Infection and Drug Resistance

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Evaluation of Commercial Products for Colistin and Polymyxin B Susceptibility Testing for *mcr*-Positive and Negative *Escherichia coli* and *Klebsiella pneumoniae* in China

Qi Zhang[1,*], Wenjuan Yan[1,*], Yingjie Zhu[2,*], Nan Jing[1], Shanmei Wang[1], Youhua Yuan[1], Bing Ma[1], Junhong Xu[1], Yafei Chu[1], Jiangfeng Zhang[1], Qiong Ma[1], Baoya Wang[1], Wenbo Xu[1], Liqiang Zhu[3], Ying Sun[2], Caiqin Shi[4], Juan Fang[5], Yi Li[1], Shengqun Liu[6]

[1]Department of Clinical Microbiology, Henan Provincial People's Hospital, People's Hospital of Zhengzhou University, and People's Hospital of Henan University, Zhengzhou, People's Republic of China; [2]Department of Clinical Microbiology, Henan No.3 Provincial People's Hospital, Zhengzhou, People's Republic of China; [3]Department of Clinical Microbiology, The Second Affiliated Hospital of Zhengzhou University, Zhengzhou, People's Republic of China; [4]Department of Microbiology Laboratory, KingMed Diagnostics Group Co., Ltd, Zhengzhou, People's Republic of China; [5]Department of Research and Development, Autobio Diagnostics Co., Ltd, Zhengzhou, People's Republic of China; [6]Department of Anesthesia and Perioperative Medicine, Henan Provincial People's Hospital, People's Hospital of Zhengzhou University, and People's Hospital of Henan University, Zhengzhou, People's Republic of China

*These authors contributed equally to this work

Correspondence: Yi Li; Shengqun Liu, Department of Anesthesia and Perioperative Medicine, Henan Provincial People's Hospital, People's Hospital of Zhengzhou University, and People's Hospital of Henan University, Weiwu Road 7, Zhengzhou, Henan, 450003, People's Republic of China, Tel +8613526882118, Fax +8637187160318, Email liyilabmed@henu.edu.cn; tim2002627@163.com

Purpose: To evaluate the performance of five widespread commercial products for colistin and polymyxin B susceptibility testing in China for *mcr*-positive and -negative *Escherichia coli* and *Klebsiella pneumoniae*.

Methods: A total of 132 *E. coli* and 83 *K. pneumoniae* strains (including 68 *mcr-1*-positive *E. coli* and 28 *mcr-8*-positive *K. pneumoniae*) were collected. We analysed the performance of colistin susceptibility (with Vitek 2 and Phoenix M50) and the performance of polymyxin B susceptibility (with DL-96II, MA120, and a Polymyxin B Susceptibility Test strip; POL E-strip). Broth microdilution was used as the gold standard. Categorical agreement (CA), essential agreement (EA), major error (ME), and very major error (VME) were calculated for comparisons.

Results: For *E. coli*, the total CA, EA, ME, and VME to colistin were as follows: Vitek 2, 98.5%/98.5%/0%/2.9%; and Phoenix M50, 98.5%/97.7%/0%/2.9%. The total CA, EA, ME, and VME to polymyxin B were as follows: POL E-strip, 99.2%/63.6%/1.6%/0%; MA120, 70.0%/-/0%/58.8%; and DL-96II, 80.2%/-/1.6%/36.8%. Only Vitek 2 and Phoenix M50 presented satisfactory performances for *mcr-1*-positive *E. coli*. For *K. pneumoniae*, the total CA, EA, ME, and VME to colistin were as follows: Vitek 2, 73.2%/72.0%/0%/61.6%; and Phoenix M50, 74.7%/74.7%/0%/58.3%. The total CA, EA, ME, and VME to polymyxin B were as follows: POL E-strip, 91.6%/74.7%/2.1%/16.7%; MA120, 92.8%/-/2.1%/13.9%; and DL-96II, 92.2%/-/2.1%/8.3%. All systems were unsatisfactory for *mcr-8*-positive *K. pneumoniae*. When the susceptibility of *mcr*-negative strains was tested, all systems presented excellent performance.

Conclusion: Vitek 2 and Phoenix M50 with colistin for *E. coli* showed acceptable performance regardless of *mcr-1* expression, while DL-96II, MA120, and the POL E-strip performed worse for *mcr-1*-positive strains. Furthermore, *mcr-8* greatly affected the performance of all systems with both colistin and polymyxin B for *K. pneumoniae* isolates.

Keywords: polymyxin B, colistin, mobilised colistin resistance, broth microdilution, susceptibility testing, semi-automated systems

Introduction

Polymyxins, including polymyxin B and polymyxin E (also known as colistin), are cyclic polypeptide antibiotics, which are long-established antimicrobials developed in the 1950s.[1] Polymyxins are currently one of the last options to treat life-

Received: 9 December 2022
Accepted: 21 February 2023
Published: 27 February 2023

threatening infections caused by multidrug-resistant gram-negative bacteria.[2] However, polymyxin resistance in gram-negative bacteria has increased worldwide, becoming a major challenge in clinical therapy.[3,4]

Despite decades of clinical use, the optimal method for rapid and accurate detection of polymyxin-resistant strains remains undefined.[1] Polymyxin susceptibility testing is methodologically challenging due to the poor diffusion of polymyxins into agar, the inherent cationic properties of polymyxins, and the occurrence of heteroresistance to polymyxins in many species. The joint CLSI-EUCAST Subcommittee on Polymyxin Susceptibility Testing and Breakpoints recommended the broth microdilution method (BMD) according to ISO standard 20776-1 as the reference method to evaluate susceptibility to polymyxins in 2016.[5] However, BMD is usually not convenient for routine clinical laboratories due to the cumbersome procedure and strict testing requirements.

