compared against BMD (colistin)	>4 (DIN), >8 (BSAC), >2 (SFM)	29 (5.2) of 401 *P. aeruginosa* isolates by BMD (colistin)	111	46	33	12	NR	Different compounds were tested against each other using different methods.
Nicodemo/2004 (18)	Polymyxin B	70 *S. maltophilia* isolates (66 isolates were tested for colistin and polymyxin)[c]	AD, DD (Oxoid, 300 IU polymyxin B), Etest	AD	≥4	15 (22.7)	NR	NR	NR	NR	DD 12/15 (80)[c]; Etest 8/15 (54.3)[c]	Disk diffusion and Etest results had unacceptable VMEs.
	Colistin		AD, DD (Oxoid, 10 μg colistin), Etest		≥4	16 (24.3)	NR	NR	NR	NR	DD 15/16 (93.7)[c]; Etest 6/16 (37.5)[c]	
Arroyo/2005 (39)	Colistin	115 *A. baumannii* isolates	Etest, BMD	BMD	≥4	22 (19.1)	3	0	0	2	2/22 (9.1)	The EA was 16.5%. The authors state that the worst agreement for strains was at 0.06 to 0.25 mg/liter and at 64 to >1,024 mg/liter by the reference method, but few isolates studied were near resistance breakpoints.
Tan/2006 (19)	Colistin	228 *Acinetobacter* species, *Pseudomonas aeruginosa*, and *Enterobacteriaceae* isolates	AD, DD (BD, 10 μg [CLSI]; Oxoid, 25 μg [BSAC]; Oxoid, 50 μg [SFM])	AD	≥4 (CLSI), >4 (BSAC), >2 (SFM)	27 (11.8)	92	107	13	4	CLSI DD 22/27 (81.5)[c]; BSAC DD 11/14 (78.6)[c]; SFM DD 24/27 (88.9)[c]	Disk diffusion is unreliable.
Goldstein/2007 (40)	Colistin	170 clinical Gram-negative bacilli and 22 *in vitro*-selected mutants	AD, Etest	AD	>4	31 (18) (clinical isolates)	31	9	43	13	7/12 (58.3) for *P. aeruginosa*	Etest MICs were 2-fold and 4- to 8-fold lower than AD MICs for 37.3% and 6.5% of isolates, respectively.
Lo-Ten-Foe/2007 (41)	Colistin and polymyxin B (DD)	102 Gram-negative bacilli (70 *Enterobacteriaceae* and 32 nonfermenters)	AD with MH, AD with Iso-Sensitest agar, BMD, DD (Rosco, 150 μg polymyxin B, 10 μg colistin), Vitek 2 with AST card N038, Etest with MH, Etest with Iso-Sensitest agar	BMD	≥4	42 (41.2)	3	6	0	9	NR	There was a high error rate and low reproducibility with DD. Authors report high levels of agreement with AD and Vitek 2 and relatively high levels of agreement with Etest, especially if Iso-Sensitest agar was used.

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TABLE 1 (Continued)

