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ORIGINAL ARTICLE

Relationship between the distribution of cefepime minimum inhibitory concentrations and detection of extended-spectrum β-lactamase production among clinically important Enterobacteriaceae isolates obtained from patients in intensive care units in Taiwan: Results from the Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART) in 2007

Shio-Shin Jean [a], Wen-Sen Lee [b], Kuan-Jen Bai [c,d], Carlos Lam [a], Chin-Wang Hsu [e], Kwok-Woon Yu [f], Chun-Hsing Liao [g], Feng-Yi Chang [h], Wen-Chien Ko [i], Jiunn-Jong Wu [j], Yen-Hsu Chen [k,l], Yao-Shen Chen [m], Jien-Wei Liu [n], Min-Chi Lu [o], Cheng-Yi Liu [p], Ray-Jade Chen [a,d], Po-Ren Hsueh [q,r,*]

[a] *Department of Emergency Medicine, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan*
[b] *Division of Infectious Diseases, Department of Internal Medicine, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan*
[c] *Department of Pulmonary, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan*
[d] *Department of Critical Care Medicine, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan*
[e] *Department of Emergency and Critical Medicine, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan*
[f] *Department of Internal Medicine, Pathology, and Laboratory Medicine, Taipei Veterans General Hospital, National Yang-Ming University, Taipei, Taiwan*
[g] *Department of Internal Medicine, Far Eastern Memorial Hospital, Taipei, Taiwan*
[h] *Department of Internal Medicine, Tri-Service General Hospital, National Defense Medical Center (NDMC), Taipei, Taiwan*

* Corresponding author. Department of Laboratory Medicine, National Taiwan University Hospital, Number 7, Chung-Shan South Road, Taipei 100, Taiwan.
E-mail address: hsporen@ntu.edu.tw (P.-R. Hsueh).

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[i] *Department of Internal Medicine, National Cheng-Kung University Hospital, Tainan, Taiwan*
[j] *School of Medical Technology, National Cheng-Kung University College of Medicine, Tainan, Taiwan*
[k] *Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan*
[l] *Graduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan*
[m] *Department of Internal Medicine, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan*
[n] *Department of Internal Medicine, Kaohsiung Chang Gung Memorial Hospital, Chang Gung Medical College, Kaohsiung, Taiwan*
[o] *Department of Laboratory Medicine and Internal Medicine, Chung Shan Medical and Dental University, Taichung, Taiwan*
[p] *Division of Infectious Diseases, Department of Internal Medicine, Taipei Veterans General Hospital, Taipei, Taiwan*
[q] *Department of Laboratory Medicine, National Taiwan University Hospital, National Taiwan University College of Medicine, Taipei, Taiwan*
[r] *Department of Internal Medicine, National Taiwan University Hospital, National Taiwan University College of Medicine, Taipei, Taiwan*

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Background: The data on susceptibility of important cephalosporins against four Enterobacteriaceae members producing potential extended-spectrum β-lactamase (ESBL) collected from Taiwanese intensive care units are lacking.
Methods: Minimum inhibitory concentrations (MICs) of cefotaxime, ceftazidime, and cefepime were determined using agar dilution method, against *Escherichia coli* ($n = 344$), *Klebsiella pneumoniae* ($n = 359$), *Enterobacter cloacae* ($n = 103$), and *Proteus mirabilis* ($n = 78$). Susceptibilities of these isolates to three cephalosporins were assessed according to MIC breakpoints recommended by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) in 2013. The double-disk synergy test using disks containing cefepime (30 μg) with or without clavulanate (10 μg) was applied to confirm production of ESBL for isolates with cephalosporin MIC ≥2 μg/mL.
Results: A total of 175 isolates were verified as ESBL producers. The rates of cefepime susceptibility among the ESBL-producing isolates, according to CLSI (EUCAST) criteria, were 56.7% (22.4%) for *E. coli*, 61.3% (12.0%) for *K. pneumoniae*, 57.9% (31.6%) for *E. cloacae*, and 71.4% (7.1%) for *P. mirabilis*. Using different cefepime MIC breakpoints (MICs ≥16 μg/mL recommended by CLSI criteria and ≥2 μg/mL by EUCAST criteria) to define nonsusceptibility, we found that both criteria were poorer at predicting ESBL producers among *K. pneumoniae* and *E. cloacae* than among the other two species. In addition, we also found that the cefepime MIC level of 1.0 μg/mL best distinguished non-ESBL- from ESBL-producing *K. pneumoniae* and *E. cloacae*.
Conclusion: To detect ESBLs, CLSI should revise the cefepime MIC breakpoint against Enterobacteriaceae.

