

1 **Supplementary data**

2 **Table S1. Home-made polymyxin containing agar**

3 **Table S2. Comparison of different polymyxin B susceptibility testing methods to detect**  
4 **polymyxin B resistance in Gram-negative clinical isolates.**

5 **Table S3. Other comparative AST methods.**

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8 **Table S1. Home-made polymyxin containing agar.** VAN: vancomycin, CTX: cefotaxime; AB: amphotericin B

Used on:	Agar	Colistin ( $\mu\text{g/mL}$ )	Other antimicrobial drugs ( $\mu\text{g/mL}$ ):	Rreference
Isolates	MH	2		[9]
Cecal samples	1-BPW	2		[26]
	2-MacConkey	2		
isolates	MacConkey	2		[27]
Stool samples	1-LB broth	2		[28]
	2-MacConkey agar	$\pm 4$		
	Or CHROMagar <sup>TM</sup> Orientation Medium	4	8 VAN $\pm$ 2 CTX	
Samples	MacConkey	2		[29]
Faecal samples	LB	2	50 VAN	[30]
Faecal samples	LB	4	5 AB	[31]
Stool samples	1-EE			[32]
	2-LB	4	10 mg/L VAN 5 mg/L AB	
Faecal samples	1- EE broth	2		[33]
	2-MacConkey	4		
Isolate	COS	2		[34]

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11 **Table S2. Comparison of different polymyxin B susceptibility testing methods to detect polymyxin B resistance in Gram-negative clinical isolates.**

Bacterial species	Reference method	MIC breakpoint	MIC range % resistant	MIC50	MIC90	Methods	CA ≥90%	EA ≥90%	ME ≤1.5%	VME ≤1.5%	MiE	Reference
76 <i>Enterobacteriaceae</i>	BMD	S≤2 µg/mL	0.06->64	4	8	Vitek2	94.7	96.1	6.1	3.7		[105]
18 <i>E.coli</i> (13 <i>mcr-1</i> )		R≥4 µg/mL	35.5%	0.12	0.5	Sensititre	94.7	96.1	6.1	3.7		
31 <i>K. pneumoniae</i> 2 <i>mcr-1</i>						Etest	89.5	48.7	1.9	26.1		
1 <i>E. aerogenes mcr-1</i>	AD	S≤2 µg/mL	0.25-16	2	4	DD 300 U	72.8	NA	0	70.6	8.6	[86]
70 <i>S. maltophilia</i> 15R		R≥4 µg/mL	22.7%			Etest	77.3	89.4	13.2	47.1	NA	
200 Gram-negative including:	BMD	S≤2 µg/mL		≤1 <sup>a</sup>	2 <sup>a</sup>	DD 10 µg <sup>1</sup>	94	NA	0	37.5	0	[88]
60 <i>A. baumannii</i>		R≥4 µg/mL	16%	≤1 <sup>b</sup>	2 <sup>b</sup>	DD 10 µg <sup>2</sup>	94		0	37.5	0	
80 <i>P. aeruginosa</i>					2 <sup>c</sup>	2 <sup>c</sup>						
c. 12 <i>S.maltophilia</i>												
35 representatives				≤1->128 40%			AD	97.1	91.4	47.6	0	
78 <i>P. aeruginosa</i>	BMD	S≤2 µg/mL	<0.25-8	0.5	1	Etest	90	33		1.	48.7	[95]
		I = 4 µg/mL	0.9%			DD	97.3	NA	11.5			
		R≥8 µg/mL										
281 <i>Enterobacteriaceae</i>	BMD	S≤2 µg/mL				Etest	49.8	40.9	0	12.5		[78]
		R≥4 µg/mL	8.5%			AD	38.1	50.2	2.7	8.3		
						DD 300U	98.9	NA	0	12.5		
48 <i>K. pneumoniae</i>	BMD	S≤2 µg/mL		≤0.25	8	Etest	75	18.7	26.8	14.3	NA	[94]
		R≥4 µg/mL	14.6%									
63 <i>E. coli</i>	BMD	S≤2 µg/mL	0.12-16		2	BMD-P80	97.6	21	0	12.5		[74]
61 <i>K. pneumoniae</i>		R≥4 µg/mL	19.4%		1							
60 <i>Acinetobacter spp.</i>					2							
63 <i>P. aeruginosa</i>					2							
501 <i>P. aeruginosa</i> (401 CF)	AD	S≤4 µg/mL	≤0.5->16 µg/mL	2	2	BMD 24h	96		22.8	1.7		[82]
50 <i>A. xylosoxidans</i>		R≥8 µg/mL	11%		4	8	BMD 48h	93.6		8.4	3.6	
50 <i>S. maltophilia</i>				4	16							
10 <i>P. aeruginosa</i>	BMD	10	≤0.25-4	0.5	1	Etest MH :						[99]
10 <i>A. baumannii</i>				0.5	4	Difco	100	100	0	0	0	
						Merck	95	80	0	1	0	
						Oxoid	80	80	0	0	2	
						HiMedia	90	90	0	0	4	