First author/yr (reference)	Antimicrobial(s) studied	Organisms studied	Methods compared (manufacturer, potency)	Reference standard	Breakpoint(s) used, resistance (mg/liter)	No. (%) of isolates resistant to polymyxin B or colistin	No. of isolates near the following resistance breakpoint (mg/liter)[b]: 1	2	4	8	VME rate (%)	Notes
Tan/2007 (42)	Colistin	172 Gram-negative bacilli	AD, Etest, Vitek 2 with AST card N032	AD	≥4	54 (31)	NR	NR	NR	NR	Etest 8/54 (14.8)[c]; Vitek 2 31/54 (57.4)[c]	Both Etest and Vitek 2 had unacceptable VMEs.
Tan/2007 (43)	Colistin	44 multidrug-resistant *Acinetobacter* spp.	AD, Vitek 2 with AST card N032	AD	>2	0 (0)	33	11	0	0		There was 100% CA, as no colistin-resistant *Acinetobacter* isolates were studied.
van der Heijden/2007 (44)	Polymyxin B	109 carbapenem-resistant *Pseudomonas aeruginosa* isolates	BMD, DD (Oxoid, 300 U), Etest	BMD	≥8	1 (0.9)	27	4	0	1	Etest 1/1 (100)[c]; DD 1/1 (100)[c]	Only 1 polymyxin B-resistant isolate was tested.
	Colistin		BMD, DD (Oxoid, 10 μg), Etest	BMD	≥8	0 (0)	38	8	0	0	Etest (NA)	No colistin-resistant isolate was tested.
Galani/2008 (45)	Colistin	778 Gram-negative bacilli (392 *Enterobacteriaceae* and 386 nonfermenters)	DD, Etest	Etest	≥8 (*P. aeruginosa*),	7	57	31	1	1	DD (R ≤ 10-mm diam, S ≥ 11-mm diam) 0 (0)	The author-proposed diam interpretations for *Enterobacteriaceae* (R ≤ 11-mm diam, S ≥ 14-mm diam) had no VME or ME, but 15.6% fell into the intermediate category. The gold standard comparator (Etest) was imperfect.
					≥4 (*Acinetobacter* spp., *S. maltophilia*, and *Enterobacteriaceae*)	104 (15.9)	29	22	8	22	DD (R ≤ 12-mm diam, S ≥ 13-mm diam) with *Acinetobacter* 1/9 (11.1)	
Behera/2010 (23)	Polymyxin B and colistin (only for DD)	281 multidrug-resistant Gram-negative bacilli and 723 multidrug-resistant Gram-negative bacilli (for DD only)	AD, DD (BD, 300 U polymyxin B, 10 μg colistin), Etest, BMD	BMD (polymyxin B only)	>4 (polymyxin B)	24 (8.5)[d]	NR	NR	NR	8	AD 3/24 (12.5)[c]; Etest 2/24 (8.3)[c]; DD with colistin 2/24 (8.3)[c]; DD with polymyxin 3/24 (12.5)[c]	Many of the resistant isolates studied were intrinsically resistant to the polymyxins (e.g., *Proteus, Morganella*, *Providencia*, and *Burkholderia cepacia*).
Nemec/2010 (46)	Colistin	154 *Acinetobacter* spp.	AD, Etest	AD	>2	18 (11.7)	18	3	4	1	NR	The Etest was 88% and 100% in agreement within 2 and 3 2-fold dilutions. There was a small no. of isolates near breakpoints.
Boyen/2010 (24)	Colistin	157 porcine *Escherichia coli* strains	AD, DD (Rosco Neo-Sensitabs, 150 μg), disk prediffusion test (Rosco Neo-Sensitabs, 10 μg), Etest	AD	≥8	14 (8.9)	20	1	1	11	DD 3/14 (21.4)[c]; Etest (0); disk prediffusion test 2/14 (14.2)[c]	Disk diffusion is unreliable. A small number of isolates were near the breakpoint. A high breakpoint was used.
Moskowitz/2010 (25)	Colistin	37 nonfermenting Gram-negative bacilli from CF patients (25 *P. aeruginosa* and 12 *S. maltophilia* isolates)	AD, BMD (MicroTech), Etest, DD (BD)	AD	≥8[e]	9 (36) (*P. aeruginosa*)	6	4	1	0	BMD 4/9 (44)[c]; Etest 5/9 (56)[c]; DD 6/9 (67)[c]	AD, instead of BMD, was used as the gold standard. Diffusion testing is unreliable.
						11 (92) (*S. maltophilia*)	0	0	1	0	BMD 1/11 (9)[c]; Etest 2/11 (18)[c]; DD 1/11 (9)[c]	
Maalej/2011 (47)	Colistin	150 clinical *Enterobacteriaceae* isolates and 50 known colistin-resistant *Enterobacteriaceae*	AD, DD (50 μg, SFM, and 10 μg, CLSI), Etest	AD	>2	7 (4.6) (clinical isolates), 57 (28.5) (all isolates)	8	2	0	6	DD (SFM) 7/57 (12.3)[c]; DD (CLSI) 5/57 (8.8)[c]; DD (product insert) 1/57 (1.7)[c] but 92.9% were "intermediate"; Etest (0)	Disk diffusion is unreliable. MICs by Etest were lower than by agar dilution (CA, 33%).