Introduction

During the past three decades, Gram-negative bacilli (GNBs) have gradually become resistant to a number of antibiotics.[1,2] Of greatest concern are the extended-spectrum β-lactamase (ESBL) producers. In 2010, the Clinical and Laboratory Standards Institute (CLSI) updated the breakpoints of minimum inhibitory concentrations (MICs) of third-generation cephalosporins against Enterobacteriaceae and omitted ESBL confirmation.[3] Nevertheless, detection of ESBL producers among clinical isolates of GNBs remains an important issue for infection control, especially in intensive care units (ICUs), where infections due to ESBL-producing bacteria are associated with dismal clinical outcome.[4,5]

Cefepime has been advocated as an alternative to carbapenems for the treatment of infections due to ESBL-producing GNBs.[6] However, Lee et al[7] demonstrated that cefepime, when used to treat patients with bacteremia caused by the ESBL-producing Enterobacteriaceae with cefepime MIC values of 2—8 μg/mL, was associated with high mortality. In addition, most studies on susceptibility of

ESBL-producing isolates to cefepime have focused on *Escherichia coli* and *Klebsiella* spp., and ignored *Enterobacter cloacae* and *Proteus mirabilis*,[8,9] which are also known to produce ESBLs.[10] Because of the inconsistency between *in vitro* susceptibility to cefepime and failure to eradicate ESBL-producing GNBs *in vivo*, we decided to investigate the cefepime MIC profile in Enterobacteriaceae with high potential for ESBL production.

The ongoing nationwide Surveillance for Monitoring Antimicrobial Resistance in Taiwan (SMART), initiated since 2000, was designed to monitor longitudinally the *in vitro* antimicrobial susceptibility of clinically important pathogens. In Taiwan, until 2011 the prevalence of important metallo-β-lactamases (VIM, IMP, NDM, etc.) was virtually low among Enterobacteriaceae.[11] In addition, *Klebsiella pneumoniae* carbapenemase-producing GNBs was first detected in 2009.[12] Therefore, we chose the ICU isolates of enteric GNBs collected in 2007 to survey the MIC distributions of third- and fourth-generation cephalosporins against ESBL-producing Enterobacteriaceae. Furthermore, with regard to predicting the ESBL production among four potentially ESBL-producing Enterobacteriaceae species, we used their cefepime MIC distribution patterns to estimate the sensitivity and specificity of different cefepime MIC nonsusceptibility breakpoints recommended in 2013. Finally, we applied the statistical analyses to find out the optimal cefepime MIC cutoff value for detecting the ESBL existence. This is part of the study of SMART 2007.

Methods

Bacterial isolates

The isolates evaluated in this study comprised 884 consecutive, nonduplicate Enterobacteriaceae isolates collected from various clinical specimens from patients (one isolate per patient) in ICUs at 10 major teaching hospitals in Taiwan (Northern Taiwan, $n = 4$; Central Taiwan, $n = 1$; Southern Taiwan, $n = 5$) from July 1, 2007 to December 31, 2007. The isolates included *E. coli* ($n = 344$), *K. pneumoniae* ($n = 359$), *E. cloacae* ($n = 103$), and *P. mirabilis* ($n = 78$). All isolates were stored at −70 °C in Trypticase soy broth (Difco Laboratories, Detroit, MI, USA) supplemented with 15% glycerol prior to testing. They were then transported to the National Taiwan University Hospital for further identification by standard methods. The isolates were identified using the Phoenix PMIC/ID-30 identification system (Becton Dickinson Systems, Sparks, MD, USA).

Antimicrobial susceptibility testing

The MICs of cefotaxime, ceftazidime, and cefepime against all isolates were determined by the agar dilution method according to CLSI guidelines.[3] *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality-control strains for each run of MIC tests. MIC testing was repeated if the results for ATCC strains were outside the expected range recommended by the CLSI.[3] The MIC breakpoint criteria recommended by the CLSI and European Committee on Antimicrobial Susceptibility Testing (EUCAST) in 2013[3,13] were applied to interpret the susceptibility profiles of bacteria under evaluation to cefotaxime, ceftazidime, and cefepime.

