Original Article

Comparative Colistin Susceptibility Testing Methods for *Escherichia coli* and *Klebsiella pneumoniae*

Darat Ruangkriengsin MD[1], Naratchaphan Pati BSc[1], Naruemon Maknakhon MSc[1], Rachanis Tan-arsuwongkul BSc[2], Chakornpat Jumderm BSc[2], Surapee Tiengrim MSc[2], Visanu Thamlikitkul MD[1]

[1] Division of Infectious Diseases and Tropical Medicine, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

[2] Department of Clinical Microbiology and Applied Technology, Faculty of Medical Technology, Mahidol University, Bangkok, Thailand

Objective: To determine the accuracy and utility of colistin susceptibility testing from the agar dilution (AD), agar gradient diffusion (AG), and disk diffusion (DD) methods compared to broth microdilution (BMD), which is considered the reference method for colistin susceptibility testing of *E. coli* and *K. pneumoniae* clinical isolates.

Materials and Methods: *E. coli* and *K. pneumoniae* isolates were evaluated for colistin susceptibility by the AD, AG, DD, and BMD methods, and the AD, AG, and DD results were compared with the minimum inhibitory concentration (MIC) of colistin from BMD. The reference value breakpoints for colistin susceptibility from BMD were 1 or less and 2 mg/L or less.

Results: Three hundred twenty-six non-duplicate clinical isolates of *E. coli* and *K. pneumoniae* were included. Of those, 16 and 214 were carbapenem-resistant *E. coli* and *K. pneumoniae*, respectively. Colistin MIC by AD was in 100% agreement with BMD. Overall agreement of AG and DD with BMD was moderate, but some values of AG and DD were found to be useful.

Conclusion: Colistin MIC measured by AG (2 or less and 4 or more mg/L), and inhibition zone diameters of colistin disk by DD (14 or more and 11 or less mm) are useful for determining colistin susceptibility in carbapenem-resistant *E. coli* and *K. pneumoniae* in settings where colistin MIC measured by BMD is unavailable.

Keywords: Colistin, Antibiotic susceptibility test, Broth microdilution, Agar dilution, Agar gradient diffusion, Disk diffusion, *E. coli*, *K. pneumoniae*

J Med Assoc Thai 2018; 101 (12): 1666-79
Website: http://www.jmatonline.com

Escherichia coli and *Klebsiella pneumoniae* are the bacteria that have most commonly caused infections in both community and hospital settings for many decades. Cephalosporins and fluoroquinolones were widely used for therapy of *E. coli* and *K. pneumoniae* infections for several decades until these bacteria developed resistance to these agents due to the production of extended-spectrum beta-lactamase (ESBL) enzymes[1-3]. Carbapenems are very active against ESBL-producing or extended-spectrum cephalosporin-resistant *E. coli* and *K. pneumoniae*, and they are used to treat infections caused by these multidrug-resistant bacteria[4,5]. However, the emergence of carbapenem-resistant Enterobacteriaceae (CRE) mediated by the production of carbapenemase enzymes, especially New Delhi metallo-bata-lactamase (NDM), *K. pneumoniae* carbapenamase (KPC), and OXA-48, has been observed in many regions of the world over the past decade[6-10]. The prevalence of CRE has also been increasing in Thailand since 2011. Carbapenem resistance is more common in *K. pneumoniae* than in *E. coli*, and the prevalence of carbapenem resistance in *K. pneumoniae* in Thailand may be as high as 27% in hospital-acquired bacteremia that is caused by *K. pneumoniae*[11]. The mortality rate was significantly higher in patients with CRE infections than in patients with carbapenem-susceptible Enterobacteriaceae infections[12].

Polymyxins, colistin, and polymyxin B are usually active against carbapenem-resistant *E. coli* and *K. pneumoniae*, and they are normally used as a backbone antibiotic for therapy of infections due to carbapenem-resistant *E. coli* and *K. pneumoniae*[13]. The results of a meta-analysis of the efficacy of polymyxins for treatment of CRE infections suggested that polymyxins may be as efficacious as other antimicrobials for the treatment of CRE infections, and that polymyxin

Correspondence to:

Thamlikitkul V. Division of Infectious Diseases and Tropical Medicine, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, 2 Wang Lang Road, Bangkoknoi, Bangkok 10700, Thailand.
Phone: +66-81-8206271, **Fax**: +66-2-4125994
Email: visanu.tha@mahidol.ac.th

How to cite this article: Ruangkriengsin D, Pati N, Maknakhon N, Tan-arsuwongkul R, Jumderm C, Tiengrim S, et al. Comparative colistin susceptibility testing methods for *Escherichia coli* and *Klebsiella pneumoniae*. J Med Assoc Thai 2018;101:1666-79.

combination regimens may achieve lower mortality than polymyxin monotherapy[14]. Therefore, in vitro susceptibility testing of polymyxin against CRE is needed to guide antimicrobial therapy for CRE infections. The broth microdilution (BMD) method is recommended by the Clinical Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) Joint Subcommittee for determining the susceptibility of Enterobacteriaceae to polymyxins[15]. However, the BMD method is time-consuming (requiring at least 24 hours), labor-intensive, and it requires fastidious attention, whereas other more convenient methods, such as disk diffusion and agar gradient diffusion (Etest) are not recommended due to poor diffusion of polymyxin into the agar[16-20]. BMD method for polymyxin susceptibility testing in Enterobacteriaceae is not available in all routine microbiology laboratories in Thailand.

Accordingly, the aim of this study was to determine the accuracy and utility of colistin susceptibility testing from the agar dilution (AD), agar gradient diffusion (AG), and disk diffusion (DD) methods compared to broth BMD method, which is considered the reference method for colistin susceptibility testing of *E. coli* and *K. pneumoniae*.

Materials and Methods

Bacterial isolates

Three hundred twenty-six non-duplicate clinical isolates of *E. coli* or *K. pneumoniae* that were collected from patients enrolled in epidemiological studies of CRE and colistin-resistant Enterobacteriaceae colonization and infection at Siriraj Hospital and stored in an antimicrobial resistant bacteria repository at the Division of Infectious Diseases and Tropical Medicine, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand were included. Forty-seven of those isolates were *E. coli* and 279 were *K. pneumoniae*. Two hundred thirty isolates were carbapenem-resistant, of which 16 were carbapenem-resistant *E. coli* and 214 were carbapenem-resistant *K. pneumoniae*. *E. coli* ATCC 25922 was used as a quality control strain.

Colistin standard powder, agar gradient diffusion strip, and susceptibility disk

Standard colistin sulfate powder with a purity of 648 µg/mg (Chem-Impex Int'l, Inc., Wood Dale, IL, USA) was used for colistin minimum inhibitory concentration (MIC) determination by BMD and AD methods. Colistin agar gradient diffusion strips (MIC Test Strip) for AG were purchased from Liofilchem (Liofilchem s.r.l, Roseto degli Abruzzi, Italy), and colistin susceptibility disks were purchased from Oxoid (Oxoid Limited, Hampshire, United Kingdom).

Colistin susceptibility test

All 326 study isolates of *E. coli* or *K. pneumoniae* were evaluated for colistin susceptibility by the BMD, AD, AG, and DD methods. All colistin susceptibility tests were performed according to the recommendation of CLSI M100, Twenty-seventh edition 2017[21]. BMD was considered the reference method for determination of colistin susceptibility.

Colistin BMD was performed using cation-adjusted Mueller-Hinton Broth (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), and AD method was performed using Mueller-Hinton Agar (Becton Dickinson) according to CLSI M100, Twenty-seventh edition 2017[21]. The concentrations of colistin for the BMD and AD tests ranged from 0.25 to 128 mg/L. Colistin AG diffusion was performed by agar gradient strip (Etest strip), with concentrations of colistin that ranged from 0.016 to 256 mg/L, according to the manufacturer's instructions (Liofilchem). Colistin DD was performed using a 10 µg colistin disk (Oxoid) onto Mueller-Hinton Agar according to CLSI M100, Twenty-seventh edition 2017[21].