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TABLE 1 (Continued)

First author/yr (reference)	Antimicrobial(s) studied	Organisms studied	Methods compared (manufacturer, potency)	Reference standard	Breakpoint(s) used, resistance (mg/liter)	No. (%) of isolates resistant to polymyxin B or colistin	No. of isolates near the following resistance breakpoint (mg/liter)[b]:				VME rate (%)	Notes
							1	2	4	8		
Lat/2011 (48)	Polymyxin B	48 KPC-producing *Klebsiella pneumoniae* isolates	BMD (Trek Diagnostics), Etest	BMD (Trek Diagnostics)	≥4	7 (15)	NR	NR	NR	NR	Etest 1/7 (14.2)	The BMD MIC_{50} was several 2-fold dilutions lower than the Etest MIC_{50} (0.25 mg/liter vs 2 mg/liter).
Giani/2012 (49)	Colistin	*P. aeruginosa*	BMD, BD Phoenix NMIC/ID-76	BMD	>4	(0)	NR	NR	NR	NR	NA	The CA was 100%, but no resistant isolates were tested.
Hindler/2013 (27)	Colistin	For phase I, 107 multidrug-resistant Gram-negative bacilli (60 *P. aeruginosa*, 20 *K. pneumoniae*, and 27 *A. baumannii* isolates)	For phase I, BMD, BMD–P-80, and Etest (BBL, Hardy Remel plates)	BMD–P-80	≥4	For phase I, 19 (17.8)[f]	NR	NR	NR	NR	For phase I, Etest (BBL plate) 6/19 (31.5); Etest (BBL, Remel, Hardy, on a subset of 50 isolates, of which 15 were resistant) (47–53); BMD (0)	The VME rates with Etest were high (they can vary with media). BMD–P-80, BMD-Trek, and AD results in this study correlated well, although a small no. of colistin-resistant isolates were studied.
		For phase II, 50 multidrug-resistant Gram-negative bacilli (11 *A. baumannii*, 15 *K. pneumoniae*, and 24 *P. aeruginosa* isolates)	For phase II, BMD, BMD-Trek (Trek Diagnostics), BMD–P-80, BMD, AD			For phase II, 10 (20)[f]	NR	NR	NR	NR	For phase II, BMD 1/10 (10); AD and BMD-Trek (0)	
Landman/2013 (50)	Polymyxin B	114 *Enterobacter cloacae* and *E. aerogenes* isolates	BMD (done in duplicate), AD (done in duplicate), Etest (of 57 select isolates on MH and Iso-Sensitest agar)	None	>2	For *E. cloacae*, 36 (32) (resistant at least once); 11 (10) (resistant in all four tests); for *E. aerogenes*, 4 (7) (resistant at least once); 1 (2) (resistant in all four tests)	NR	NR	NR	NR	Not determined	Skip wells are a problem for interpretation for this genus, and the problem tends to emerge at concentrations around 4 μg/ml. The categorical agreement within BMD and AD (done in duplicate) was 77% only.
Lee/2013 (51)	Colistin	213 *A. baumannii* isolates	AD, Vitek 2, Etest, MicroScan	AD	≥4	13 (6.1)	109	46	1	2	Vitek 2 and MicroScan 2/13 (15.4)[c]; Etest (0)	The categorical agreement between AD, Vitek 2, and Etest was ≥99% but 87.3% for MicroScan.
Albur/2014 (13)	Colistin	146 Gram-negative bacilli (56 *P. aeruginosa*, 29 *Acinetobacter* species, 61 *Enterobacteriaceae* isolates)	BMD (2 different microtiter trays: V-bottom and tissue culture-coated round-bottom trays) with or without P-80	None	None	8 (5.5)[g]	50	11	8	0	Not assessed	V-bottom microtiter trays with P-80 showed a more dramatic and significant reduction (25.6-fold) in colistin MICs than tissue culture-coated round-bottom trays with P-80.
Piewngam/2014 (28)	Colistin	290 *A. baumannii* isolates	DD (10 μg colistin), Etest, Vitek 2, BMD	BMD	≥4	27 (9.3)	57	206	14	0	Etest 15/27 (55.5); DD (S was indicated by a ≥12-mm diam; R was indicated by a ≤9-mm diam) (no VME); Vitek 2 16/27 (59.2)	Etest and Vitek 2 results had unacceptably high VMEs. Modified MIC interpretive criteria for DD and Etest may reduce these errors.