12 CA: Categorical agreement, EA: Essential agreement, VME: very major error, ME: major error, MiE: minor error, AD: Agar Dilution, BMD: Broth Microdilution, DD: disk  
13 diffusion, MH: Mueller Hinton, ISO: ISO Sensitest agar. Italic values indicate number of error and not the percentage for VME or ME when a too few number of resistant  
14 susceptible strains were tested respectively.

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**Table S3. Other comparative AST methods.**

Bacterial species	Reference method	MIC breakpoint	MIC range % resistant	MIC50	MIC90	Methods	CA ≥90%	EA ≥90%	ME ≤1.5%	VME ≤1.5%	MiE	Reference
101 KP	Etest	S≤2 µg/mL R≥4 µg/mL	0.25-48 16.8%	0.75	16	Microscan	76.3	88.1	11.9	11.8		[93]
146 isolates 56 <i>P. aeruginosa</i> 29 <i>Acinetobacter</i> spp. 61 <i>Enterobacteriaceae</i>	NA					BMD V-bottom +P80 U bottom + P-80						[75]
169 <i>E. coli</i> 157 <i>K. pneumoniae</i> 163 <i>P. aeruginosa</i>	BMD					BMD-P80						[73]
778 Gram-negative 226 <i>A. baumannii</i> 124 <i>P. aeruginosa</i> 250 <i>K. pneumoniae</i> 102 <i>E. coli</i> 60 <i>Enterobacter</i> spp. 36 <i>S. maltophilia</i>	Etest	S≤2 µg/mL I = 4 µg/mL R≥8 µg/mL	≤0.5->16 13.1%	0.5 1 0.5 0.5 0.5 8	0.5 2 16 0.5 16 128	DD 10 µg	87.8	NA	0	0.8	12.1	[102]
200 Gram-negative including	Etest					DD E DD B	75.8 78.8	NA NA	0 0	75 47.1	5.7 8.6	[86]
228 isolates: 61 <i>Acinetobacter</i> spp 43 <i>Enterobacter</i> spp 26 <i>E. coli</i> 42 <i>K. pneumoniae</i> 56 <i>P. aeruginosa</i>	AD	S≤2 µg/mL R≥4 µg/mL <sup>3</sup> S≤4 µg/mL R≥8 µg/mL	0.5->128 µg/mL 11.8%	1 1 2 1 2	2 ≥128 2 2 4	DD MH- 10µg <sup>11</sup> S≤8- R>11mm DD MH- 10µg <sup>22</sup> S≤10- R>14mm DD ISO- 25µg <sup>33</sup> R≤14mm <i>P. aeruginosa</i> R≤13mm DD MH-50 µg <sup>44</sup> R≤14mm		ND		81.5 44.4 <sup>#</sup> 78.6 88.9	70.6	[80]
246 isolates 0 mcr 1 2	MacroD Colistin	S≤2 µg/mL R≥4 µg/mL	≤0.5-8 µg/mL 11.8%			Etest Polymyxin B	97.5	96.7	0.5	17.2		[121]

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