Thus far, few studies have assessed the performance of polymyxin susceptibility testing methods. The gradient diffusion test,[6,7] the disc diffusion test,[8] the agar dilution[9] susceptibility method, some commercial systems such as Vitek 2[9] and Phoenix,[10] and colistin broth disc elution (CBDE)[11] were evaluated in previous studies. However, these studies displayed controversial results due to different proportions of genera or species, a limited number of resistant strains, and different mechanisms of resistance. The increasing number of *mcr*-mediated polymyxin-resistant strains is a more challenging issue since the isolates containing the *mcr* gene usually present minimum inhibitory concentrations (MICs) close to the EUCAST breakpoint, and some commercial products like Vitek 2 showed poor reliability in colistin susceptibility testing for *mcr-1*-positive *E. coli*.[12]

At present, VITEK 2® COMPACT (BioMérieux), Phoenix™ M50 (Becton Dickson Diagnostics), DL-96II (Zhuhai DL Biotech Co., Ltd.), MA120 (Zhuhai Meihua Medical Technology Co., Ltd.), and E-strip (Autobio Diagnostics Co., Ltd.) are the most common commercial systems used in China. In the current study, the performance of these systems for polymyxin B and colistin was evaluated on isolates of *mcr*-positive and *mcr*-negative *E. coli* and *K. pneumoniae* strains in order to determine the potential usefulness of these methods in routine clinical tests.

Materials and Methods

Strains

Between February 2018 and December 2020, 215 strains were collected from the Henan and Zhejiang provinces in China, including 132 *E. coli* strains (68 *mcr-1*-positive) and 83 *K. pneumonia* strains (28 *mcr-8*-positive). The *mcr-8*-positive *K. pneumoniae* were obtained from livestock, and other strains were isolated from bacterial cultures of clinical specimens (including blood, respiratory tract samples, lumbar puncture fluid, urine, and wound samples) collected at Henan Provincial People's Hospital. All isolates were incubated for 18–24 hours at 35 ± 1 °C using tryptic soy agar with 5% sheep's blood prior to being identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Daltonics, Billerica, MA, USA) according to the manufacturer's instructions. As a matrix, α-Cyano-4-hydroxycinnamic acid was used. Protein spectra were analysed using Bruker Biotyper 3.1 software and library v5.0 5898.

Antimicrobial Susceptibility Testing

The BMD method, as the standard reference method for antimicrobial susceptibility testing (AST), was performed in strict accordance with the CLSI M7-A10 document.[13] The antibiotic drugs polymyxin B and colistin were obtained from the National Institutes for Food and Drug Control of China (polymyxin B lot: 130313-202111, colistin lot: 130327-200906). *E. coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853 were used as polymyxin-susceptible control strains, while *E. coli* NCTC 13846 (*mcr-1*-positive) and *K. pneumoniae* CCUG59348 (colistin resistant, *mcr*-negative) served as the polymyxin-resistant control strains.[14] The performance of five commercial methods, including VITEK 2® COMPACT (BioMérieux, Marcy l'Etoile, France) with an AST-N335 card (colistin), Phoenix™ M50 (Becton Dickinson Diagnostics, Sparks, MD, USA) with a NMIC-502 card (colistin), DL-96II (Zhuhai DL Biotech Co., Ltd., Zhuhai, China) with a DL-E card (polymyxin B), MA120 (Zhuhai Meihua Medical Technology Co., Ltd., Zhuhai, China) with a MA card (polymyxin B), and Polymyxin B Susceptibility Test strip (Autobio Diagnostics Co., Ltd., Zhengzhou, China), which were performed according to the manufacturer's instructions, were evaluated.

The possible ranges of MIC readings for each method were as follows: BMD (colistin and polymyxin B), ≤ 0.5 to ≥ 32mg/L; Vitek 2, ≤ 0.5 to ≥ 16 mg/L; Phoenix M50, ≤ 1 to ≥ 8 mg/L; DL-96II, ≤ 2 to ≥ 4 mg/L; MA120, ≤ 1 to ≥ 4 mg/L; Polymyxin B Susceptibility Test Strip (POL E-Strip), ≤ 0.06 to ≥ 256 mg/L. All POL E-Strip results were recorded up to the nearest MIC measured in the BMD category (eg, 0.75 mg/L was recorded as 1 mg/L).