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TABLE 1 (Continued)

First author/yr (reference)	Antimicrobial(s) studied	Organisms studied	Methods compared (manufacturer, potency)	Reference standard	Breakpoint(s) used, resistance (mg/liter)	No. (%) of isolates resistant to polymyxin B or colistin	No. of isolates near the following resistance breakpoint (mg/liter)[b]: 1	2	4	8	VME rate (%)	Notes
Dafopoulou/2015 (21)	Colistin	41 non-carbapenem-susceptible *K. pneumoniae* isolates	BMD, BMD–P-80, AD, Etest, MIC test strip (MTS; Liofilchem), Vitek 2	BMD	>2	58 (95.1)	0	1	12	16	Etest 24/58 (41.3)[c]; MTS 19/58 (32.8)[c]; BMD–P-80 11/58 (19)[c]; AD 2/58 (3.3); Vitek 2 (0)	Diffusion methods are inaccurate. Authors recommend Vitek 2 or dilution methods for colistin susceptibility.
		20 non-carbapenem-susceptible *A. baumannii* isolates					0	2	8	6		
Perez/2015 (52)	Colistin and polymyxin B	101 KPC-2-producing *K. pneumoniae* isolates	MicroScan WalkAway (NC66, colistin), Etest (polymyxin B), BMD (only for discrepant results, using polymyxin B)	Etest	>2	For Etest, 17 (16.8)	12	13[h]	0	2[h]	Unable to determine	An imperfect comparator standard and different drugs were used. There were 10 discrepant results (10 major errors, 2 VMEs) between Etest (polymyxin B) and MicroScan (colistin).
Rojas/2017 (53)	Colistin and polymyxin B	246 carbapenem-resistant *K. pneumoniae* isolates	"Reference laboratory" broth macrodilution, "clinical laboratory susceptibility" (based on a combination of MicroScan, Vitek 2, BMD Sensititre [GN4F], and Etest)	Broth macrodilution	>2	For colistin, 31 (13) by broth macrodilution (compared to only 21 or 9% by routine lab testing). For 31 colistin-resistant isolates, 25 (10) were polymyxin resistant, 6 were indeterminate (skipped wells and trailing endpoints)	9	3	3	6	"Clinical laboratory susceptibility" 11/31 (35)	The "clinical laboratory susceptibility" major error rate was 1/215 (0.1%). BMD resulted in different interpretations in 12 of 246 (5%) isolates. Patients with colistin-resistant isolates had an increased risk of 30-day mortality (adjusted OR, 3.48), although specific treatments were not associated with mortality.
Chew/2017 (31)	Colistin and polymyxin B	76 *Enterobacteriaceae* (21 *mcr-1*-positive) isolates	BMD, BMD-Trek (Trek Sensititre, GNX3F), Vitek 2 (N315, N275), MicroScan (NM44, colistin only), Etest	BMD	>2	For colistin, 25 (32.9)	1	4	11	5	BMD-Trek 1/25 (4); Vitek 2 9/25 (36); MicroScan 1/25 (4); Etest 3/25 (12)	BMD-Trek with a susceptibility breakpoint of ≤2 mg/liter detected 100% and 95.2% of *mcr*-1-positive isolates, compared to 71.4% and 81% for the reference BMD.
					>2	For polymyxin B, 27 (35.6)	2	1	10	6	BMD-Trek 1/27 (3.7); Vitek 2 1/27 (3.7); Etest 6/27 (26.1)	All commercial methods at current breakpoints had unacceptable VME rates, but for some methods, this was likely a function of the small no. of resistant isolates studied.

[a]BMD, broth microdilution; AD, agar dilution; DD, disk diffusion; MH, Muller-Hinton agar; BMD–P-80, BMD with P-80; CF, cystic fibrosis; NR, not reported; NA, not applicable; OR, odds ratio; SFM, Société Française de Microbiologie; DIN, Deutsches Institut für Normung; BSAC, British Society for Antimicrobial Chemotherapy; S, sensitive; R, resistant; VME, very major error; EA, essential agreement.

[b]As determined by the gold standard comparator used in the study.

[c]VMEs were presented with the denominator as the total number of isolates tested but were recalculated from figures presented in the paper in this table and presented as a percentage, with the denominator as the number of total resistant isolates tested.

[d]Comprising *Burkholderia cepacia* ($n = 7$), *S. maltophilia* ($n = 3$), *P. aeruginosa* ($n = 3$), *A. baumannii* ($n = 2$), *K. pneumoniae* ($n = 3$), *P. mirabilis* ($n = 2$), *M. morganii* ($n = 1$), *Providencia* ($n = 1$), *Enterobacter* ($n = 1$).

[e]Intermediate, 4 mg/liter.

