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ORIGINAL ARTICLE

Comparison of commonly used antimicrobial susceptibility testing methods for evaluating susceptibilities of clinical isolates of *Enterobacteriaceae* and nonfermentative Gram-negative bacilli to cefoperazone–sulbactam

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Vitek 2 system

Abstract *Background/Purpose:* The aim of this study was to investigate the cefoperazone–sulbactam (CFP–SUL) susceptibilities of important Gram-negative bacteria (GNB) by agar dilution (reference method), disk diffusion, and two automated methods.
Methods: A total of 799 GNB isolates, including *Enterobacteriaceae* ($n = 500$) and nonfermentative GNB (NFGNB, $n = 299$), were recovered from various clinical specimens collected at National Taiwan University Hospital, Taipei, Taiwan from November 2013 to December 2014. The agar dilution method, disk diffusion method, and two automated susceptibility systems (Phoenix and Vitek 2) were used for testing susceptibility of the isolates to CFP–SUL.

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Categories of susceptibility (susceptible, intermediate, or resistant) to CFP—SUL yielded from each method were interpreted according to CFP—SUL interpretive breakpoints proposed previously. The results of categorical agreement and errors obtained between the agar dilution method and the other three methods were analyzed.

Results: The Vitek 2 system had the highest error rates against *Escherichia coli* ($n = 150$) and *Enterobacter cloacae* ($n = 77$) isolates, i.e., 6.7% and 11.7% minor errors, 8.5% and 1.7% major errors, and 40% and 20% very major errors, respectively. Additionally, the Vitek 2 system was also found to have a significantly lower sensitivity (44.4%) and lower positive predictive value (18.2%) for detecting CFP—SUL nonsusceptible *E. coli* isolates than other methods. For carbapenem-nonsusceptible *Enterobacteriaceae* isolates, the Vitek 2 system failed to detect correct susceptibility to CFP—SUL. The three methods failed to correctly detect CFP—SUL susceptibility categories against all NFGNB isolates except *Pseudomonas aeruginosa*.

Conclusion: The Vitek 2 system is a suboptimal method in correctly detecting CFP—SUL susceptibility categories for *E. coli*, *E. cloacae*, and carbapenem-nonsusceptible *Enterobacteriaceae* isolates.

Introduction

Antimicrobial drug resistance is a serious health care problem worldwide, and nosocomial infections due to multidrug-resistant (MDR) Gram-negative bacilli (GNB) are of particular concern.[1,2] Initiation of appropriate antibiotics for treatment of severe infections, however, depends on the rapid identification of the pathogen and an understanding of the susceptibility profiles of pathogens to various antimicrobials.

Resistance to major antibiotics is often conferred by the expression of β-lacatmases.[2,3] Sulbactam (SUL), an important β-lactamase inhibitor, was noted to possess good activity against some extended-spectrum β-lactamase (ESBL)-producing pathogens.[4] Furthermore, this agent has been shown to augment the activity of cefoperazone (CFP) against a variety of *Enterobacteriaceae* species (especially *Escherichia coli*, *Morganella morganii*, and *Klebsiella pneumoniae*) which include some AmpC β-lactamase producers as well (*Enterobacter cloacae*, *Enterobacter aerogenes*, and *Citrobacter freundii*),[5–7] and some important nonfermentative GNB species that are originally resistant to CFP.[7–11]

There are no recently validated minimum inhibitory concentration (MIC) interpretive breakpoints for CFP (with or without SUL) against GNB isolates by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing.[12–14] CFP itself has fallen into disuse in the United States (US) and European Union and CFP—SUL had never been licensed in the US or anywhere in Western Europe. However, this combination agent is still extensively used in many hospitals of the Asian countries and has been reported to successfully treat several nosocomial infections, including mild-to-moderate nosocomial pneumonia, intra-abdominal infections, and sepsis in patients with febrile neutropenia.[11,15,16] Nevertheless, there were only a few studies comparing the accuracy of various susceptibility testing methods for detection of *in vitro* susceptibility of important GNB species to CFP or CFP—SUL.[5,6,17,18]

In this study, we conducted an *in vitro* study to investigate the performance of the agar dilution method, disk diffusion method, automated BD Phoenix (Becton Dickinson, Sparks, MD, USA), and Vitek 2 (bioMérieux, Marcy l'Etoile, France) systems for evaluating susceptibility of clinically important GNB isolates to CFP—SUL. Correlations between the susceptibility profiles obtained by the agar dilution method and the other three methods were also explored.