Interpretation of colistin susceptibility tests

CLSI does not recommend colistin or polymyxin B susceptibility breakpoints for Enterobacteriaceae. The epidemiological cut-off values of colistin to define wild-type and non-wild-type Enterobacteriaceae are colistin MIC of 2 mg/L or less and 4 mg/L or more, respectively[21]. EUCAST recommends a colistin MIC of 2 mg/L or less as a susceptible isolate, and 4 mg/L or more as a resistant isolate for Enterobacteriaceae[22]. Therefore, arbitrary colistin MIC breakpoints of 1 mg/L or less for susceptible and 2 mg/L or more for resistant, and colistin MIC breakpoints of 2 mg/L or less for susceptible and 4 mg/L or more for resistant were used to analyze the data in the present study.

Data analysis

The MICs of colistin measured by BMD were used as reference values to determine colistin susceptibility or colistin resistance. The MICs of colistin measured by AD and AG methods were compared with those of BMD method. The inhibition zone diameters of colistin disks were compared with the MICs of colistin

Table 1. Distribution of colistin MICs from the study *E. coli* and *K. pneumoniae* using BMD method

	n	MIC range (mg/L)	MIC (mg/L)									
			0.25	0.5	1	2	4	8	16	32	64	128	>128
All isolates	326												
E. coli	47	0.5 to 32		20	4		12	5	4	2			
K. pneumoniae	279	0.25 to >128	2	37	25	3	6	11	17	57	44	26	51
Carbapenem-resistant isolates	230												
E. coli	16	0.5 to 32		3	1		6	3	2	1			
K. pneumoniae	214	0.25 to >128	2	19	21	3	6	9	13	35	31	24	51

MIC = minimum inhibitory concentration; BMD = broth microdilution method; *E. coli* = *Escherichia coli*; *K. pneumoniae* = *Klebsiella pneumoniae*

measured by BMD method. Data analyses were performed using descriptive statistics, scattergrams, inter-method agreement, and error rates. Data are presented as number or number and percentage. Essential agreement (EA) was calculated for isolates tested by the AD and AG methods that had a MIC within ±1 two-fold dilution when compared with the reference BMD method. Categorical agreement (CA) was calculated as the percentage of isolates from the results of the AD, AG, and DD methods that had the same interpretative criteria for susceptibility or resistance as the BMD method. Very major error (VME) was defined as isolates that were found to be susceptible by the AD, AG, or DD methods, but that were found to be resistant by BMD method (false-susceptible result). Major error (ME) was defined as isolates that were found to be resistant by AD, AG, or DD, but that were found to be susceptible by BMD (false-resistant result). The acceptable values of EA and CA are more than 90%, VME is less than 1.5% and ME is less than 3%. Sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) were calculated for the results of the AD, AG, and DD methods compared to the BMD reference values. Correlation between MICs of colistin measured by AD and AG and those measured by BMD method were calculated using intraclass correlation coefficient (ICC). Correlation between inhibition zone diameters of DD and MICs of BMD were calculated using Spearman's rank correlation coefficient. All statistical analyses were performed using SPSS Statistics version 18.0 (SPSS, Inc., Chicago, IL, USA).

Results

MICs of colistin for the study of E. coli and K. pneumoniae isolates

The distribution of colistin MICs from the study *E. coli* and *K. pneumoniae* isolates is shown in Table 1. The ranges of MICs of colistin against 326 *E. coli* and *K. pneumoniae* isolates were similar to those of colistin against 230 carbapenem-resistant *E. coli* and *K. pneumoniae* isolates. The range of MICs of colistin for all *E. coli* isolates and carbapenem-resistant *E. coli* isolates was 0.5 to 32 mg/L, and the range of MICS of colistin for all *K. pneumoniae* isolates and carbapenem-resistant *K. pneumoniae* isolates was 0.25 to more than 128 mg/L.

Comparison of colistin MICs between BMD and AD methods for 326 E. coli and K. pneumoniae isolates

A scattergram showing colistin MICs performed by BMD method and colistin MICs performed by AD method for 326 *E. coli* and *K. pneumoniae* isolates is shown in Figure 1, with an ICC of 0.96 (95% CI 0.94 to 0.96). When a colistin MIC breakpoint of 1 mg/L or less from BMD method was considered susceptible to colistin, the VME and ME values were very low (less than 1%), and the EA, CA, Se, Sp, PPV, and NPV values were very high (more than 95%) (Table 2). When a colistin MIC breakpoint of 2 mg/L or less from BMD method was considered susceptible to colistin, the VME and ME values were zero, and the EA, CA, Se, Sp, PPV, and NPV values were also very high (more

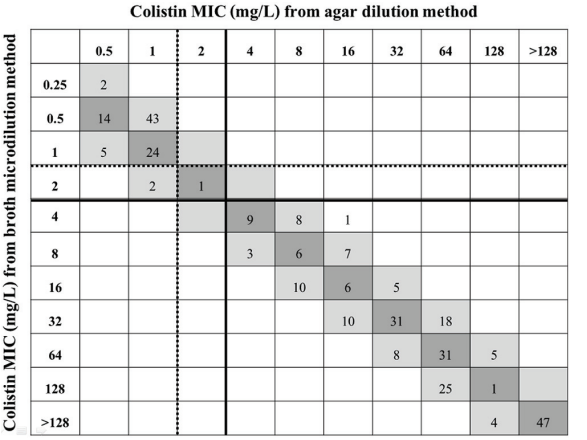

Figure 1. Scattergram of colistin MICs by BMD method and AD method for 326 *E. coli* or *K. pneumoniae* isolates.

Table 2. EA, CA, VME, ME, Se, Sp, PPV, and NPV values from the results of AD method at colistin MIC breakpoints of ≤1 mg/L and ≤2 mg/L from BMD method for 326 *E. coli* or *K. pneumoniae* isolates

Susceptibility criteria	EA	CA	VME	ME	Se	Sp	PPV	NPV
All isolates (n = 326)								
BMD: MIC ≤1 mg/L = susceptible	99.7%	99.4%	0.6%	0.0%	100%	99.2%	97.8%	100%
BMD: MIC ≤2 mg/L = susceptible		100%	0.0%	0.0%	100%	100%	100%	100%

EA = essential agreement; CA = categorical agreement; VME = very major error; ME = major error; Se = sensitivity; Sp = specificity; PPV = positive predictive value; NPV = negative predictive value; AD = agar dilution method; MIC = minimum inhibitory concentration; BMD = broth microdilution method; *E. coli* = *Escherichia coli*; *K. pneumoniae* = *Klebsiella pneumoniae*

Table 3. Colistin MICs from AD method to predict colistin susceptibility at colistin MIC breakpoints of ≤1 mg/L and ≤2 mg/L from BMD method for 326 *E. coli* or *K. pneumoniae* isolates

MIC (mg/L) from AD	No.	BMD: MIC ≤1 mg/L = susceptible		BMD: MIC ≤2 mg/L = susceptible	
		Predict susceptible	Predict resistant	Predict susceptible	Predict resistant
0.5	21	100% (21/21)	0.0%	100% (21/21)	0.0%
1	69	97.1% (67/69)	2.9% (2/69)	100% (69/69)	0.0%
2	1	0.0%	100% (1/1)	100% (1/1)	0.0%
4	12	0.0%	100% (12/12)	0.0%	100% (12/12)
8	24	0.0%	100% (24/24)	0.0%	100% (24/24)
16	24	0.0%	100% (24/24)	0.0%	100% (24/24)
32	44	0.0%	100% (44/44)	0.0%	100% (44/44)
64	74	0.0%	100% (74/74)	0.0%	100% (74/74)
≥128	57	0.0%	100% (57/57)	0.0%	100% (57/57)

MIC = minimum inhibitory concentration; AD = agar dilution method; BMD = broth microdilution method; *E. coli* = *Escherichia coli*; *K. pneumoniae* = *Klebsiella pneumoniae*

than 95%) (Table 2).

The accuracy of colistin MICs from AD method to predict colistin susceptibility at colistin MIC breakpoints of 1 mg/L or less and 2 mg/L or less from BMD method for 326 *E. coli* and *K. pneumoniae* isolates is shown in Table 3. Colistin MICs from AD method were very accurate for predicting colistin susceptibility at colistin MIC breakpoints of 1 mg/L or less and 2 mg/L or less from BMD method.