[f]By BMD–P-80.

[g]The MIC was >4 mg/liter with V-bottom trays without P-80.

[h]MICs were determined by Etest. For 13 isolates, the MIC was 1.5 mg/liter, and for 2 isolates, the MIC was 6 mg/liter (rounded up in the table to the next higher dilution).

standard (8, 9). These comprise a commercial BMD system (Sensititre), gradient diffusion (Etest), and two automated AST systems: Vitek 2 and MicroScan (colistin only). A total of 76 *Enterobacteriaceae* were studied, including 21 isolates harboring *mcr-1*. Using the EUCAST colistin resistance breakpoint of >2 mg/liter for *Enterobacteriaceae* and applying the same to polymyxin B, the authors found high levels of essential and categorical agreement (EA and CA, respectively) for polymyxin B with Sensititre and Vitek 2 (both >90%); for colistin, EA was 89.5% for Sensititre and 93.4% for Vitek 2. Due to limited dilutions, EA was not assessed for MicroScan. CA for colistin was 88.2% for MicroScan and Vitek 2 and 90.1% for Sensititre. VME rates for all commercial systems assessed, however, were in excess of the 1.5% recommended by the FDA (12), although in this comparison, Sensititre and MicroScan achieved the lowest VME rate for colistin (VME rate, 4%), and Sensititre and Vitek 2 achieved the lowest VME rate for polymyxin B (VME rate, 3.7%) (only 1 VME for both compounds with these systems). Using FDA requirements, the Etest did not meet acceptance criteria for EA, CA, VMEs, and MEs for polymyxin B and met CA criteria (92.1%) for colistin only. As the authors point out, the VME rates of >1.5% may be in part due to the relatively small (although comparable to those of previous studies) number of resistant isolates in their study. Larger studies with more resistant isolates may allow a more comprehensive assessment.

Overall, the study by Chew et al. (31) found Sensititre to be generally reliable for polymyxin B and colistin, although it tended to overcall resistance, and it found Vitek 2 to have an unacceptable false-susceptibility (VME) rate with colistin, although interestingly, it performed similarly to Sensititre with polymyxin B. MicroScan with colistin (despite having limited dilutions) performed similarly to Sensititre. The findings with the Sensititre panel are in keeping with previously published data (27) and a recent evaluation by EUCAST, which also examined other commercial BMD methods (e.g., Micronaut-S and Micronaut MIC Strip, SensiTest [Liofilchem], and UMIC [Biocentric]) (32, 33). It should be noted that not all commercial BMD systems perform alike and that few data are available for some systems, like the BD Phoenix; unpublished data from one evaluation with this system found an unacceptable VME rate of 15% (4).

The strengths of Chew et al.'s study include the utilization of the ISO-20776 reference BMD as the gold standard and the multiple commercial AST methods studied, which will be of interest to clinical laboratories seeking to evaluate a method for AST of the polymyxins. This is also the largest multimethod comparison, to date, on *mcr-1*-positive *Enterobacteriaceae*. Interestingly, the authors note that, if a colistin susceptibility breakpoint of ≤2 mg/liter is used, the Sensititre and MicroScan systems would detect 100% of the *mcr-1*-positive isolates included in their study (compared to only 71.4% by the reference method, BMD) and that the Sensititre and Vitek systems would detect 95.2% (compared to 81% by BMD) using the same breakpoint with polymyxin B. By reference BMD, six and four *mcr-1*-positive isolates, respectively, had colistin and polymyxin B MICs of ≤2 mg/liter. In an analysis with lower simulated breakpoints for polymyxin B and colistin (susceptible [S], ≤1 mg/liter; intermediate [I], 2 mg/liter; resistant [R], ≥4 mg/liter), the authors found reductions in MEs for all methods and VMEs for Vitek 2 (colistin) and Etest (colistin and polymyxin B), although again, FDA acceptance criteria were not fully met.