Methods

Bacterial isolates

From November 2013 to December 2014, a total of 799 non-duplicate GNB isolates (1 isolate per patient) were randomly collected from various clinical specimens of patients who were hospitalized at National Taiwan University Hospital, Taipei, Taiwan, a 2500-bed university-affiliated tertiary care hospital. This study was approved by the Institutional Review Boards of the National Taiwan University Hospital (NTUH 201307067RINA). The GNB isolates comprised *Enterobacteriaceae* species, namely *E. coli* ($n = 150$), *K. pneumoniae* ($n = 150$), *E. cloacae* ($n = 77$), *C. freundii* ($n = 13$), *Serratia marcescens* ($n = 30$), *Salmonella* species ($n = 50$), *Proteus mirabilis* ($n = 30$), and nonfermentative GNB (NFGNB) species, namely *Pseudomonas aeruginosa* ($n = 110$), *Acinetobacter calcoaceticus—Acinetobacter baumannii* (Acb) complex ($n = 120$), *Stenotrophomonas maltophilia* ($n = 39$), *Chyrseobacterium indologenes* ($n = 10$), *Elizabethkingia meningoseptica* ($n = 12$), and *Burkholderia cepacia* complex ($n = 8$). The isolates were identified using conventional identification methods as well as the Phoenix PMIC/ID-30 identification system (Becton Dickinson).

Antimicrobial susceptibility testing

Antimicrobial susceptibilities of all isolates were determined concomitantly by the agar dilution, disk diffusion method, and two commercial automated susceptibility systems, namely the Phoenix system (Becton Dickinson) and the Vitek 2 system (bioMérieux). The disks (75 μg CFP and 30 μg SUL) used in the disk diffusion method were obtained from Becton Dickinson. For susceptibility testing by the agar dilution method, CFP—SUL at a fixed 2:1 ratio of CFP:SUL and serial two-fold CFP concentrations ranging from 0.015 μg/mL to 128 μg/mL in combination with SUL were used together with cation-adjusted Mueller-Hinton agar (Becton Dickinson). The inoculated agar plates were incubated in ambient air at 35°C for 16—20 hours. The MIC of each antimicrobial agent was defined as the lowest concentration that inhibited visible growth of the organism.

For evaluation of the two automated susceptibility systems, a Vitek AST-N310 card (bioMérieux) and a BD Phoenix Gram Negative Combo Panel (NMIC/ID-99; Becton Dickinson Diagnostics) were used. For the Vitek AST-N310 card, the ratio of concentration of CFP to SUL was 2:1 and the concentrations of CFP tested were $\leq$ 8 μg/mL, 16 μg/mL, 32 μg/mL, and $\geq$ 64 μg/mL. By contrast, with regard to the Phoenix NMIC/ID99 panel, as Barry and Jones[5] demonstrated that the 8 μg/mL SUL concentration addition would convert more CFP-nonsusceptible (MIC $>$ 16 μg/mL) isolates of *Enterobacteriaceae* and *Pseudomonas* spp. into CFP-susceptible ones than the 4 μg/mL SUL concentration use, the CFP concentrations were also tested in serial two-fold dilutions ranging from 0.5 μg/mL to 32 μg/mL in combination with a fixed SUL concentration of 8 μg/mL. Control strains of *E. coli* ATCC 25922, *E. coli* ATCC 35218, and *P. aeruginosa* ATCC 27853, were included in each set of tests.

Tests were repeated for isolates showing discrepant results between the agar dilution and the other three evaluated methods. If the results yielded by the initial and repeated tests were not identical, all of the susceptibility methods were repeated. The susceptibility results which were obtained two out of three times were recorded as the final results.