Comparison of colistin MICs between BMD and AD methods for 230 carbapenem-resistant E. coli and K. pneumoniae isolates

A scattergram of colistin showing MICs performed by BMD method and colistin MICs performed by AD method for 230 carbapenem-resistant *E. coli* and *K. pneumoniae* isolates is shown in Figure 2, with an ICC of 0.96 (95% CI 0.94 to 0.97). When a colistin MIC breakpoint of 1 mg/L or less from BMD method was considered susceptible to colistin, the VME and ME values were very low (less than 1%), and the EA, CA, Se, Sp, PPV, and NPV values were very high (more than 95%) (Table 4). When a colistin MIC breakpoint of 2 mg/L or less from BMD method was considered susceptible to colistin, the VME and ME values were zero, and the EA, CA, Se, Sp, PPV, and NPV values were also very high (more than 95%) (Table 4).

The accuracy of colistin MICs from AD method for predicting colistin susceptibility at colistin MIC breakpoints of 1 mg/L or less and 2 mg/L or less from BMD method for 230 carbapenem-resistant *E. coli* and *K. pneumoniae* isolates are shown in Table 5. Colistin MICs from AD method were also very accurate for predicting colistin susceptibility at colistin MIC

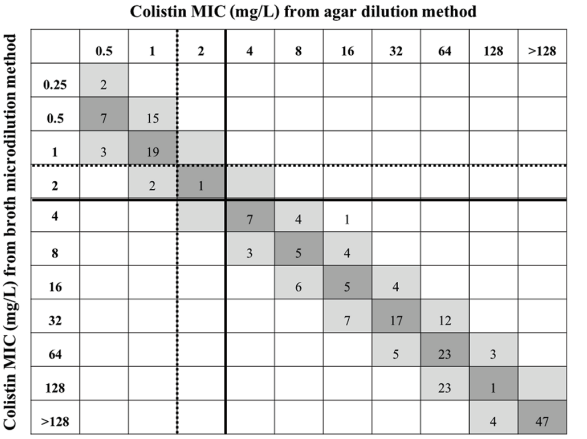

Figure 2. Scattergram of colistin MICs by BMD method and AD method for 230 carbapenem-resistant *E. coli* or *K. pneumoniae* isolates.

Table 4. EA, CA, VME, ME, Se, Sp, PPV, and NPV values from the results of AD method at colistin MIC breakpoints of ≤1 mg/L and ≤2 mg/L from BMD method for 230 carbapenem-resistant *E. coli* or *K. pneumoniae* isolates

Susceptibility criteria	EA	CA	VME	ME	Se	Sp	PPV	NPV
Carbapenem-resistant isolates (n = 230)								
BMD: MIC ≤1 mg/L = susceptible	99.6%	99.1%	0.9%	0.0%	100%	98.9%	95.8%	100%
BMD: MIC ≤2 mg/L = susceptible		100%	0.0%	0.0%	100%	100%	100%	100%

EA = essential agreement; CA = categorical agreement; VME = very major error; ME = major error; Se = sensitivity; Sp = specificity; PPV = positive predictive value; NPV = negative predictive value; AD = agar dilution method; MIC = minimum inhibitory concentration; BMD = broth microdilution method; *E. coli* = *Escherichia coli*; *K. pneumoniae* = *Klebsiella pneumoniae*

Table 5. Colistin MICs from AD method to predict colistin susceptibility at colistin MIC breakpoints of ≤1 mg/L and ≤2 mg/L from BMD method for 230 carbapenem-resistant *E. coli* or *K. pneumoniae* isolates

MIC (mg/L) from AD	No.	BMD: MIC ≤1 mg/L = susceptible		BMD: MIC ≤2 mg/L = susceptible	
		Predict susceptible	Predict resistant	Predict susceptible	Predict resistant
0.5	12	100% (12/12)	0.0%	100% (12/12)	0.0%
1	36	94.4% (34/36)	5.6% (2/36)	100% (36/36)	0.0%
2	1	0.0%	100% (1/1)	100% (1/1)	0.0%
4	10	0.0%	100% (10/10)	0.0%	100% (10/10)
8	15	0.0%	100% (15/15)	0.0%	100% (15/15)
16	17	0.0%	100% (17/17)	0.0%	100% (17/17)
32	26	0.0%	100% (26/26)	0.0%	100% (26/26)
64	58	0.0%	100% (58/58)	0.0%	100% (58/58)
≥128	55	0.0%	100% (55/55)	0.0%	100% (55/55)

MIC = minimum inhibitory concentration; AD = agar dilution method; BMD = broth microdilution method; *E. coli* = *Escherichia coli*; *K. pneumoniae* = *Klebsiella pneumoniae*

breakpoints of 1 mg/L or less and 2 mg/L or less from BMD method.

Comparison of colistin MICs between BMD and AG methods for 326 E. coli and K. pneumoniae isolates

A scattergram of colistin MICs performed by BMD method and of colistin MICs performed by AG method for 326 *E. coli* and *K. pneumoniae* isolates is shown in Figure 3, with an ICC of 0.60 (95% CI 0.52 to 0.66). The performance of AG method for 326 *E. coli* and *K. pneumoniae* isolates when colistin MIC breakpoint of 1 mg/L or less from BMD method was considered susceptible to colistin is shown in Table 6. The ME value was 23.3% with no VMEs, and the EA and CA values were moderate. The Se was very low with a moderate NPV value, and the Sp and PPV values were both very high. The performance of AG method for 326 *E. coli* and *K. pneumoniae* isolates when a colistin MIC breakpoint of 2 mg/L or less from BMD method was considered susceptible to colistin is shown in Table 6. The VME and ME values were 1.2% and 4.3%, respectively, and the EA and CA values were moderate to high. The Se, Sp, PPV, and NPV values were all higher than 80%.

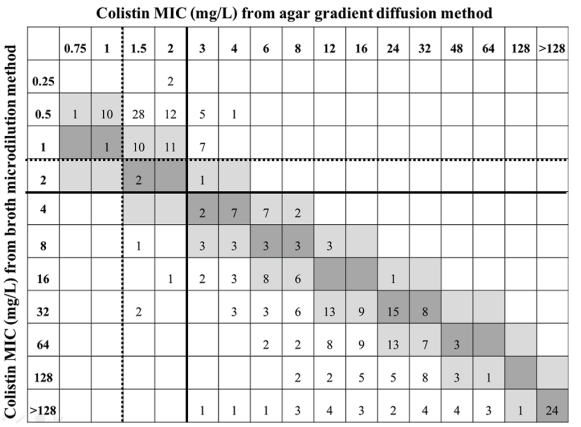

Figure 3. Scattergram of colistin MICs by BMD method and AG method for 326 *E. coli* or *K. pneumoniae* isolates.

The accuracy of colistin MICs from AG method for predicting colistin susceptibility at colistin MIC breakpoints of 1 mg/L or less and 2 mg/L or less from BMD method for 326 *E. coli* and *K. pneumoniae* isolates are shown in Table 7. Colistin MICs 2 mg/L or less and 4 mg/L or more from AG method were accurate for predicting colistin susceptibility at colistin MIC breakpoints of 1 mg/L or less and 2 mg/L or less from BMD method. However, colistin MIC 3 mg/L from AG method had 57.1% probability of susceptibility to colistin MIC breakpoint of 1 mg/L or less from BMD

Table 6. EA, CA, VME, ME, Se, Sp, PPV, and NPV values from the results of AG method at colistin MIC breakpoints of ≤1 mg/L and ≤2 mg/L from BMD method for 326 *E. coli* or *K. pneumoniae* isolates

Susceptibility criteria	EA	CA	VME	ME	Se	Sp	PPV	NPV
All isolates (n = 326)								
BMD: MIC ≤1 mg/L = susceptible	55.5%	76.7%	0.0%	23.3%	13.6%	100%	100%	75.8%
BMD: MIC ≤2 mg/L = susceptible		94.5%	1.2%	4.3%	84.4%	98.3%	95.0%	94.3%