Evaluation Method

EUCAST clinical breakpoints-bacteria (v 12.0)[15] was used for the interpretation of colistin and polymyxin B MIC results (susceptible, ≤ 2 mg/L; resistant, > 2 mg/L). BMD results were considered the reference standard. Essential agreement (EA) was defined as the percentage of MICs within a single doubling dilution of the corresponding reference MICs. Categorical agreement (CA) was the proportion of isolates classified in the same susceptibility category by BMD and the compared methods. Very major error (VME) was defined as false susceptible results compared to BMD. VME rates were calculated using the number of resistant isolates reported by BMD as the denominator. Major error (ME) was defined as false resistant results compared to BMD. ME rates were calculated using the number of susceptible isolates by BMD as the denominator. According to CLSI recommendations, a new system can be acceptable when it meets the standards as follows: CA > 90%, EA > 90%, VME < 1.5%, and ME < 3%.[16]

Results

Antimicrobial Susceptibility Test

MICs for quality control strains by all testing methods were within the expected ranges. All 215 strains were tested for susceptibility to polymyxin B and colistin by BMD. Due to differences in AST cards, susceptibility to colistin was reported by Vitek 2 and Phoenix M50, and susceptibility to polymyxin B was reported by DL-96II, MA120, and the POL E-strip. The colistin and polymyxin B reference MICs for the 215 isolates ranged from ≤ 0.5 to ≥ 32 mg/L, with 51.5% susceptible isolates and 48.4% resistant isolates. The reference MIC of *mcr-1*-positive *E. coli* was mainly distributed between 4 and 8 mg/L, and the reference MIC of *mcr-8*-positive *K. pneumoniae* was mainly found to be ≥ 32 mg/L. The reference MICs of the 215 isolates between polymyxin B and colistin were slightly different (Table 1).

Susceptibility to Colistin; Agreements and Errors for *E. coli* and *K. pneumoniae* Isolates

The general agreement between BMD and two commercial AST systems for colistin is shown in Figure 1. The performance of Phoenix M50 and Vitek 2 for colistin showed overall comparable CAs (89.3% and 88.7%, respectively), EAs (89.3% and 87.8%, respectively), and VMEs (22.1% and 23.1%, respectively).

Both the Phoenix M50 and Vitek 2 systems performed best for *E. coli* with only two false-susceptible results (2.9% VME), a high CA of 98.5%, and high EAs of 98.5% and 97.7%, respectively. No isolates tested as false-resistant in either system (Table 2).

Table 1 Colistin and Polymyxin B Reference Minimum Inhibitory Concentrations for 215 Isolates

Drug	Organism	Number of Isolates	MIC (mg/L)						
			≤0.5	1	2	4	8	16	≥32
Colistin	Total	215	107	3	1	43	31	7	23
	Escherichia coli	132	63	0	1	42	26	0	0
	Klebsiella pneumoniae	83	44	3	0	1	5	7	23
Polymyxin B	Total	215	108	2	1	48	27	8	21
	Escherichia coli	132	63	1	0	46	21	1	0
	Klebsiella pneumoniae	83	45	1	1	2	6	7	21

Abbreviation: MIC, minimum inhibitory concentration.

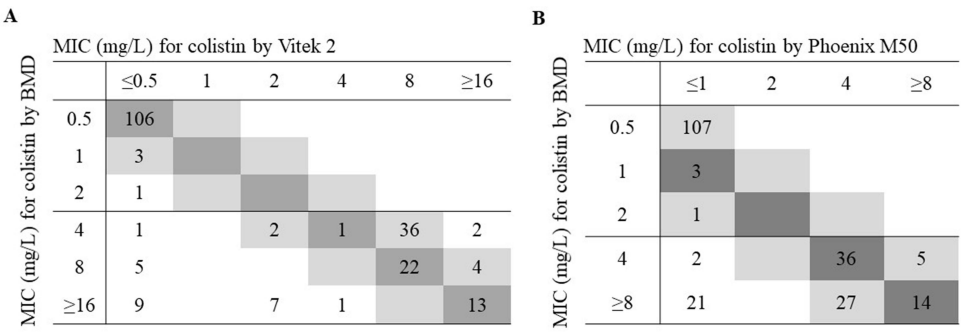

Figure 1 Comparison of two commercial products for colistin susceptibility testing against the reference method. Minimum inhibitory concentrations (MICs) identical to the reference broth microdilution (BMD) are highlighted in dark grey. MICs within the essential agreement (±1 dilution compared to the reference method) are highlighted in light grey. EUCAST breakpoints (susceptible ≤ 2 mg/L, resistant > 2 mg/L) are indicated as lines. (**A**) Scatterplot of Vitek 2 versus BMD. (**B**) Scatterplot of Phoenix M50 versus BMD.

However, the two AST systems performed poorly for *K. pneumoniae* with high VME rates of 58.3% for Phoenix M50 and 61.1% for Vitek 2 and lower CAs (74.7% and 73.2%, respectively) and EAs (74.2% and 72.0%, respectively). No isolates tested as false-resistant in either system (Table 2).

Susceptibility to Polymyxin B; Agreements and Errors for *E. coli* and *K. pneumoniae* Isolates

The performance of the POL E-strip for polymyxin B showed an acceptable CA (96.3%), an unacceptable EA (69.7%), and only two false-resistant results and six false-susceptible results for all strains. The DL-96II system performed poorly, with only 86.0% of results classified as CA and 28 false-susceptible results (VME 26.9%) for polymyxin B, while the MA120 system showed a significantly higher VME (43.3%) and a lower CA (78.6%) (Table 3).

Compared with BMD for *E. coli*, the total CA was 80.2% for DL-96II, 70.0% for MA120, and 99.2% for the POL E-strip. Both DL-96II and MA120 had very high VMEs (36.8% and 58.8%, respectively). The ME was 1.6% for both DL-96II and the POL E-strip.

As for *K. pneumoniae*, the total CA was 95.2% for DL-96II, 92.8% for MA120, and 91.6% for the POL E-strip. All three methods showed high VMEs (8.3% for DL-96II, 13.9% for MA120, and 16.7% for the POL E-strip, respectively). The ME was 2.1% for all three methods.