In this study we also investigated the susceptibilities of all isolates to imipenem and meropenem by the agar dilution method to delineate carbapenem susceptibility.[13]

Interpretation of susceptibility results

There are no CLSI-recommended MIC or disk diffusion interpretive criteria for susceptibility of *Enterobacteriaceae* spp., *P. aeruginosa,* species of Acb complex, *B. cepacia* complex, *S. maltophilia* and other non-*Enterobacteriaceae* isolates to CFP—SUL.[13] Furthermore, these MIC judgment criteria were also not documented in the expert rules of the Vitek AST-N310 card or Phoenix NMIC/ID99 panel. Nevertheless, in this study, isolates were considered susceptible, intermediate, or resistant to CFP—SUL in accordance with the previous MIC breakpoints of CFP—SUL recommended by Jones et al[6] and Barry and Jones,[5] which are just consistent with the CLSI 2015 guidelines for *Enterobacteriaceae* spp.,[13] and the CLSI 2010 guidelines for *P. aeruginosa* for CFP alone.[12] For the disk (CFP/SUL, 75/30 μg) diffusion method, the isolates with zone diameters $\geq$ 21 mm were defined as susceptible; those with diameters ranging from 16 mm to 20 mm were defined as intermediate; and isolates with zone diameters $\leq$ 15 mm were defined as resistant, as stated elsewhere.[5,19] For the three dilution methods, after SUL was added, isolates with CFP MIC values $\leq$ 16 μg/mL were defined as susceptible; those with a MIC value of 32 μg/mL were defined as intermediate; and isolates with CFP MIC values $\geq$ 64 μg/mL were defined as resistant. Isolates showing no susceptibility to either imipenem or meropenem, as defined by the CLSI, were considered nonsusceptible to carbapenems.[13]

Data analysis

The rates of accuracy (categorical agreement) of the three evaluation modalities (disk diffusion, BD Phoenix system, and Vitek 2 system) were examined using GNB isolates. Using the results of the agar dilution method as the reference, we analyzed categorical agreement (CA) of the susceptibility (i.e., susceptible, intermediate, and resistant) categories between the evaluation system and agar dilution method. Errors were defined as follows: a minor error (mE) indicated that the result was intermediate in one system and susceptible or resistant in the other; a major error (ME) indicated a false-resistant result; and a very major error (VME) indicated a false-susceptible result. When calculating the rates of error, we applied the following denominators in respective species for estimation: the number of reference resistant isolates for the VME rate, the number of reference susceptible isolates for the ME rate, and the number of all reference isolates for the mE rate, respectively.[17] The acceptable intermethod error rates of VME, ME, and mE is $\leq$ 1.5%, $\leq$ 3%, and $\leq$ 10%, respectively.[20]

Statistical analysis

Categorical variables are expressed as percentages of total isolates required for specific purposes in respective species, and were compared between different systems by the Chi-square test with Yate's correction or Fisher's exact test, as appropriate. Continuous variables were compared using the Student's *t* test or Mann—Whitney *U* test, depending on the validity of the normality assumption. In addition, sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV) were calculated to compare the accuracy (i.e., degree of categorical agreement) of the disk diffusion, Vitek 2, and Phoenix systems with that of the reference method (agar dilution) in detecting CFP—SUL nonsusceptibilities. Finally, because only a few nonfermentative GNB isolates (*C. indologenes*, *E. meningoseptica*) have CLSI 2015-defined CFP MIC breakpoints,[13] the discriminatory powers of the methods under evaluation were surveyed only for *Enterobacteriaceae* species by estimating the area under the receiver-operating characteristic (ROC) curve. In this way, we were able to explore the differences in accuracy between the three comparator methods and the agar dilution method for correctly classifying the tested strains of enterobacterial GNB species with low CA rates ($<$ 90%) into

Table 1 The range of minimum inhibitory concentrations (MICs) and disk diffusion diameter (in millimeters), MIC_{50}, MIC_{90} levels, and susceptibility profiles (percentages of susceptible, intermediate, and resistant categories) versus cefoperazone—sulbactam for Gram-negative bacteria, and their concordance and discordance between the susceptibility results evaluated by the agar dilution method (reference data) and the disk diffusion method, the BD Phoenix system, as well as the Vitek 2 system, respectively.