EA = essential agreement; CA = categorical agreement; VME = very major error; ME = major error; Se = sensitivity; Sp = specificity; PPV = positive predictive value; NPV = negative predictive value; AG = agar gradient dilution method; MIC = minimum inhibitory concentration; BMD = broth microdilution method; *E. coli* = *Escherichia coli*; *K. pneumoniae* = *Klebsiella pneumoniae*

Table 7. Colistin MICs from AG method to predict colistin susceptibility at colistin MIC breakpoints of ≤1 mg/L and ≤2 mg/L from BMD method for 326 *E. coli* or *K. pneumoniae* isolates

MIC (mg/L) from AG	No.	BMD: MIC ≤1 mg/L = susceptible		BMD: MIC ≤2 mg/L = susceptible	
		Predict susceptible	Predict resistant	Predict susceptible	Predict resistant
≤1	12	100% (12/12)	0.0%	100% (12/12)	0.0%
1.5	43	88.4% (38/43)	11.6% (5/43)	93.0% (40/43)	7.0% (3/43)
2	26	96.2% (25/26)	3.8% (1/26)	96.2% (25/26)	3.8% (1/26)
3	21	57.1% (12/21)	42.9% (9/21)	61.9% (13/21)	38.1% (8/21)
4	18	5.6% (1/18)	94.4% (17/18)	5.6% (1/18)	94.4% (17/18)
6	24	0.0%	100% (24/24)	0.0%	100% (24/24)
8	24	0.0%	100% (24/24)	0.0%	100% (24/24)
12	30	0.0%	100% (30/30)	0.0%	100% (30/30)
16	26	0.0%	100% (26/26)	0.0%	100% (26/26)
24	36	0.0%	100% (36/36)	0.0%	100% (36/36)
32	27	0.0%	100% (27/27)	0.0%	100% (27/27)
48	10	0.0%	100% (10/10)	0.0%	100% (10/10)
64	4	0.0%	100% (4/4)	0.0%	100% (4/4)
≥128	25	0.0%	100% (25/25)	0.0%	100% (25/25)

MIC = minimum inhibitory concentration; AG = agar gradient dilution method; BMD = broth microdilution method; *E. coli* = *Escherichia coli*; *K. pneumoniae* = *Klebsiella pneumoniae*

method, and 61.9% probability of susceptibility to colistin MIC breakpoint of 2 mg/L or less from BMD method.

Comparison of colistin MICs between BMD and AG methods for 230 carbapenem-resistant E. coli and K. pneumoniae isolates

A scattergram of colistin MICs performed by BMD method and of colistin MICs performed by AG method for 230 carbapenem-resistant *E. coli* and *K. pneumoniae* isolates is shown in Figure 4, with an ICC of 0.57 (95% CI 0.48 to 0.65). The performance of AG method for 230 carbapenem-resistant *E. coli* and *K. pneumoniae* isolates when a colistin MIC breakpoint of 1 mg/L or less from BMD method was considered susceptible to colistin is shown in Table 8. The ME value was 18.7% with no VMEs, and the EA and CA values were moderate to high. The Se was very low with an NPV value more than 80%, and the Sp and PPV values were both very high. The performance of AG method for 230 carbapenem-resistant *E. coli* and *K. pneumoniae* isolates when a colistin MIC breakpoint of 2 mg/L or less from BMD method was considered susceptible to colistin is shown in Table 8. The VME and ME values were 1.7% and 3.5%, respectively, and

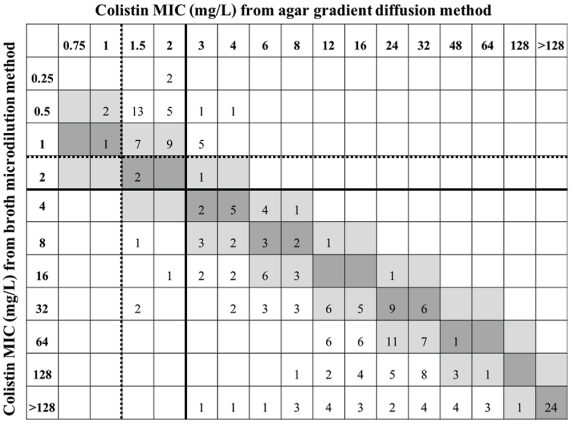

Figure 4. Scattergram of colistin MICs by BMD method and AG method for 230 carbapenem-resistant *E. coli* or *K. pneumoniae* isolates.

Table 8. EA, CA, VME, ME, Se, Sp, PPV, and NPV from the results of AG method at colistin MIC breakpoints of ≤1 mg/L and ≤2 mg/L from BMD method for 230 carbapenem-resistant *E. coli* or *K. pneumoniae* isolates

Susceptibility criteria	EA	CA	VME	ME	Se	Sp	PPV	NPV
Carbapenem-resistant isolates (n = 230)								
BMD: MIC ≤1 mg/L = susceptible	56.1%	81.3%	0.0%	18.7%	6.5%	100%	100%	81.1%
BMD: MIC ≤2 mg/L = susceptible		94.8%	1.7%	3.5%	83.7%	97.8%	91.1%	95.7%

EA = essential agreement; CA = categorical agreement; VME = very major error; ME = major error; Se = sensitivity; Sp = specificity; PPV = positive predictive value; NPV = negative predictive value; AG = agar gradient dilution method; MIC = minimum inhibitory concentration; BMD = broth microdilution method; *E. coli* = *Escherichia coli*; *K. pneumoniae* = *Klebsiella pneumoniae*

Table 9. Colistin MICs from AG method to predict colistin susceptibility at colistin MIC breakpoints of ≤1 mg/L and ≤2 mg/L from BMD method for 230 carbapenem-resistant *E. coli* or *K. pneumoniae* isolates

MIC (mg/L) from AG	No.	BMD: MIC ≤1 mg/L = susceptible		BMD: MIC ≤2 mg/L = susceptible	
		Predict susceptible	Predict resistant	Predict susceptible	Predict resistant
≤1	3	100% (3/3)	0.0%	100% (3/3)	0.0%
1.5	25	80.0% (20/25)	20.0% (5/25)	88.0% (22/25)	12.0% (3/25)
2	17	94.1% (16/17)	5.9% (1/17)	94.1% (16/17)	5.9% (1/17)
3	15	40% (6/15)	60% (9/15)	46.7% (7/15)	53.3% (8/15)
4	13	7.7% (1/13)	92.3% (12/13)	7.7% (1/13)	92.3% (12/13)
6	17	0.0%	100% (17/17)	0.0%	100% (17/17)
8	13	0.0%	100% (13/13)	0.0%	100% (13/13)
12	19	0.0%	100% (19/19)	0.0%	100% (19/19)
16	18	0.0%	100% (18/18)	0.0%	100% (18/18)
24	28	0.0%	100% (28/28)	0.0%	100% (28/28)
32	25	0.0%	100% (25/25)	0.0%	100% (25/25)
48	8	0.0%	100% (8/8)	0.0%	100% (8/8)
64	4	0.0%	100% (4/4)	0.0%	100% (4/4)
≥128	25	0.0%	100% (25/25)	0.0%	100% (25/25)

MIC = minimum inhibitory concentration; AG = agar gradient dilution method; BMD = broth microdilution method; *E. coli* = *Escherichia coli*; *K. pneumoniae* = *Klebsiella pneumoniae*

the EA and CA values were moderate to high. The Se, Sp, PPV, and NPV values were all higher than 80%.

The accuracy of colistin MICs from AG method for predicting colistin susceptibility at colistin MIC breakpoints of 1 mg/L or less and 2 mg/L or less from BMD method for 230 carbapenem-resistant *E. coli* and *K. pneumoniae* isolates is shown in Table 9. Colistin MICs 2 mg/L or less and 4 mg/L or more from AG method were accurate for predicting colistin susceptibility at colistin MIC breakpoints of 1 mg/L or less and 2 mg/L or less from BMD method. However, colistin MIC 3 mg/L from AG method had 40% probability of susceptibility to colistin MIC breakpoint of 1 mg/L or less from BMD method, and 46.7% probability of susceptibility to colistin MIC breakpoint of 2 mg/L or less from BMD method.