Detection of ESBL production

The potential ESBL producers (*E. coli*, *K. pneumoniae*, *E. cloacae*, and *P. mirabilis*) with phenotypically cefotaxime or ceftazidime MICs ≥2 μg/mL were tested for ESBL production. Traditional ESBL confirmation methods, using a disk containing 30-μg ceftazidime (or cefotaxime), alone or in combination with a 10-μg clavulanate disk, are known to have low sensitivity for detecting bacteria that produce AmpC β-lactamase.[14] Therefore, in this study we used the modified double-disk synergy test (DDST), which involves a disk containing 30 μg cefepime, with or without clavulanic acid (10-μg disk; with a center-to-center distance of 30 mm) instead of a disk containing 4-μg clavulanic acid,[15] to detect ESBL producers. The production of ESBL was considered positive if the diameter of the cefepime disk increased by ≥5 mm, or the zone expansion was ≥50% of the original size. The latter standard was suggested by M'Zali et al[16] who used third-generation cephalosporin ± clavulanate (10-μg) to detect ESBLs among enteric GNBs.

Statistical analysis

The sensitivity and specificity of detecting ESBL production among four ESBL-producing Enterobacteriaceae species (*E. coli*, *K. pneumoniae*, *E. cloacae*, and *P. mirabilis*) were calculated using CLSI 2013 and EUCAST 2013 MIC breakpoints for nonsusceptibility to cefepime. If low ESBL detection sensitivity was recognized for specific enteric GNBs, the accuracy of the cefepime MIC profile to predict ESBL was further investigated by calculating the area under the receiver operating characteristic (ROC) curve, which provides an overall index of diagnostic accuracy from a plot of sensitivity against the false-positive rate (i.e., 1 − specificity) for all cefepime MIC values. Then, the Youden index [sensitivity − (1 − specificity)] was calculated to determine the optimal MIC cutoff point, that is, selecting the maximum result of sensitivity plus specificity, which corresponded to a cefepime MIC level that best detected ESBL production. If appropriate, the Fisher exact test was applied to compare differences in percentages of categorical variables. A p value <0.05 was considered to indicate statistical significance. All analyses were conducted using the statistical package SPSS version 17 for Windows (SPSS Inc., Chicago, IL, USA).

Results

The rate of ESBL production among Enterobacteriaceae species in 2007 was 19.5% for *E. coli* ($n = 67$), 20.9% for *K. pneumoniae* ($n = 75$), 18.4% for *E. cloacae* ($n = 19$), and 17.9% for *P. mirabilis* ($n = 14$). A bar plot comparing the susceptibility of ESBL-producing GNBs species with that of non-ESBL-producing species to cefepime is shown in Fig. 1. Although all GNBs species, regardless of status of ESBL production, showed cefepime susceptibility rates greater than 55%, there were significant differences in rates of susceptibility to that of antibiotics among all members of

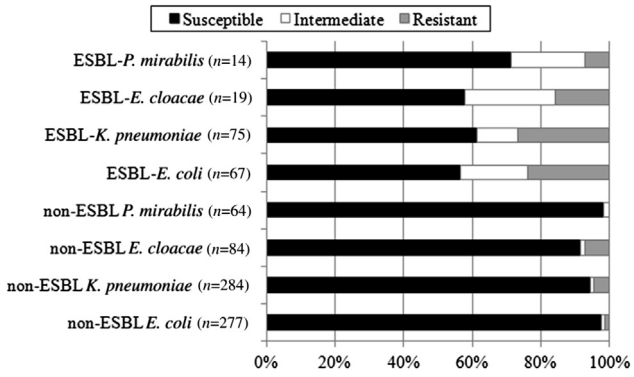

Figure 1. Proportions of susceptibilities of cefepime against ESBL- and non-ESBL-producing *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Proteus mirabilis*. ESBL = extended-spectrum β-lactamase.

the Enterobacteriaceae family. The MIC distributions of three antimicrobials against the four members of Enterobacteriaceae with ESBL production are illustrated in Table 1. We noted that marked differences between rates of susceptibility to cefepime based on CLSI 2013 criteria and rates based on EUCAST 2013 criteria existed among *E. coli*, *K. pneumoniae*, and *P. mirabilis*. In addition, more than 70% of the ESBL-producing *P. mirabilis* strains were susceptible to ceftazidime. The majority of isolates of all four species showed poor susceptibility to cefotaxime. Results of the DDST of all Enterobacteriaceae isolates that were tested positive for ESBL production revealed that the CLSI and EUCAST nonsusceptibility breakpoints for cefepime were less sensitive at detecting ESBL production among *K. pneumoniae* (60—68%) and *E. cloacae* (28—54%) than among the other two GNBs species (Table 2). In addition, better positive predictive values of EUCAST 2013 criteria for detecting ESBL production are noted among *E. coli*, *K. pneumoniae*, and *P. mirabilis* strains than those of CLSI 2013 criteria (Table 2).