Species, results	Range (μg/mL)	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	Susceptibilities			% of susceptibility results with:			
				S (%)	I (%)	R (%)	CA	mE	ME	VME
Escherichia coli (n = 150)										
Agar dilution	0.03—64	1	16	94	2.7	3.3				
Disk diffusion (diameter, mm)	13—35			92.7	5.3	2	97.3*	2.7	0*	0
BD Phoenix	≤0.5/8—>32/8	≤0.5/8	4/8	94	1.3	4.7	97.3*	2.7	0*	0
Vitek 2	≤8—≥64	≤8	≤8	85.3	5.3	9.3	84*,[a(1)]	6.7	8.5*,[b(1)]	40
Klebsiella pneumoniae (n = 150)										
Agar dilution	0.12—>128	0.25	16	90.4	1.9	7.7				
Disk diffusion (diameter, mm)	13—28			86.5	5.8	7.7	96.7	3.3	0	0
BD Phoenix	≤0.5/8—>32/8	≤0.5/8	32/8	88.5	1.9	9.6	96	3.3	0.7	0
Vitek 2	≤8—≥64	≤8	≤8	94.2	0	5.8	95.3	4	0	16.7
Enterobacter cloacae (n = 77)										
Agar dilution	0.03—128	0.5	64	76.6	10.4	13				
Disk diffusion (diameter, mm)	11—40			76.6	20.8	2.6	84.4[b(2)]	15.6[b(2)]	0	0
BD Phoenix	≤0.5/8—>32/8	≤0.5/8	>32/8	76.6	3.9	19.5	87[b(3)]	11.7[b(3)]	0	10
Vitek 2	≤8—≥64	≤8	≥64	77.9	6.5	15.6	84.4	11.7[b(4)]	1.7	20
Citrobacter freundii (n = 13)										
Agar dilution	0.25—32	0.5	2	92.3	7.7	0				
Disk diffusion (diameter, mm)	16—28			92.3	7.7	0	100	0	0	0
BD Phoenix	≤0.5/8—>32/8	≤0.5/8	≤0.5/8	92.3	0	7.7	92.3[c(3)]	7.7	0	0
Vitek 2	≤8—≥64	≤8	≤8	92.3	0	7.7	92.3	7.7	0	0
Serratia marcescens (n = 30)										
Agar dilution	0.5—32	2	16	96.7	3.3	0				
Disk diffusion (diameter, mm)	18—26			86.7	13.3	0	90	10	0	0
BD Phoenix	≤0.5/8—>32/8	2/8	16/8	96.7	0	3.3	93.3	3.3	3.4	0
Vitek 2	≤8—32	≤8	≤8	96.7	3.3	0	100	0	0	0[c(4)]
Salmonella spp. (n = 50)										
Agar dilution	0.25—32	1	8	98	2	0				
Disk diffusion (diameter, mm)	18—28			98	2	0	100	0	0	0
BD Phoenix	≤0.5/8—>32/8	≤0.5/8	1/8	98	0	2	98	2	0	0
Vitek 2	≤8—32	≤8	≤8	98	2	0	100[b(5)]	0	0	0[c(4)]
Proteus mirabilis (n = 30)										
Agar dilution	0.5—8	1	2	100	0	0				
Disk diffusion (diameter, mm)	25—34			100	0	0	100*	0	0	0
BD Phoenix	≤0.5/8—>32/8	≤0.5/8	4/8	93.3	3.3	3.3	93.3*	3.3	3.3	0
Vitek 2	≤8	≤8	≤8	100	0	0	100*	0	0	0[b(4)]
Pseudomonas aeruginosa (n = 110)										
Agar dilution	2—128	8	64	80	13.6	6.4				
Disk diffusion (diameter, mm)	12—29			82.7	6.4	10.9	92.7	7.3	0	0
BD Phoenix	4/8—>32/8	8/8	>32/8	80	7.3	12.7	94.5[b(6)]	5.5[c(5)]	0[c(7)]	0
Vitek 2	≤8—≥64	≤8	≥64	81.8	7.3	10.9	93.6[b(7)]	6.4[c(6)]	0	0[c(8)]
Species of *Acinetobacter calcoaceticus—Acinetobacter baumannii* complex (n = 120)										
Agar dilution	1—128	4	64	78.3	6.7	15				
Disk diffusion (diameter, mm)	6—34			76.7	10	13.3	91.7*	8.3	0*	0*
BD Phoenix	≤0.5/8—>32/8	≤0.5/8	>32/8	67.5	1.7	30.8	79.2*	8.3	13.8*	11.1
Vitek 2	≤8—≥64	≤8	32	89.2	5.8	5	82.5*	12.5	0*	33.3*
Stenotrophomonas maltophilia (n = 39)										
Agar dilution	8—>128	64	>128	5.1	23.1	71.8				
Disk diffusion (diameter, mm)	8—26			12.8	51.3	35.9	56.4*	43.6	0	0*
BD Phoenix	1/8—>32/8	>32/8	>32/8	30.8	15.4	53.8	53.8*	35.9	0	14.3*
Vitek 2	≤8—≥64	≤8	16	94.9	2.6	2.6	10.3*	25.6	0	89.3*
Other nonfermentative GNB[a] (n = 30)										
Agar dilution	4—>128	32	>128	46.7	36.7	16.7				