Comparison of colistin MICs from BMD method and inhibition zone diameters from DD method for 326 E. coli and K. pneumoniae isolates

A scattergram of colistin MICs performed by BMD method and the inhibition zone diameters from DD method for 326 *E. coli* and *K. pneumoniae* isolates is shown in Figure 5, with a negative correlation coefficient (Spearman's rank correlation coefficient)

Inhibition zone diameter (mm) of colistin disk from disk diffusion method

Colistin MIC (mg/L) from broth microdilution method	6	7	8	9	10	11	12	13	14	15	16
0.25									1	1	
0.5							15	26	14	2	
1							2	11	10	5	1
2							1	1	1		
4						8	6	3	1		
8					3	6	1	4	2		
16			1	1	2	11	4	2			
32		3	4	15	17	17	1	2			
64	4	4	7	11	10	6	2				
128	3	7	6	6	1	1	2				
>128	16	8	10	4		1	5	7			

Figure 5. Scattergram of colistin MICs by BMD method, and inhibition zone diameters from DD method for 326 *E. coli* or *K. pneumoniae* isolates .

Table 10. CA, VME, ME, Se, Sp, PPV, and NPV values from the results of DD method at colistin MIC breakpoints of ≤1 mg/L and ≤2 mg/L from BMD method for 326 *E. coli* or *K. pneumoniae* isolates

Susceptibility criteria (zone diameter)	BMD: MIC ≤1 mg/L = susceptible							BMD: MIC ≤2 mg/L = susceptible						
	CA	VME	ME	Se	Sp	PPV	NPV	CA	VME	ME	Se	Sp	PPV	NPV
≥11 mm = susceptible	70.9%	29.1%	0.0%	100%	60.1%	48.1%	100%	71.8%	28.2%	0.0%	100%	60.9%	49.7%	100%
≤10 mm = resistant				60.1%	100%	100%	48.1%				60.9%	100%	100%	49.7%
≥12 mm = susceptible	86.2%	13.8%	0.0%	100%	81.1%	66.2%	100%	87.1%	12.9%	0.0%	100%	82.1%	68.4%	100%
≤11 mm = resistant				81.1%	100%	100%	66.2%				82.1%	100%	100%	68.4%
≥13 mm = susceptible	87.7%	7.1%	5.2%	80.7%	90.3%	75.5%	92.7%	88.0%	6.4%	5.5%	80.2%	91.1%	77.7%	92.2%
≤12 mm = resistant				90.3%	80.7%	92.7%	75.5%				91.1%	80.2%	92.2%	77.7%
≥14 mm = susceptible	82.2%	1.2%	16.6%	38.6%	98.3%	89.5%	81.3%	81.9%	0.9%	17.2%	38.5%	98.7%	92.1%	80.6%
≤13 mm = resistant				98.3%	38.6%	81.3%	89.5%				98.7%	38.5%	80.6%	92.1%

EA = essential agreement; CA = categorical agreement; VME = very major error; ME = major error; Se = sensitivity; Sp = specificity; PPV = positive predictive value; NPV = negative predictive value; DD = disk diffusion method; MIC = minimum inhibitory concentration; BMD = broth microdilution method; *E. coli* = *Escherichia coli*; *K. pneumoniae* = *Klebsiella pneumoniae*

Table 11. Colistin inhibition zone diameters from DD method to predict colistin susceptibility at colistin MIC breakpoints of ≤1 mg/L and ≤2 mg/L from BMD method for 326 *E. coli* or *K. pneumoniae* isolates

DD (mm)	No.	BMD: MIC ≤1 mg/L = susceptible		BMD: MIC ≤2 mg/L = susceptible	
		Predict susceptible	Predict resistant	Predict susceptible	Predict resistant
6	23	0.0%	100% (23/23)	0.0%	100% (23/23)
7	22	0.0%	100% (22/22)	0.0%	100% (22/22)
8	28	0.0%	100% (28/28)	0.0%	100% (28/28)
9	37	0.0%	100% (37/37)	0.0%	100% (37/37)
10	33	0.0%	100% (33/33)	0.0%	100% (33/33)
11	50	0.0%	100% (100/100)	0.0%	100% (100/100)
12	39	43.6% (17/39)	56.4% (22/39)	46.2% (18/39)	53.8% (21/39)
13	56	66.1% (37/56)	33.9% (19/56)	67.9% (38/56)	32.1% (18/56)
14	29	86.2% (25/29)	13.8% (4/29)	89.7% (26/29)	10.3% (3/29)
15	8	100% (8/8)	0.0%	100% (8/8)	0.0%
16	1	100% (1/1)	0.0%	100% (1/1)	0.0%

DD = disk diffusion method; MIC = minimum inhibitory concentration; BMD = broth microdilution method; *E. coli* = *Escherichia coli*; *K. pneumoniae* = *Klebsiella pneumoniae*

of -0.77. The performance of each inhibition zone diameter of DD method for 326 *E. coli* and *K. pneumoniae* isolates when a colistin MIC breakpoint of 1 mg/L or less from BMD method was considered susceptible to colistin is shown in Table 10. When an inhibition zone diameter of 11 mm or more was considered susceptible to colistin, the VME value was 29.1% with no MEs, and the CA value was moderate. The Se and NPV values were very high, and the Sp and PPV values were moderate (60.1% versus 48.1%, respectively). When an inhibition zone diameter of 12 mm or more was considered susceptible to colistin, the VME value was 13.8% with no MEs, and the CA value greater than 80%. The Se and NPV values were very high, and the Sp and PPV values were moderate to high (88.1% versus 66.2%, respectively). When an inhibition zone diameter of 13 mm or more was considered susceptible to colistin, the VME and ME values were 7.1% and 5.2%, respectively, and the CA value was more than 80%. The Se, Sp, and NPV values were all higher than 80%, and the PPV value was 75.5%. When an inhibition zone diameter of 14 mm or more was considered susceptible to colistin, the VME and ME values were 1.2% and 16.6%, respectively, and the CA value was more than 80%. The Se value was very low, and the Sp, PPV, and NPV values were higher than 80%.

The performance of each inhibition zone diameter of DD method for 326 *E. coli* and *K. pneumoniae* isolates when a colistin MIC breakpoint of 2 mg/L or less from BMD method was considered susceptible to colistin is shown in Table 10. When an inhibition zone diameter of 11 mm or more was considered susceptible to colistin, the VME value was 28.2%

Table 12. CA, VME, ME, Se, Sp, PPV, and NPV from the results of DD method at colistin MIC breakpoints of ≤1 mg/L and ≤2 mg/L from BMD method for 230 carbapenem-resistant *E. coli* or *K. pneumoniae* isolates

Susceptibility criteria (zone diameter)	BMD: MIC ≤1 mg/L = susceptible							BMD: MIC ≤2 mg/L = susceptible						
	CA	VME	ME	Se	Sp	PPV	NPV	CA	VME	ME	Se	Sp	PPV	NPV
≥11 mm = susceptible	67.8%	32.2%	0.0%	100%	59.8%	38.3%	100%	69.1%	30.9%	0.0%	100%	60.8%	40.8%	100%
≤10 mm = resistant				59.8%	100%	100%	38.3%				60.8%	100%	100%	40.8%
≥12 mm = susceptible	82.6%	17.4%	0.0%	100%	78.3%	53.5%	100%	83.9%	16.1%	0.0%	100%	79.6%	57.0%	100%
≤11 mm = resistant				78.3%	100%	100%	53.5%				79.6%	100%	100%	57.0%
≥13 mm = susceptible	89.1%	10%	0.9%	95.7%	87.5%	65.7%	98.8%	89.6%	9.1%	1.3%	93.9%	88.4%	68.7%	98.2%
≤12 mm = resistant				87.5%	95.7%	98.8%	65.7%				88.4%	93.9%	98.2%	68.7%
≥14 mm = susceptible	92.2%	1.7%	6.1%	69.6%	97.8%	88.9%	92.8%	91.7%	1.3%	7.0%	67.3%	98.3%	91.7%	91.8%
≤13 mm = resistant				97.8%	69.6%	92.8%	88.9%				98.3%	67.3%	91.8%	91.7%