Based on the results of the area under the ROC curve analysis, we found that cefepime MIC values were more predictive of ESBL production among isolates of *K. pneumoniae* [area under ROC curve: 0.892; 95% confidence interval (CI): 0.852—0.940] than among isolates of *E. cloacae* (area under ROC curve: 0.743; 95% CI: 0.622—0.864) (Fig. 2A and B). In addition, cefepime MIC values with a cutoff value ≥1.0 μg/mL provided the best compromise between sensitivity and specificity for detecting ESBLs in both *K. pneumoniae* and *E. cloacae* (Table 3).

Discussion

In this study, a number of ESBL-producing enteric GNBs isolates were found to be susceptible to cefepime. In addition, among the *E. cloacae* and *K. pneumoniae* isolates with ESBL production, the current CLSI and EUCAST cefepime MIC nonsusceptible breakpoints were not robust enough to differentiate between non-ESBL producers and ESBL producers. Among Enterobacteriaceae spp., although the CLSI does not consider the importance of ESBL detection, the EUCAST considers it mandatory for epidemiological purposes. We found that the rates of susceptibility to cefepime for ESBL-producing *E. coli* and *K. pneumoniae* species were significantly higher among isolates obtained from the patients in ICUs during 2007 in Taiwan (56.7% for *E. coli* and 61.3% for *K. pneumoniae*) than those among isolates collected from the patients in two recently reported surveys conducted in the Asia-Pacific region.[9,17]

In one study conducted in China, Wang et al[18] reported that CTX-M-type ESBLs were predominant amongst isolates of Enterobacteriaceae species (>90% for *E. coli* and *P. mirabilis*, and approximately two-thirds for *K. pneumoniae*), which showed a high degree of nonsusceptibility to

Table 1 Susceptibilities of 175 ESBL-producing Enterobacteriaceae isolates against three cephalosporins

Bacteria (no. of isolates)	Agent	No. of isolates with indicated MIC (μg/mL)														MIC (μg/mL)		Susceptible rates (%), evaluated by	
		0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128	50%	90%	CLSI 2013 (M100-S23)	EUCAST 2013
Escherichia coli (*n* = 67)	Cefotaxime	0	1	0	1	1	2	1	4	4	5	9	8	11	20	64	>128	7.5	7.5
	Ceftazidime	0	0	0	1	3	6	8	4	10	13	15	5	1	1	16	64	32.8*	14.9
	Cefepime	0	0	0	5	9	1	5	8	10	13	9	4	3	0	8	64	56.7*	22.4
Klebsiella pneumoniae (*n* = 75)	Cefotaxime	0	2	0	0	0	2	1	3	9	12	17	9	10	10	32	>128	5.3	5.3
	Ceftazidime	0	0	0	0	5	3	3	4	4	8	3	6	7	32	128	>128	20	10.7
	Cefepime	0	1	2	1	3	2	9	12	16	9	6	8	4	2	8	64	61.3*	12.0
Enterobacter cloacae (*n* = 19)	Cefotaxime	0	0	0	0	0	0	0	3	4	1	2	3	3	3	32	>128	0	0
	Ceftazidime	0	0	0	1	0	0	0	2	0	0	3	3	2	8	128	>128	15.8	5.3
	Cefepime	0	0	0	0	3	3	0	4	1	5	1	0	0	2	4	>128	57.9	31.6
Proteus mirabilis (*n* = 14)	Cefotaxime	0	0	0	0	0	0	1	3	3	4	2	0	1	0	8	32	0	0
	Ceftazidime	0	0	7	3	0	0	1	0	0	0	2	1	0	0	0.12	32	78.6	71.4
	Cefepime	0	0	0	0	1	0	0	6	3	3	1	0	0	0	4	16	71.4*	7.1

* $p < 0.05$.
CLSI = Clinical and Laboratory Standards Institute; ESBL = extended-spectrum β-lactamase; EUCAST = European Committee on Antimicrobial Susceptibility Testing; MIC = minimum inhibitory concentration.