Currently, the CLSI and EUCAST share harmonized colistin breakpoints for *Pseudomonas aeruginosa* and *Acinetobacter* spp. (susceptible, ≤2 mg/liter). For the *Enterobacteriaceae*, EUCAST adopts the same colistin breakpoints, but the CLSI has yet to set these, although it adopts 2 mg/liter as the epidemiologic cutoff value (ECOFF) for *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Enterobacter cloacae*, and *Raoultella ornithinolytica*. Clinical breakpoints are meaningful if they delineate an MIC for which there is a high probability of clinical response to properly dosed antibiotics. However, robust data correlating MICs, drug concentrations, and clinical outcomes of the polymyxins are very limited, and a clear correlation has not always been demonstrated (34). Retrospective correlative studies are also difficult to perform because polymyxin resistance may be lost upon subculture and storage (4). Less than 40% of patients with normal or augmented renal function, however, are able to achieve a

steady-state plasma colistin concentration of 2 mg/liter, which has been proposed as a surrogate target for the optimization of the area under the curve of free or unbound drug at the MIC for the bacterial pathogen (*f*AUC/MIC), the PK/PD parameter that predicts treatment success (29). PK data lend support to lowering the current colistin breakpoints. The simulated lowered breakpoint analyses (with the introduction of an "intermediate" category) by Chew et al. was also found to improve CA and diminished VME and ME rates. Lowering breakpoints would bring about some technical challenges, however, as these would fall within the wild-type MIC distribution of isolates ($\leq$2 mg/liter), and there remain concerns over the reliability of current testing systems to parse out S and R adequately. Perhaps, as the authors suggest, adding an intermediate category would help reflect these uncertainties in testing and also address the concerns over current dosing strategies and target attainment. More importantly, actual clinical correlative data are sorely needed.

With polymyxin B, there are even fewer comparative data for AST and PK, although the CLSI has breakpoints for *Acinetobacter* and *P. aeruginosa*. This lack of data is somewhat historic, as traditionally, colistin has been more widely used in North America and Europe, with correspondingly more studies performed, despite the fact it is administered as a prodrug, colistimethate, of which only about up to a quarter is converted to active colistin *in vivo* (4). In contrast, polymyxin B is administered in its active form, and target concentrations are more easily achieved, with less interpatient variability, and its PK is independent of renal function (30), making it a more attractive option for clinical use than colistin. Although polymyxin B MICs generally trend within $\pm$1 dilution with colistin (10, 31, 35), they are different drugs, and AST should ideally be performed individually. Besides considering the format of the assay adopted, individual laboratories should carefully weigh, with clinician input, the preferred polymyxin at their institutions when introducing susceptibility testing for this class of antibiotics.

The study by Chew et al. included 21 *mcr-1*-positive isolates, for a number of which strains MICs were $\leq$2 mg/liter. Laboratories performing surveillance should note that surveillance criteria by current breakpoints may not always identify all isolates with *mcr-1*. The implications of polymyxin therapy for infections caused by the isolates for which MICs are low are uncertain, as there are currently no clinical outcome data. While the promoter sequences upstream of *mcr-1* in the current study were examined and found to be intact, the actual level of *mcr-1* expression was not determined in their study, and it is plausible that phenotypic resistance may become apparent only after polymyxin exposure. A recent study found that for transformants with *mcr-1*, the colistin MICs were usually 16-fold or higher (except for *Pseudomonas aeruginosa*, for which MICs were only modestly increased) (36). The possibility that *mcr-1* isolates for which polymyxin MICs are low may remain undetected and silently spread is somewhat disconcerting given that *mcr-1* and *-2* are plasmid borne and may be shared by horizontal gene transfer. A lower breakpoint was found to increase the rate of detection of *mcr-1*-positive isolates in the authors' study across all methods, although the utility of such an approach would need to be examined with further *in vitro* and clinical data, which will hopefully also help shed light on the extent and clinical significance of this phenomenon.

The study performed by Chew et al. (31) indicates that commercial and automated BMD systems, which are in reach of most routine microbiology laboratories, may provide results fairly comparable to those of the reference, BMD. Future assessments will be strengthened by including larger numbers of isolates, especially those with polymyxin resistance and MICs straddling existing or putative breakpoints. It is also anticipated that impending regulatory changes will soon lead to FDA-cleared tests for the polymyxins (16). Future studies should focus on correlating microbiologic, PK, and clinical outcome data. For this to happen, quality assurance standards utilized in determining the potencies of clinically administered drugs also need to be reassessed and updated, given that these still rely on derivatives of diffusion-based methods (37). Nevertheless, the adoption of a standard reference methodology and continued harmonization of breakpoints by the CLSI and EUCAST, along with work such as that by

Chew et al. (31) and other groups, are steps in the right direction toward further clarity in susceptibility testing for the polymyxins.

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