(*continued on next page*)

Table 1 (*continued*)

Species, results	Range (μg/mL)	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	Susceptibilities			% of susceptibility results with:			
				S (%)	I (%)	R (%)	CA	mE	ME	VME
Disk diffusion (diameter, mm)	6–31			60	16.7	23.3	73.3*	26.7	0	0
BD Phoenix	≤0.5/8–>32/8	32/8	>32/8	26.7	26.7	46.7	40*,[c(9)]	50[b(8)]	21.4	0
Vitek 2	≤8–≥64	32	≥64	16.7	56.7	26.7	53.3[c(10)]	33.3[b(9)]	21.4[b(10)]	20

[a] Other nonfermentative GNB comprises *Chyrseobacterium indologenes* ($n = 10$), *Elizabethkingia meningoseptica* ($n = 12$), and *Burkholderia cepacia* complex ($n = 8$).
[b] Above average (for all *Enterobacteriaceae* isolates, with statistically significant differences): [1] 5.0%, [2] 4.8%, [3] 4.4%, [4] 5.2%, and [5] 91.2%, (and for all nonfermentative GNB, also with statistically significant differences): [6] 77.6%, [7] 74.2%, [8] 15.1%, [9] 14.0%, and [10] 1.5%.
[c] Below the average (for all *Enterobacteriaceae* isolates, with statistically significant differences): [1] 91.2%, [2] 95.2%, [3] 94.8%, and [4] 20.0%, (and for all nonfermentative GNB, also with statistically significant differences): [5] 15.1%, [6] 14.0%, [7] 8.0%, [8] 50.8%, [9] 77.6%, and [10] 74.2%.
CA = categorical agreement; GNB = Gram-negative bacteria; I = intermediate; mE = minor error; ME = major error; MIC = minimum inhibitory concentration; R = resistant; S = susceptible; VME = very major. error.
*The difference of rates of CA, mE, ME as well as VME in CFP–SUL susceptibilities yielded between three methods under evaluation is significant ($p < 0.05$) by statistical analysis.

categories of CFP–SUL susceptibility. The method was also applied to test the discriminatory power of the susceptibility systems against carbapenem-nonsusceptible (i.e., nonsusceptibility to either of the 2 carbapenem agents) enteric GNB isolates. All statistical calculations were two-tailed, and $p < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA).

Results

The comparisons of CFP–SUL susceptibility data, and categorical agreement rates between the agar dilution method and the other three in vitro methods for respective GNB species