Table 2 Colistin Susceptibility Rates Determined by BMD, Vitek 2, and Phoenix M50, and the EAs, CAs, and Errors of Each AST Method Compared with BMD

Species	Method	Total	Results [n (%)]		Performance [n (%)]			
			S	R	EA	CA	VME	ME
Total	BMD	215	111 (51.6)	104 (48.4)				
	Phoenix M50	215	134 (62.3)	81 (37.7)	192 (89.3)	192 (89.3)	23 (22.1)	0 (0)
	Vitek 2	213	134 (62.9)	79 (37.1)	187 (87.8)	189 (88.7)	24 (23.1)	0 (0)
Escherichia coli	BMD	132	64 (48.5)	68 (51.5)				
	Phoenix M50	132	66 (50.0)	66 (50.0)	130 (98.5)	130 (98.5)	2 (2.9)	0 (0)
	Vitek 2	131	66 (50.4)	65 (49.6)	128 (97.7)	129 (98.5)	2 (2.9)	0 (0)
Klebsiella pneumoniae	BMD	83	47 (56.6)	36 (43.4)				
	Phoenix M50	83	68 (81.9)	15 (18.1)	62 (74.7)	62 (74.7)	21 (58.3)	0 (0)
	Vitek 2	82	68 (82.9)	14 (17.1)	59 (72.0)	60 (73.2)	22 (61.1)	0 (0)

Abbreviations: BMD, broth microdilution; CA, categorical agreement; EA, essential agreement; ME, major error; VME, very major error; S, susceptible; R, resistant.

Table 3 Polymyxin B Susceptibility Rates Determined by BMD, DL-96II, MA120, and POL E-Strip, and the EAs, CAs, and Errors of Each Method Compared with BMD

Species	Method	Total	Results [n (%)]		Performance [n (%)]			
			S	R	EA	CA	VME	ME
Total	BMD	215	111 (51.6)	104 (48.4)				
	DL-96II	214	137 (64.0)	77 (36.0)	NA	184 (86.0)	28 (26.9)	2 (1.8)
	MA120	215	155 (72.1)	60 (27.9)	NA	169 (78.6)	45 (43.3)	1 (0.9)
	POL E-strip	215	115 (53.5)	100 (46.5)	146 (67.9)	207 (96.3)	6 (5.7)	2 (1.8)
Escherichia coli	BMD	132	64 (48.5)	68 (51.5)				
	DL-96II	131	88 (67.2)	43 (32.8)	NA	105 (80.2)	25 (36.8)	1 (1.6)
	MA120	132	104 (78.8)	28 (21.2)	NA	92 (70.0)	40 (58.8)	0 (0)
	POL E-strip	132	63 (47.7)	69 (52.3)	84 (63.6)	131 (99.2)	0 (0)	1 (1.6)
Klebsiella pneumoniae	BMD	83	47 (56.6)	36 (43.4)				
	DL-96II	83	49 (59.0)	34 (41.0)	NA	79 (95.2)	3 (8.3)	1 (2.1)
	MA120	83	51 (61.4)	32 (38.6)	NA	77 (92.8)	5 (13.9)	1 (2.1)
	POL E-strip	83	52 (62.7)	31 (37.3)	62 (74.7)	76 (91.6)	6 (16.7)	1 (2.1)

Abbreviations: BMD, broth microdilution; CA, categorical agreement; EA, essential agreement; ME, major error; VME, very major error; S, susceptible; R, resistant; NA, not applicable.

Performance Evaluation on *mcr*-Positive and -Negative Strains for Colistin and Polymyxin B

Among the 215 isolates, 68 strains were *mcr-1*-positive *E. coli* and 28 strains were *mcr-8*-positive *K. pneumoniae*, which were verified by PCR. The performance of Phoenix M50 and Vitek 2 for colistin showed comparable overall CAs (76.0% and 74.7%, respectively), EAs (76.0% and 73.7%, respectively), and VMEs (24.0% and 25.0%, respectively) for *mcr*-positive isolates. Both the Phoenix M50 and the Vitek 2 systems produced excellent CAs (97.1% and 97.0%, respectively) and EAs (97.1% and 97.0%, respectively) for *mcr-1*-positive *E. coli* strains with only two false-susceptible results (2.9% VME). However, both systems performed poorly for *mcr-8*-positive *K. pneumoniae* strains, with high VME rates (75.0% for Phoenix M50 and 78.5% for Vitek 2), low CAs (25.0% and 21.4%, respectively), and low EAs (25.0% and 17.9%, respectively) (Table 4).

As for polymyxin B, only the POL E-strip showed an acceptable CA (93.8%) and an unacceptable EA (35.4%) for all *mcr*-positive isolates. DL-96II and MA120 systems performed poorly with lower CAs (70.5% and 53.1%, respectively) and higher VMEs (29.2% and 46.9%, respectively). For *mcr-1*-positive *E. coli* strains, the POL E-strip showed excellent CAs (100%) and VMEs (0%), while the DL-96II and MA120 systems presented unacceptable EAs (62.7% and 41.2%, respectively) and VMEs (36.8% and 58.8%, respectively). For *mcr-8*-positive *K. pneumoniae* strains, all three systems (DL-96II, MA120, and the POL E-strip) showed comparable overall CAs (89.3%, 82.1%, and 78.6%, respectively) and high VMEs (10.7%, 17.9%, and 21.4%, respectively) (Table 4).