EA = essential agreement; CA = categorical agreement; VME = very major error; ME = major error; Se = sensitivity; Sp = specificity; PPV = positive predictive value; NPV = negative predictive value; DD = disk diffusion method; MIC = minimum inhibitory concentration; BMD = broth microdilution method; *E. coli* = *Escherichia coli*; *K. pneumoniae* = *Klebsiella pneumoniae*

with no MEs, and the CA value was moderate. The Se and NPV values were very high, and the Sp and PPV values were moderate (60.9% versus 49.7%, respectively). When an inhibition zone diameter of 12 mm or more was considered susceptible to colistin, the VME value was 12.9% with no MEs, and the CA value was more than 80%. The Se and NPV values were very high, and the Sp and PPV values were moderate to high (82.1% versus 68.4%, respectively). When an inhibition zone diameter of 13 mm or more was considered susceptible to colistin, the VME and ME values were 6.4% and 5.5%, respectively, and the CA value was higher than 80%. The Se, Sp, and NPV values were higher than 80%, and the PPV value was 77.7%. When an inhibition zone diameter of 14 mm or more was considered susceptible to colistin, the VME and ME values were 0.9% and 17.2%, respectively, and the CA value was more than 80%. The Se value was very low, and the Sp, PPV, and NPV values were all higher than 80%.

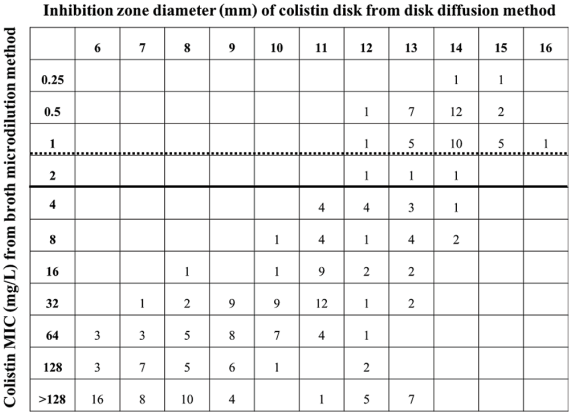

Figure 6. Scattergram of colistin MICs by BMD method, and inhibition zone diameters from DD method for 230 carbapenem-resistant *E. coli* or *K. pneumoniae* isolates.

The accuracy of colistin inhibition zone diameters from DD method for predicting colistin susceptibility at colistin MIC breakpoints of 1 mg/L or less and 2 mg/L or less from BMD method for 326 *E. coli* and *K. pneumoniae* isolates is shown in Table 11. Colistin inhibition zone diameters of 11 mm or less or 14 mm or more from DD method were accurate for predicting colistin susceptibility at colistin MICs of 1 mg/L or less and 2 mg/L or less from BMD method. However, inhibition zone diameters 12 mm and 13 mm had 43.6% to 66.1% probability of susceptibility to colistin at MIC 1 mg/L or less from BMD method, and had 46.2% to 67.9% probability of susceptibility to colistin at MIC 2 mg/L or less from BMD method.

Comparison of colistin MICs from BMD method and inhibition zone diameters from DD method for 230 carbapenem-resistant E. coli and K. pneumoniae isolates

A scattergram of colistin MICs performed by BMD method and the inhibition zone diameters from DD method for 230 carbapenem-resistant *E. coli* and *K. pneumoniae* isolates is shown in Figure 6, with a negative correlation coefficient (Spearman's rank correlation coefficient) of -0.76. The performance of each inhibition zone diameter of the DD method for 230 carbapenem-resistant *E. coli* and *K. pneumoniae* isolates when a colistin MIC breakpoint of 1 mg/L or less from BMD method was considered susceptible to colistin is shown in Table 12. When an inhibition zone diameter of 11 mm or more was considered susceptible to colistin, the VME value was 32.2% with no MEs, and the CA value was moderate. The

Table 13. Colistin inhibition zone diameters from DD method to predict colistin susceptibility at colistin MIC breakpoints of ≤1 mg/L and ≤2 mg/L from BMD method for 230 carbapenem-resistant *E. coli* or *K. pneumoniae* isolates

DD (mm)	No.	BMD: MIC ≤1 mg/L = susceptible		BMD: MIC ≤2 mg/L = susceptible	
		Predict susceptible	Predict resistant	Predict susceptible	Predict resistant
6	22	0.0%	100% (22/22)	0.0%	100% (22/22)
7	19	0.0%	100% (19/19)	0.0%	100% (19/19)
8	23	0.0%	100% (23/23)	0.0%	100% (23/23)
9	27	0.0%	100% (27/27)	0.0%	100% (27/27)
10	19	0.0%	100% (19/19)	0.0%	100% (19/19)
11	34	0.0%	100% (34/34)	0.0%	100% (34/34)
12	19	10.5% (2/19)	89.5% (17/19)	15.8% (3/19)	84.2% (16/19)
13	31	38.7% (12/31)	61.3% (19/31)	41.9% (13/31)	58.1% (18/31)
14	27	85.2% (23/27)	14.8% (4/27)	88.9% (24/27)	11.1% (3/27)
15	8	100% (8/8)	0.0%	100% (8/8)	0.0%
16	1	100% (1/1)	0.0%	100% (1/1)	0.0%

DD = disk diffusion method; MIC = minimum inhibitory concentration; BMD = broth microdilution method; *E. coli* = *Escherichia coli*; *K. pneumoniae* = *Klebsiella pneumoniae*

Se and NPV values were very high, and the Sp and PPV values were moderate (59.8% versus 38.3%, respectively). When an inhibition zone diameter of 12 mm or more was considered susceptible to colistin, the VME value was 17.4% with no MEs, and the CA value was greater than 80%. The Se and NPV values were very high, and the Sp and PPV values were moderate to high (78.3% versus 53.5%, respectively). When an inhibition zone diameter of 13 mm or more was considered susceptible to colistin, the VME and ME values were 10% and 0.9%, respectively, and the CA value was greater than 80%. The Se, Sp, and NPV values were higher than 80%, and the PPV value was 65.7%. When an inhibition zone diameter of 14 mm or more was considered susceptible to colistin, the VME and ME values were 1.7% and 6.1%, respectively, and the CA value was higher than 90%. The Se value was moderate (69.6%), and the Sp, PPV, and NPV values were all higher than 80%.

The performance of each inhibition zone diameter of the DD method for 230 carbapenem-resistant *E. coli* and *K. pneumoniae* isolates when a colistin MIC breakpoint of 2 mg/L or less from BMD method was considered susceptible to colistin is shown in Table 12. When an inhibition zone diameter of 11 mm or more was considered susceptible to colistin, the VME value was 30.9% with no MEs, and the CA value was moderate. The Se and NPV values were very high, and the Sp and PPV values were moderate (60.8% versus 40.8%, respectively). When an inhibition zone diameter of 12 mm or more was considered susceptible to colistin, the VME value was 16.1% with no MEs, and the CA value was greater than 80%. The Se and NPV values were very high, and the Sp and PPV values were moderate to high (79.6% versus 57%, respectively). When an inhibition zone diameter of 13 mm or more was considered susceptible to colistin, the VME and ME values were 9.1% and 1.3%, respectively, and the CA value was higher than 80%. The Se, Sp, and NPV values were higher than 80%, and the PPV value was 68.7%. When an inhibition zone diameter of 14 mm or more was considered susceptible to colistin, the VME and ME values were 1.3% and 7%, respectively, and the CA value was more than 90%. The Se value was a moderate 67.3%, and the Sp, PPV, and NPV values were all higher than 90%.

The accuracy of colistin inhibition zone diameters from the DD method to predict colistin susceptibility at colistin MIC breakpoints of 1 mg/L or less and 2 mg/L or less from BMD method for 230 carbapenem-resistant *E. coli* and *K. pneumoniae* isolates is shown in Table 13. Colistin inhibition zone diameters of ≤11 mm or ≥14 mm from DD method were accurate for predicting colistin susceptibility at colistin MICs breakpoints of 1 mg/L or less and 2 mg/L or less from BMD method. However, inhibition zone diameters 12 mm and 13 mm had 10.5% to 38.7% probability of susceptibility to colistin at MIC 1 mg/L or less from BMD method, and 15.8% to 41.9% probability of susceptibility to colistin at MIC 2 mg/L or less from BMD method.