Table 2 Performance parameters of estimating the sensitivity, specificity, positive, and negative predictive values after use of various nonsusceptibility MIC breakpoints to cefepime for detection of ESBL production, among 884 clinical isolates of Enterobacteriaceae (*Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Proteus mirabilis*)

Bacterial spp. (no. of isolates)	Judgment breakpoints (cefepime MIC, μg/mL)	True		False		Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
		Positive (no.)	Negative (no.)	Positive (no.)	Negative (no.)				
E. coli (344)	CLSI, R + I (non-S, ≥16)	29	271	38	6	82.9	87.7	62.0	95.5
	CLSI, R (≥32)	16	273	51	4	80.0	84.3	55.2	94.6
	EUCAST, non-S (≥2)	52	260	15	17	75.4	94.5	76.8	94.1
K. pneumoniae (359)	CLSI, R ± I (non-S, ≥16)	29	268	46	16	64.4	85.4	53.8	90.1
	CLSI, R (≥32)	20	271	55	13	60.6	83.1	48.6	88.9
	EUCAST, non-S (≥2)	66	253	9	31	68.0	96.6	84.1	92.0
E. cloacae (103)	CLSI, R ± I (non-S, ≥16)	8	77	11	7	53.3	87.5	49.1	89.2
	CLSI, R (≥32)	3	78	16	6	33.3	83.0	30.7	84.6
	CLSI, R (≥32)	13	52	6	32	28.8	89.7	38.7	84.8
P. mirabilis (78)	CLSI, R ± I (non-S, ≥16)	4	63	10	1	80.0	86.3	56.0	95.2
	CLSI, R (≥32)	1	64	13	0	100	81.0	53.4	100
	EUCAST, non-S (≥2)	13	61	1	3	81.3	98.4	89.9	96.0
Overall (884)	CLSI, R ± I (non-S, ≥16)	70	679	105	30	70.0	86.6	56.3	92.1
	CLSI, R (≥32)	40	686	135	23	63.5	83.6	48.9	90.3
	EUCAST, non-S (≥2)	144	626	83	83	63.4	88.3	57.2	90.7

CLSI = Clinical and Laboratory Standards Institute; EUCAST = European Committee on Antimicrobial Susceptibility Testing; I = intermediate; MIC = minimum inhibitory concentration; non-S = non-susceptible; R = resistant.

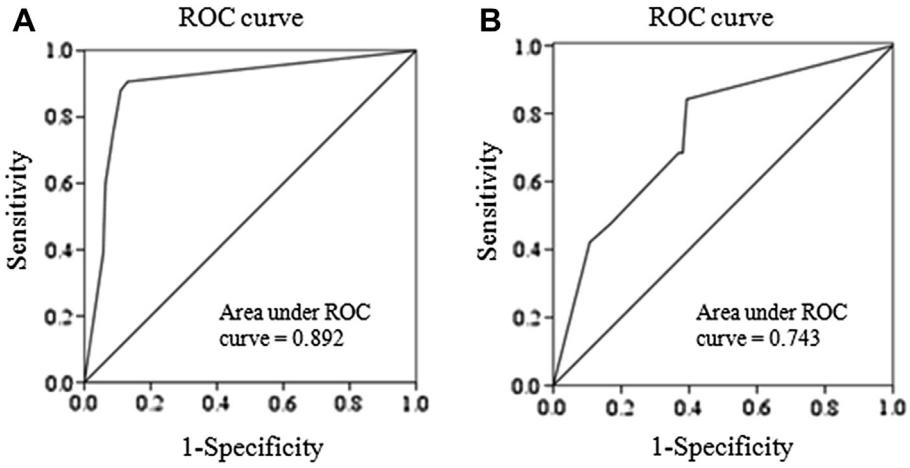

Figure 2. (A) Discrimination power (area under the receiver operating characteristic curve) of the distributions of cefepime minimum inhibitory concentrations (MICs) for predicting the extended-spectrum β-lactamase (ESBL) phenotype of *Klebsiella pneumoniae*, and (B) *Enterobacter cloacae* isolates. ROC = receiver operating characteristic.