All five systems showed excellent rates of CAs with few errors for *mcr*-negative strains. Phoenix M50 and Vitek 2 to colistin demonstrated 100% CA rates with no false-resistant or false-susceptible strains. DL-96II, MA120, and POL E-strip to polymyxin B showed high CA rates (98.3%, 99.2%, and 98.3%, respectively), no false-susceptible strains, and no more than two false-resistant strains (Table 4).

Table 4 Comparison of Performance Characteristics to Colistin and Polymyxin B Between AST Systems and BMD Method for Strains in Different *mcr* Gene Conditions

Drug	*mcr* Gene	Species	Method	Total	Results [n (%)]		Performance [n (%)]			
					S	R	EA	CA	VME	ME
Colistin	*mcr*-positive	Total	BMD	96	0 (0)	96 (100.0)				
			Phoenix M50	96	23 (24.0)	73 (76.0)	73 (76.0)	73 (76.0)	23 (24.0)	NA
			Vitek 2	95	24 (25.3)	71 (74.7)	70 (73.7)	71 (74.7)	24 (25.0)	NA
	mcr-1-positive	*Escherichia coli*	BMD	68	0 (0)	68 (100.0)				
			Phoenix M50	68	2 (2.9)	66 (97.1)	66 (97.1)	66 (97.1)	2 (2.9)	NA
			Vitek 2	67	2 (3.0)	65 (97.0)	65 (97.0)	65 (97.0)	2 (2.9)	NA
	mcr-8-positive	*Klebsiella pneumoniae*	BMD	28	0 (0)	28 (100.0)				
			Phoenix M50	28	21 (75.0)	7 (25.0)	7 (25.0)	7 (25.0)	21 (75.0)	NA
			Vitek 2	28	22 (78.6)	6 (21.4)	5 (17.9)	6 (21.4)	22 (78.5)	NA
	mcr-negative	Total	BMD	119	111 (93.3)	8 (6.7)				
			Phoenix M50	119	111 (93.3)	8 (6.7)	119 (100.0)	119 (100.0)	0 (0)	0 (0)
			Vitek 2	118	110 (93.2)	8 (6.8)	117 (99.2)	118 (100.0)	0 (0)	0 (0)
		Escherichia coli	BMD	64	64 (100.0)	0 (0)				
			Phoenix M50	64	64 (100.0)	0 (0)	64 (100.0)	64 (100.0)	NA	0 (0)
			Vitek 2	64	64 (100.0)	0 (0)	63 (98.4)	64 (100.0)	NA	0 (0)
		Klebsiella pneumoniae	BMD	55	47 (85.5)	8 (14.5)				
			Phoenix M50	55	47 (85.5)	8 (14.5)	55 (100.0)	55 (100.0)	0 (0)	0 (0)
			Vitek 2	54	46 (85.2)	8 (14.8)	54 (100.0)	54 (100.0)	0 (0)	0 (0)

Polymyxin B	*mcr*-positive	Total	BMD	96	0 (0)	96 (100.0)				
			DL-96II	95	28 (29.5)	67 (70.5)	NA	67 (70.5)	28 (29.2)	NA
			MA120	96	45 (46.9)	51 (53.1)	NA	51 (53.1)	45 (46.9)	NA
			POL E-strip	96	6 (6.2)	90 (93.8)	34 (35.4)	90 (93.8)	6 (6.3)	NA
	mcr-1-positive	*Escherichia coli*	BMD	68	0 (0)	68 (100.0)				
			DL-96II	67	25 (37.3)	42 (62.7)	NA	42 (62.7)	25 (36.8)	NA
			MA120	68	40 (58.9)	28 (41.1)	NA	28 (41.2)	40 (58.8)	NA
			POL E-strip	68	0 (0)	68 (100.0)	23 (33.8)	68 (100.0)	0 (0)	NA
	mcr-8-positive	*Klebsiella pneumoniae*	BMD	28	0 (0)	28 (100.0)				
			DL-96II	28	3 (10.7)	25 (89.3)	NA	25 (89.3)	3 (10.7)	NA
			MA120	28	5 (17.9)	23 (82.1)	NA	23 (82.1)	5 (17.9)	NA
			POL E-strip	28	6 (21.4)	22 (78.6)	11 (39.3)	22 (78.6)	6 (21.4)	NA
	mcr-negative	Total	BMD	119	111 (93.3)	8 (6.7)				
			DL-96II	119	109 (91.6)	10 (8.4)	NA	117 (98.3)	0 (0)	2 (1.8)
			MA120	119	110 (92.4)	9 (7.6)	NA	118 (99.2)	0 (0)	1 (0.9)
			POL E-strip	119	109 (91.6)	10 (8.4)	112 (94.1)	117 (98.3)	0 (0)	2 (1.8)
		Escherichia coli	BMD	64	64 (100.0)	0 (0)				
			DL-96II	64	63 (98.4)	1 (1.6)	NA	63 (98.4)	NA	1 (1.6)
			MA120	64	64 (100.0)	0 (0)	NA	64 (100.0)	NA	0 (0)
			POL E-strip	64	63 (98.4)	1 (1.6)	61 (95.3)	63 (98.4)	NA	1 (1.6)
		Klebsiella pneumoniae	BMD	55	47 (85.5)	8 (14.5)				
			DL-96II	55	46 (83.6)	9 (16.4)	NA	54 (98.2)	0 (0)	1 (2.1)
			MA120	55	46 (83.6)	9 (16.4)	NA	54 (98.2)	0 (0)	1 (2.1)
			POL E-strip	55	46 (83.6)	9 (16.4)	51 (92.7)	54 (98.2)	0 (0)	1 (2.1)