Discussion

Only *E. coli* and *K. pneumoniae* isolates were included in the present study, because they are the most common species of Enterobacteriaceae that

cause infections in humans. The authors included many isolates of *E. coli* and *K. pneumoniae* that are resistant to carbapenems, because polymyxins are only indicated for treatment of infections caused by carbapenem-resistant *E. coli* and *K. pneumoniae*. Seventy percent of the isolates in the present study were carbapenem-resistant *E. coli* and *K. pneumoniae*. Since species of Enterobacteriaceae other than *E. coli* and *K. pneumoniae* were not included in the present study, the in vitro colistin susceptibility results reported herein may not be generalizable to other species of Enterobacteriaceae.

Colistin MICs determined by BMD method are used as reference values for colistin susceptibility according to the recommendations of CLSI[21] and EUCAST[22]. Two sets of colistin susceptibility breakpoints (MIC 1 mg/L or less for susceptibility and 2 mg/L or more for resistance; and, MIC 2 mg/L or less for susceptibility and 4 mg/L or more for resistance) were used for comparative data analysis in the present study. Two value sets were selected because the conventional colistin MIC breakpoint of 2 mg/L or less for susceptibility and 4 mg/L or more for resistance recommended by CLSI and EUCAST may be too high since the colistin plasma level at steady state in patients who received colistin at the recommended dosing ranged from 2 to 2.5 mg/L[23-25]. Therefore, a colistin MIC breakpoint of 1 mg/L or less for susceptibility and 2 mg/L or more for resistance may be more appropriate.

The MICs of colistin determined by AD method are in nearly perfect agreement with those from BMD method, and they have very high performance for predicting colistin susceptibility of carbapenem-susceptible and carbapenem-resistant *E. coli* and *K. pneumoniae* isolates with negligible VME and no ME. Therefore, the AD method should be considered an accurate and reliable alternative method to BMD method for determination of colistin MICs against carbapenem-susceptible and carbapenem-resistant *E. coli* and *K. pneumoniae*.

The MICs of colistin determined by AG method were found to be in moderate agreement with those from BMD method, but they have high ME and low performance for predicting colistin susceptibility when colistin MIC 1 mg/L or less for susceptibility and 2 mg/L or more for resistance breakpoint values from the BMD method were used. However, the MIC values of 2 mg/L or less and 4 mg/L or more from the AG method were found to be useful for predicting colistin susceptibility and colistin resistance, respectively. Colistin MIC value of 3 mg/L in the AG method should not be used since that MIC value was shown to have low predictive value for colistin susceptibility. If the AG method is the only available method for colistin susceptibility testing of *E. coli* and *K. pneumoniae*, the MIC values of 2 mg/L or less and 4 mg/L or more can be used as a guide to provide or withhold colistin treatment for infections due to carbapenem-resistant *E. coli* and *K. pneumoniae*.

The inhibition zone diameters of colistin determined by DD method were also found to be in moderate agreement with the colistin MICs determined by BMD method, but some inhibition zone diameter values have high VME and/or ME, and low performance for predicting colistin susceptibility. However, the inhibition zone diameters of colistin 11 mm or less and 14 mm or more were shown to be useful for predicting colistin resistance and colistin susceptibility, respectively. However, the inhibition zone diameters of colistin of 12 mm and 13 mm should not be used since these diameter values were found to have low predictive value for colistin susceptibility. If the DD method has to be used for colistin susceptibility testing of *E. coli* and *K. pneumoniae*, the inhibition zone diameters of 11 mm or less and 14 mm or more can be used as a guide to withhold or provide colistin treatment for infections caused by carbapenem-resistant *E. coli* and *K. pneumoniae*.

Based on the aforementioned observations from in vitro colistin susceptibility testing for *E. coli* or *K. pneumoniae* using the AG and DD methods, the authors partnered with the microbiology laboratory at Siriraj Hospital to perform colistin susceptibility testing for CRE and to report the results as a MIC value from the AG method or inhibition zone diameter for the DD method without interpretation if such isolate was susceptible or resistant to colistin since neither CLSI nor EUCAST have recommended breakpoints relative to colistin susceptibility results from the AG and DD methods. However, the authors report the findings of our investigation and offer suggestions regarding how to interpret colistin MIC determined by AG, and the inhibition zone diameter from DD. Specifically, if the colistin MIC determined by AG is 2 mg/L or less or the inhibition zone diameters of colistin are 14 mm or more for carbapenem-resistant *E. coli* or *K. pneumoniae*, the evaluated isolate is very likely to be susceptible to colistin. In contrast, a MIC of 4 mg/L or more by AG or inhibition zone diameters of colistin 11 mm or less from DD suggest a high likelihood of colistin resistance. However, if the colistin MIC determined by AG is 3 mg/L, or the inhibition zone diameters of colistin are

12 or 13 mm for carbapenem-resistant *E. coli* or *K. pneumoniae*, there is only a 46.2% to 67.9% probability that that isolate will be susceptible to colistin.

Previous reports on the performance of colistin susceptibility testing in Enterobacteriaceae performed by the AG and DD methods compared with the BMD method found the AG method to have VME values of 7% to 39.3% and ME values of 2.4% to 5.9%[19,24], whereas the DD method had VME and ME values of 1% to 44%[26-29]. Those studies concluded that both methods were unreliable for detecting colistin resistance in Enterobacteriaceae, but that they might be useful for initial screening in diagnostic laboratories; however, they did not provide any suggested criteria for interpretation of the test results.

In vitro colistin susceptibility testing for Enterobacteriaceae could be performed by Rapid Polymyxin NP test[30-32]. The results from a locally-made version of the Rapid Polymyxin NP test were compared with the colistin MICs determined by BMD method in 327 non-duplicate isolates of Enterobacteriaceae (37 *E. coli* and 280 *K. pneumoniae*) that were recovered from patients hospitalized at Siriraj Hospital[33]. The locally-made Rapid Polymyxin NP test was shown to be an accurate, convenient, and inexpensive method for rapid detection of colistin susceptibility in Enterobacteriaceae isolates, with Se, Sp, PPV, and NPV values for detecting 231 carbapenem-resistant *E. coli* or *K. pneumoniae* isolates of 100%, 95.9%, 98.9%, and 100%, respectively, with only 0.9% ME and no VME.

Another method that can be used to detect colistin resistance to Enterobacteriaceae is molecular method to detect resistance genes, including the mcr-1 gene[20,34]. However, this method is sophisticated and it cannot detect all colistin-resistant genes in Enterobacteriaceae.

Conclusion

Although AG and DD are not recommended for colistin susceptibility testing for Enterobacteriaceae by CLSI and EUCAST, the colistin MIC measured by the AG method (2 mg/L or less and 4 mg/L or more), and the inhibition zone diameters of colistin disk by DD method (inhibition zone diameters 14 mm or more and 11 mm or less) are useful for determining colistin susceptibility in carbapenem-resistant *E. coli* and *K. pneumoniae* in clinical settings where colistin MIC measured by BMD method is not available.

What is already known on this topic?

The agar gradient diffusion and disk diffusion methods are not recommended for in vitro colistin susceptibility testing due to poor diffusion of colistin into the agar. BMD is considered the reference method for colistin susceptibility testing of *E. coli* and *K. pneumoniae*. However, the BMD method for colistin susceptibility testing is not available in almost all microbiology laboratories in Thailand.

What this study adds?

Although they are not recommended by CLSI and EUCAST guidelines, the agar gradient diffusion and disk diffusion methods were still found to be useful for colistin susceptibility testing in a resource-limited setting. Colistin MICs measured by agar gradient diffusion method of 2 mg/L or less and 4 mg/L or more, and inhibition zone diameters by disk diffusion method of 14 mm or more and 11 mm or less can be used to determine colistin susceptibility in carbapenem-resistant *E. coli* and *K. pneumoniae* in clinical settings where colistin MIC measured by BMD method is not available.