cefepime. High rates of susceptibility to cefepime were also reported for ESBL-producing *E. coli* and *K. pneumoniae* in Taiwan, and for other ESBL producers without CTX-M predominance in the United States.[19] In addition, we found that 67% of ESBL-producing *E. cloacae* isolates were susceptible to cefepime, which is comparable with the results reported by Szabó et al.[20] Therefore, based on the present CLSI MIC interpretive criteria, the susceptibility to cefepime did not parallel with excluding the existence of ESBL production in Enterobacteriaceae. According to the pattern of cefepime MIC distributions of Enterobacteriaceae in this SMART study conducted in 2007, ESBLs of the non-CTX-M type sparing CTX-M-9 were dominant among the ESBL-producing isolates of *K. pneumoniae*,[18,21] and the majority of *E. cloacae* strains harbored the SHV type of β-lactamase. The findings regarding *E. cloacae* were similar to those reported in Taiwan by Yu et al[22] and Liu et al.[23] Besides, we observed that the *P. mirabilis* isolates in our study were highly susceptible to cefepime and ceftazidime, which is consistent with data reported by Wang et al.[18] Grossly, a substantial number of ESBL-producing species were sensitive to cefepime, which reflects the considerably variable hydrolyzing spectra of ESBLs against a variety of cephalosporins.

Table 3 The sensitivities, specificities, and Youden indexes of various categories of cefepime MICs for recognizing the existence of ESBL phenotype among *Klebsiella pneumoniae* and *Enterobacter cloacae* isolates

Cefepime MIC level (μg/mL)	Sensitivity	1 − Specificity	Youden index
K. pneumoniae			
<0.50	1.000	1.000	0.000
≥0.50	0.933	0.179	0.754
≥1.0	0.927	0.130	0.797
≥2.0	0.880	0.109	0.771
≥4.0	0.760	0.088	0.672
≥8.0	0.600	0.063	0.537
≥16.0	0.387	0.056	0.331
≥32.0	0.000	0.000	0.000
E. cloacae			
<0.50	1.000	1.000	0.000
≥0.50	0.861	0.441	0.420
≥1.0	0.842	0.393	0.449
≥2.0	0.684	0.381	0.303
≥4.0	0.684	0.369	0.315
≥8.0	0.474	0.167	0.307
≥16.0	0.421	0.107	0.314
≥32.0	0.000	0.000	0.000

CLSI = Clinical and Laboratory Standards Institute; ESBL = extended-spectrum β-lactamase; MIC = minimum inhibitory concentration.

In general, for *E. cloacae* and other Enterobacteriaceae species of potentially plasmidic AmpC β-lactamase producers (including *E. coli*, *P. mirabilis*, and *K. pneumoniae*),[24] cefepime is considered *in vitro* effective against them.[10] Polsfuss et al[8] found that DDST performed against cefepime plus clavulanate had an overall sensitivity of 96.6% and a specificity of 89.8% at detecting ESBL producers among Enterobacteriaceae species. Similar findings were also reported by Stürenburg et al.[14] By contrast, Tzelepi et al[15] demonstrated that the same DDST (spaced 30 mm) was not sensitive at detecting ESBL-producing *Enterobacter* species. However, they found that 10 (32%) of 31 *Enterobacter* isolates were non-SHV-type ESBL producers,[15] indicating that DDST performed against cefepime is not sensitive at detecting ESBL production for *Enterobacter* species. Reduction of the disk distance from 30 mm to 20 mm in DDST has been shown to improve the detection rate of ESBL among *Enterobacter* species.[15] Interestingly, we found that the current EUCAST MIC breakpoint of 1.0 μg/mL for cefepime increased the sensitivity of detecting ESBL producers among isolates of *E. cloacae* and *K. pneumoniae*, indicating that the CLSI should re-evaluate its MIC breakpoints for detecting ESBLs. Similar suggestion was also proposed by Chin et al[25] previously.

In conclusion, using the MIC nonsusceptibility criteria for cefepime recommended by CLSI and EUCAST in 2013, we found that both criteria had poor sensitivity in detecting ESBL production among *E. cloacae* and *K. pneumoniae* isolates, although the criteria provided acceptable sensitivity for detecting ESBL production among *E. coli* and *P. mirabilis* isolates. Analyses of the area under the ROC curve revealed that a cefepime MIC value of 1 μg/mL was a reliable cutoff level for differentiating ESBL-producing from non-ESBL-producing *K. pneumoniae* and *E. cloacae* isolates.

Conflicts of interest

All contributing authors declare no conflicts of interest.

Acknowledgments

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