Abbreviations: AST, antimicrobial susceptibility testing; BMD, broth microdilution; CA, categorical agreement; EA, essential agreement; ME, major error; VME, very major error; S, susceptible; R, resistant; NA, not applicable.

https://doi.org/10.2147/IDR.S400772

Discussion

Polymyxins are considered to be last-resort antibiotics for the treatment of serious infections caused by multidrug-resistant gram-negative bacteria. However, susceptibility testing with polymyxins is challenging. Recently, BMD, CBDE, and colistin agar tests have been recommended by CLSI as acceptable methods. For polymyxin B, BMD is the only approved method. However, BMD is not commonly carried out in most clinical microbiology laboratories in China due to the strict testing requirements, laborious methods, and requirement for manual preparation of antibiotic solutions. In this study, we evaluated the performance of four commonly used commercial AST systems and one polymyxin B susceptibility test strip for colistin and polymyxin B susceptibility testing in China.

Phoenix M50 and Vitek 2 are the most widely used microbial identification and drug sensitivity analysis systems in the world. Phoenix M50 uses the colorimetry and fluorescent methods for identification and turbidimetry and the oxidation-reduction method for susceptibility. The principle of Vitek 2 is photoelectric colorimetry for identification and the turbidimetry method for susceptibility. As for colistin, the two semi-automated systems, Phoenix M50 and Vitek 2, exhibited unacceptable rates of CAs, EAs, and false-susceptible results, especially with *K. pneumoniae* isolates. Both Phoenix M50 and Vitek 2 exhibited excellent CAs and EAs and no false-resistant results for *E. coli* isolates, regardless of the *mcr* gene. These two systems showed relatively low rates of VMEs (2.9%). As a result, Phoenix M50 and Vitek 2 may be alternative choices for clinical *E. coli* strains in colistin AST. However, it was inconsistent with previous reports. In a study conducted by Chew et al, which included 21 *mcr-1*-positive isolates, a high VME of 36% by Vitek 2 was demonstrated.[7] In the Pfennigwerth et al study, the CA (92.0%) and EA (76.1%) for Phoenix, and the CA (90.5%) and EA (75.9%) for Vitek 2 were reported in 325 carbapenemase-producing Enterobacterales isolates, and high VMEs (26 for BD Phoenix, and 31 for Vitek 2) were also detected.[10] The performance of Vitek 2 and Phoenix for colistin susceptibility testing of 117 carbapenem-resistant *Acinetobacter baumannii* clinical isolates has been estimated by Vourli et al, with CA (88.9%) and EA (91.5%) for Phoenix and Vitek 2 (89.7% and 88.9%, respectively), and high rates of VMEs (41.4% for Phoenix and 37.9% for Vitek 2).[17] There are also controversial results. In the blind testing of the colistin susceptibilities of 20 colistin-resistant and 10 colistin-susceptible Enterobacterales, Phoenix provided accurate and reproducible categorical results. However, the Vitek 2 system showed poor performance in the detection of colistin-resistant isolates.[18] Recently, Zhu et al reported that the Vitek 2 system yielded a high VME (25.5%) in 55 *mcr-1*-positive *E. coli* isolates, while Phoenix had an excellent CA (100%) and no ME or VME, which is in line with our results for Phoenix M50.[12] The variable performance of Vitek 2 and Phoenix for colistin susceptibility testing may result from the diversity of the species included and the different frequencies of *mcr*-positive isolates. Anantharajah et al reported that Vitek 2 performed poorly for *E. coli* isolates with MICs 4 mg/L by BMD and *Enterobacter spp*. isolates.[12] Pfennigwerth et al also reported a high rate of VMEs with *E. cloacae* isolates in Phoenix and Vitek 2.[10] *Enterobacter asburiae and Enterobacter cloacae* have been verified for the development of *acrAB-tolC* efflux pump-based high level colistin heteroresistance.[19] More studies are needed to further interpret the poor reliability of colistin susceptibility testing by the Phoenix M50 and Vitek 2 systems. Notably, Phoenix M50 and Vitek 2 performed poorly for *K. pneumoniae* isolates with low EAs, low CAs, and very high VMEs (58.3% for Phoenix M50 and 61.1% for Vitek 2) in our study. These VMEs all occurred in *mcr-8*-positive strains, and this is the first study to evaluate the performance of colistin susceptibility testing for *mcr-8*-positive Enterobacterales isolates. No MEs were observed with either system for all isolates, suggesting that resistant results can be considered valid. On the other hand, the high VMEs for *mcr-8*-positive strains implied that the susceptible results from these two systems should be confirmed by the reference method. However, the *mcr-8* gene was mainly found in animal-isolated strains, with a relatively low prevalence (0.3%) in clinical isolates.[20] Therefore, it seems to have a very weak effect on clinical colistin susceptibility testing.