Acknowledgement

The authors gratefully acknowledge Asst. Prof. Dr. Chulaluk Komoltri of the Division of Clinical Epidemiology, Department of Research and Development, Faculty of Medicine Siriraj Hospital, Mahidol University for assistance with statistical analysis.

Funding disclosure

This study was funded by grants from the Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, and the Health Systems Research Institute, Nonthaburi, Thailand.

Potential conflicts of interest

The authors declare no conflict of interest.

References

1. Chayakulkeeree M, Junsriwong P, Keerasuntonpong A, Tribuddharat C, Thamlikitkul V. Epidemiology of extended-spectrum beta-lactamase producing gram-negative bacilli at Siriraj Hospital, Thailand, 2003. Southeast Asian J Trop Med Public Health 2005;36:1503-9.
2. Apisarnthanarak A, Kiratisin P, Saifon P, Kitphati R, Dejsirilert S, Mundy LM. Clinical and molecular epidemiology of community-onset, extended-spectrum beta-lactamase-producing *Escherichia coli* infections in Thailand: a case-case-control study. Am J Infect Control 2007;35:606-12.

3. Quan J, Zhao D, Liu L, Chen Y, Zhou J, Jiang Y, et al. High prevalence of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* in community-onset bloodstream infections in China. J Antimicrob Chemother 2017;72:273-80.
4. Paterson DL, Ko WC, Von Gottberg A, Mohapatra S, Casellas JM, Goossens H, et al. Antibiotic therapy for *Klebsiella pneumoniae* bacteremia: implications of production of extended-spectrum beta-lactamases. Clin Infect Dis 2004;39:31-7.
5. Tamma PD, Han JH, Rock C, Harris AD, Lautenbach E, Hsu AJ, et al. Carbapenem therapy is associated with improved survival compared with piperacillin-tazobactam for patients with extended-spectrum beta-lactamase bacteremia. Clin Infect Dis 2015;60:1319-25.
6. van Duin D, Perez F, Rudin SD, Cober E, Hanrahan J, Ziegler J, et al. Surveillance of carbapenem-resistant *Klebsiella pneumoniae*: tracking molecular epidemiology and outcomes through a regional network. Antimicrob Agents Chemother 2014;58:4035-41.
7. Centers for Disease Control and Prevention. Tracking CRE [Internet]. [updated 2018 Mar 3; cited 2018 Mar 30]. Available from: http://www.cdc.gov/hai/organisms/cre/TrackingCRE.html.
8. Albiger B, Glasner C, Struelens MJ, Grundmann H, Monnet DL. Carbapenemase-producing Enterobacteriaceae in Europe: assessment by national experts from 38 countries, May 2015. Euro Surveill 2015;20. doi: 10.2807/1560-7917.ES.2015.20.45.30062.
9. Kazi M, Drego L, Nikam C, Ajbani K, Soman R, Shetty A, et al. Molecular characterization of carbapenem-resistant Enterobacteriaceae at a tertiary care laboratory in Mumbai. Eur J Clin Microbiol Infect Dis 2015;34:467-72.
10. van Duin D, Doi Y. The global epidemiology of carbapenemase-producing Enterobacteriaceae. Virulence 2017;8:460-9.
11. Sirijatuphat R, Sripanidkulchai K, Boonyasiri A, Rattanaumpawan P, Supapueng O, Kiratisin P, et al. Implementation of global antimicrobial resistance surveillance system (GLASS) in patients with bacteremia. PLoS One 2018;13:e0190132.
12. Falagas ME, Tansarli GS, Karageorgopoulos DE, Vardakas KZ. Deaths attributable to carbapenem-resistant Enterobacteriaceae infections. Emerg Infect Dis 2014;20:1170-5.
13. Iovleva A, Doi Y. Carbapenem-Resistant Enterobacteriaceae. Clin Lab Med 2017;37:303-15.
14. Ni W, Cai X, Wei C, Di X, Cui J, Wang R, et al. Efficacy of polymyxins in the treatment of carbapenem-resistant Enterobacteriaceae infections: a systematic review and meta-analysis. Braz J Infect Dis 2015;19:170-80.
15. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Recommendations for MIC determination of colistin (polymyxin E) as recommended by the joint CLSI-EUCAST Polymyxin Breakpoints Working Group. Basel, Switzerland: EUCAST; 2016.
16. Hindler JA, Humphries RM. Colistin MIC variability by method for contemporary clinical isolates of multidrug-resistant Gram-negative bacilli. J Clin Microbiol 2013;51:1678-84.
17. Humphries RM. Susceptibility testing of the polymyxins: where are we now? Pharmacotherapy 2015;35:22-7.
18. Vasoo S. Susceptibility Testing for the Polymyxins: Two Steps Back, Three Steps Forward? J Clin Microbiol 2017;55:2573-82.
19. Dafopoulou K, Zarkotou O, Dimitroulia E, Hadjichristodoulou C, Gennimata V, Pournaras S, et al. Comparative evaluation of colistin susceptibility testing methods among carbapenem-nonsusceptible *Klebsiella pneumoniae* and *Acinetobacter baumannii* clinical isolates. Antimicrob Agents Chemother 2015;59:4625-30.
20. Poirel L, Jayol A, Nordmann P. Polymyxins: Antibacterial activity, susceptibility testing, and resistance mechanisms encoded by plasmids or chromosomes. Clin Microbiol Rev 2017;30:557-96.
21. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing, 27th ed. CLSI document M100-S27. Wayne, PA: CLSI; 2017.
22. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoint tables for interpretation of MICs and zone diameters, Version 8.0 [Internet]. 2018 [cited 2018 Jan 1]. Available from: http://www.eucast.org/clinical_breakpoints/.
23. Garonzik SM, Li J, Thamlikitkul V, Paterson DL, Shoham S, Jacob J, et al. Population pharmacokinetics of colistin methanesulfonate and formed colistin in critically ill patients from a multicenter study provide dosing suggestions for various categories of patients. Antimicrob Agents Chemother 2011;55:3284-94.
24. Chew KL, La MV, Lin RTP, Teo JWP. 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;55:2609-16.
25. Nation RL, Garonzik SM, Thamlikitkul V, Giamarellos-Bourboulis EJ, Forrest A, Paterson DL, et al. Dosing guidance for intravenous colistin in critically-ill patients. Clin Infect Dis 2017;64:565-71.
26. Gales AC, Reis AO, Jones RN. Contemporary assessment of antimicrobial susceptibility testing methods for polymyxin B and colistin: review of available interpretative criteria and quality control guidelines. J Clin Microbiol 2001;39:183-90.
27. Tan TY, Ng LS. Comparison of three standardized disc susceptibility testing methods for colistin. J Antimicrob Chemother 2006;58:864-7.
28. Behera B, Mathur P, Das A, Kapil A, Gupta B, Bhoi S, et al. Evaluation of susceptibility testing methods for polymyxin. Int J Infect Dis 2010; 14:e596-601.
29. 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;53:546-51.
30. Nordmann P, Jayol A, Poirel L. Rapid detection of polymyxin resistance in Enterobacteriaceae. Emerg Infect Dis 2016;22:1038-43.
31. Jayol A, Nordmann P, Lehours P, Poirel L, Dubois V. Comparison of methods for detection of plasmid-mediated and chromosomally encoded colistin resistance in Enterobacteriaceae. Clin Microbiol Infect 2018;24:175-9.
32. Poirel L, Larpin Y, Dobias J, Stephan R, Decousser JW, Madec JY, et al. Rapid Polymyxin NP test for the detection of polymyxin resistance mediated by the mcr-1/mcr-2 genes. Diagn Microbiol Infect Dis 2018;90:7-10.
33. Yainoy S, Hiranphan M, Phuadraksa T, Eiamphungporn W, Tiengrim S, Thamlikitkul V. Evaluation of the Rapid Polymyxin NP test for detection of colistin susceptibility in Enterobacteriaceae isolated from Thai patients. Diagn Microbiol Infect Dis 2018;92:102-6.
34. Bontron S, Poirel L, Nordmann P. Real-time PCR for detection of plasmid-mediated polymyxin resistance (mcr-1) from cultured bacteria and stools. J Antimicrob Chemother 2016;71:2318-20.