Regarding polymyxin B, DL-96II and MA120 are the two local microbial identification and drug sensitivity analysis systems most commonly used in Chinese secondary hospitals. The principles of these two systems are the same: colorimetry for identification and turbidimetry for susceptibility testing. There are limited reports on them. In this study, the total CAs for DL-96II and MA120 in *E. coli* were 80.2% and 70.0%, respectively. A high VME of 36.8% for DL-96II and 58.8% for MA120 was observed. Additionally, in *K. pneumoniae* isolates, the total CA for the DL-96II and MA120 was 95.2% and 92.8%, respectively. VME showed 8.3% for DL-96II and 13.9% for MA120, which were also

very high. DL-96II and MA120 showed higher VEM rates and lower CAs for *mcr*-positive isolates when compared to *mcr*-negative isolates. One reason could be that the plate had too few drug concentration gradient holes for the two local systems, resulting in low EAs and CAs. It remains unclear whether the poor performance of polymyxin B susceptibility testing by DL-96II and MA120 for *mcr*-positive *E. coli* and *K. pneumoniae* is a systematic error or an occasional occurrence. As a result, they were not recommended for polymyxin B susceptibility testing for clinical isolates, particularly *mcr*-positive isolates.

The POL E-strip is similar to the E-test based on gradient drug diffusion techniques. The performance of the E-test for polymyxin B susceptibility testing against Enterobacterales has been estimated in several studies. The CA, EA, and VME were 80%, 10%, and 88%, respectively, in 70 isolates, primarily *Klebsiella* spp. and *Enterobacter* spp. CRE.[21] E-test results yielded one VME (2%) and 11 MEs (23%) for polymyxin B in 48 KPC-producing *K. pneumoniae* isolates.[22] Chew et al confirmed the same trend in the E-test for polymyxin B, and the CA, EA, and VMEs showed values of 89.5%, 48.7%, and 26.1%, respectively, in 76 CRE isolates, among which 21 strains were *mcr-1*-positive.[7] However, the above studies all focused on CRE isolates. A high VME was also reported for the E-test and MIC Test Strip (MTS) in 75 gram-negative bacteria with varying levels of colistin susceptibility by Matuschek et al.[6] In a recent study, the CA, EA, ME, and VME of MTS for polymyxin B were 95.2%, 97.8%, 0.9%, and 10.7%, respectively, for 185 *E. coli* isolates that contained 78 *mcr-1*-positive strains.[23] In our study, the POL E-strip showed very good CA (99.2%), ME (1.6%), and VME (0%) in *mcr-1*-negative or *mcr-1*-positive *E. coli* isolates but a low EA (63.6%). In contrast, in the *K. pneumoniae* isolates, a considerably high VME of 16.7% was confirmed, along with a low EA (74.7%).

The limitations of this study should be considered. We expected to perform all susceptibility tests on the same day and from the same inoculum suspension; however, this was impossible as the workload was too great. Indeed, we finished the study in several days, and each batch of tested isolates underwent the six methods on the same day. Furthermore, we performed replication in our study when accidental errors happened, such as the failed results due to an insufficient indicator for BD Phoenix. For result validation, quality control strains were used for each batch of experiments for both the original tests and repeated tests. Additionally, only *mcr-1*-positive and *mcr-8*-positive isolates were collected in this study, and our findings may not extrapolate to other *mcr*-positive strains. In future studies, we need to collect more different *mcr*-positive strains from clinical samples. Lastly, herein, we presented our findings in numbers and percentages to make them more understandable. We would like to conduct more statistical analysis in future studies.

Conclusions

In conclusion, the performances of the Vitek 2 and Phoenix M50 systems for colistin susceptibility testing were poor in *mcr-8*-positive *K. pneumoniae* but acceptable in *E. coli* regardless of *mcr-1* gene expression compared to BMD. The *mcr*-positive *E. coli* and *K. pneumoniae* isolates might be poorly identified by the DL-96II and MA120 systems, and the main shortcoming of the POL E-strip was a low EA value and high VMEs in *mcr-8*-positive *K. pneumoniae* isolates.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Approval and Informed Consent

This study was approved by the Ethics Committee of Henan Provincial People's Hospital, Henan, China (2022-1-233). No personally identifiable information was collected in this study. The requirement for informed consent from patients was also waived.

Acknowledgments

We would like to thank Pr. Rong Zhang for providing us with mcr-positive strains, and we would like to thank Editage (www.editage.cn) for English language editing.

Funding

This work was supported by Henan Provincial Key Programs in Science and Technology [grant number: 202102310355].

Disclosure

The authors declare no conflicts of interest in this work.

References

1. Ezadi F, Ardebili A, Mirnejad R, Kraft CS. Antimicrobial susceptibility testing for polymyxins: challenges, issues, and recommendations. *J Clin Microbiol*. 2019;57(4). doi:10.1128/JCM.01390-18
2. El-Sayed Ahmed MAE, Zhong LL, Shen C, Yang Y, Doi Y, Tian GB. Colistin and its role in the Era of antibiotic resistance: an extended review (2000–2019). *Emerg Microbes Infect*. 2020;9(1):868–885. doi:10.1080/22221751.2020.1754133
3. Stefaniuk EM, Tyski S. Colistin resistance in Enterobacterales strains – a current view. *Pol J Microbiol*. 2019;68(4):417–427. doi:10.33073/pjm-2019-055
4. Bialvaei AZ, Samadi Kafil H. Colistin, mechanisms and prevalence of resistance. *Curr Med Res Opin*. 2015;31(4):707–721. doi:10.1185/03007995.2015.1018989
5. The European Committee on Antimicrobial Susceptibility Testing and Clinical and Laboratory Standards Institute. Recommendations for MIC determination of colistin (polymyxin E) as recommended by the joint CLSI-EUCAST Polymyxin Breakpoints Working Group; 2016. Available from: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/General_documents/Recommendations_for_MIC_determination_of_colistin_March_2016.pdf. Accessed August 18, 2022.
6. Matuschek E, Åhman J, Webster C, Kahlmeter G. Antimicrobial susceptibility testing of colistin – evaluation of seven commercial MIC products against standard broth microdilution for Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter spp. *Clin Microbiol Infect*. 2018;24(8):865–870. doi:10.1016/j.cmi.2017.11.020
7. Chew KL, La MV, Lin RTP, Teo JWP, Munson E. Colistin and Polymyxin B susceptibility testing for carbapenem-resistant and mcr-positive Enterobacteriaceae: comparison of sensititre, MicroScan, Vitek 2, and Etest with Broth Microdilution. *J Clin Microbiol*. 2017;55(9):2609–2616. doi:10.1128/JCM.00268-17
8. Maalej SM, Meziou MR, Rhimi FM, Hammami A. Comparison of disc diffusion, Etest and agar dilution for susceptibility testing of colistin against Enterobacteriaceae. *Lett Appl Microbiol*. 2011;53(5):546–551. doi:10.1111/j.1472-765X.2011.03145.x
9. Dafopoulou K, Zarkotou O, Dimitroulia E, et al. Comparative evaluation of colistin susceptibility testing methods among carbapenem-nonsusceptible Klebsiella pneumoniae and Acinetobacter baumannii clinical isolates. *Antimicrob Agents Chemother*. 2015;59(8):4625–4630. doi:10.1128/AAC.00868-15
10. Pfennigwerth N, Kaminski A, Korte-Berwanger M, et al. Evaluation of six commercial products for colistin susceptibility testing in Enterobacterales. *Clin Microbiol Infect*. 2019;25(11):1385–1389. doi:10.1016/j.cmi.2019.03.017
11. Humphries RM, Green DA, Schuetz AN, et al. Multicenter evaluation of colistin broth disk elution and colistin agar test: a report from the Clinical and Laboratory Standards Institute. *J Clin Microbiol*. 2019;57(11). doi:10.1128/JCM.01269-19
12. Zhu Y, Jia P, Zhou M, et al. Evaluation of the clinical systems for polymyxin susceptibility testing of clinical Gram-negative bacteria in China. *Front Microbiol*. 2021;11:610604. doi:10.3389/fmicb.2020.610604
13. Clinical and Laboratory Standards Institute. *Methods for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically M07-A10*. 10th ed. Wayne (PA): Clinical and Laboratory Standards Institute; 2015.
14. García-Fernández S, García-Castillo M, Ruiz-Garbajosa P, et al. Performance of CHROMID(R) Colistin R agar, a new chromogenic medium for screening of colistin-resistant Enterobacterales. *Diagn Microbiol Infect Dis*. 2019;93(1):1–4. doi:10.1016/j.diagmicrobio.2018.07.008
15. European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters, version 12.0; 2022. Available from: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_12.0_Breakpoint_Tables.pdf. Accessed August 18, 2022.
16. Clinical and Laboratory Standards Institute. *Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems M52*. 1st ed. Wayne (PA): Clinical and Laboratory Standards Institute; 2015.
17. Vourli S, Dafopoulou K, Vrioni G, Tsakris A, Pournaras S. Evaluation of two automated systems for colistin susceptibility testing of carbapenem-resistant Acinetobacter baumannii clinical isolates. *J Antimicrob Chemother*. 2017;72(9):2528–2530. doi:10.1093/jac/dkx186
18. Anantharajah A, Glupczynski Y, Hoebeke M, et al. Multicenter study of automated systems for colistin susceptibility testing. *Eur J Clin Microbiol Infect Dis*. 2021;40(3):575–579. doi:10.1007/s10096-020-04059-4
19. Telke AA, Olaitan AO, Morand S, Rolain JM. soxRS induces colistin hetero-resistance in Enterobacter asburiae and Enterobacter cloacae by regulating the acrAB-tolC efflux pump. *J Antimicrob Chemother*. 2017;72(10):2715–2721. doi:10.1093/jac/dkx215
20. Farzana R, Jones LS, Barratt A, et al. Emergence of mobile colistin resistance (mcr-8) in a highly successful Klebsiella pneumoniae sequence type 15 clone from clinical infections in Bangladesh. *mSphere*. 2020;5(2). doi:10.1128/mSphere.00023-20
21. Kulengowski B, Ribes JA, Burgess DS. Polymyxin B Etest® compared with gold-standard broth microdilution in carbapenem-resistant Enterobacteriaceae exhibiting a wide range of polymyxin B MICs. *Clin Microbiol Infect*. 2019;25(1):92–95. doi:10.1016/j.cmi.2018.04.008
22. Lat A, Clock SA, Wu F, et al. Comparison of polymyxin B, tigecycline, cefepime, and meropenem MICs for KPC-producing Klebsiella pneumoniae by broth microdilution, Vitek 2, and Etest. *J Clin Microbiol*. 2011;49(5):1795–1798. doi:10.1128/JCM.02534-10
23. Wang Y, Berglund B, Zhu Y, Luo Q, Xiao Y. Performance of different methods for testing polymyxin B: comparison of broth microdilution, agar dilution and MIC test strip in mcr-1 positive and negative Escherichia coli. *Lett Appl Microbiol*. 2021;73(2):197–205. doi:10.1111/lam.13492