The MIC range, MIC_{50} and MIC_{90} values, range of disk diffusion diameters, and susceptibility profiles of the GNB isolates are presented in Table 1. Nonparametric statistical analyses revealed that isolates of *E. cloacae* and *Salmonella* species had significantly smaller disk diffusion diameters than the other enteric GNB species isolates with the exception of *K. pneumoniae* (data not shown). In addition, CFP–SUL susceptibility evaluated by the Vitek 2 system had much higher ME rates for *E. coli* isolates than the disk diffusion method and the BD Phoenix system ($p < 0.001$). The CA rates ranged from 84.0% to 100% for *Enterobacteriaceae*. Comparison of the results of antimicrobial susceptibility testing (AST) to CFP–SUL obtained from the agar dilution method with those from the other three methods revealed high rates of categorical disagreement for three enterobacterial species, i.e., 10.0% by disk diffusion for *S. marcescens*, 13.0–15.6% by the three evaluation methods for *E. cloacae*, and 16.0% by the Vitek 2 system for *E. coli* isolates. Furthermore, high mE rates were obtained by the Vitek 2 system for *E. coli*, *E. cloacae*, and *P. aeruginosa* isolates (6.7%, 11.7%, and 6.4%, respectively) as well as by the disk diffusion test for *S. marcescens* isolates (10%). The Vitek 2 system had a very high rate of VME for isolates of *E. coli*, *E. cloacae*, and *K. pneumoniae* (40%, 20%, and 16.7%, respectively; Table 1). Of note, great deviations (with statistically significant differences) from the average values in CFP–SUL susceptibility results against overall *Enterobacteriaceae* organisms were also seen in the Vitek 2 system for *E. coli* and in the three comparator methods for *E. cloacae* isolates (Table 1). By contrast with the enterobacterial isolates, there were complex differences in the AST results in CA and in error rates between the three comparator testing methods, and significant deviations from the overall averages of the organisms for isolates of the nonfermentative GNB species with the exception of *P. aeruginosa*. Nevertheless, the disk diffusion test was reliable (i.e., CA rates >90%) for correctly categorizing CFP–SUL susceptibility for *P. aeruginosa* and species of Acb complex (Table 1).

Performance of detection of CFP–SUL nonsusceptibility for three *in vitro* methods

Fisher's exact test revealed no significant differences in detecting CFP–SUL nonsusceptibility of *C. freundii* and *Salmonella* species, and *S. marcescens* and *P. mirabilis* isolates between the agar dilution method and the other three methods. By contrast, significant differences were found between the agar dilution method and disk diffusion test [7.3% vs. 6.0%; $p < 0.001$, odds ratio (OR) 0.014, and 95% confidence interval (CI) 0.004–0.056], as well as the BD Phoenix (7.3% vs. 6.0%; $p < 0.001$, OR 0.008, 95% CI 0.001–0.057) and Vitek 2 systems (7.3% vs. 14.7%; $p = 0.027$, OR 0.287, 95% CI 0.123–0.671) for *E. coli* isolates. As demonstrated in Table 2, there were also prominent differences in detecting CFP–SUL nonsusceptibility for *K. pneumoniae*, *E. cloacae*, *P. aeruginosa*, and species of Acb complex, as well as *S. maltophilia* and the other miscellaneous nonfermentative GNB isolates between the agar dilution method and the other three evaluation systems.

The performance of the three comparator methods with respect to susceptibility profiles (nonsusceptibility vs. susceptibility) of the isolates of respective GNB species,

overall *Enterobacteriaceae* and nonfermentative GNB species are illustrated in Table 3. The Vitek 2 evaluation system had a significantly lower sensitivity (44.4%), PPV (18.2%) and CA rate (84.7%) for clinical *E. coli* isolates than the other two methods. The Vitek 2 system also had markedly low sensitivity (45.5%) for *K. pneumoniae* isolates. Moreover, the Vitek 2 system performed poorly (sensitivity 65.9%, PPV 57.4%) for all isolates of the seven *Enterobacteriaceae* species tested. Wide diversities were found in all of the performance parameters of the different testing methods for detecting CFP—SUL nonsusceptible strains of nonfermentative GNB species other than *P. aeruginosa* (Table 3).

The ROC curve analysis of the capacity of Vitek 2 system to identify correct susceptibility categories among different Enterobacteriaceae spp

The ROC curve analysis demonstrated that the Vitek 2 system had a significantly poorer discriminatory power to detect the correct categories of susceptibility of *E. coli* isolates [area under the ROC curve (AUC) 0.654; $p = 0.123$, 95% CI 0.448—0.859] than those of isolates of *E. cloacae* (AUC 0.903; $p < 0.001$, 95% CI 0.786—1.000) and overall isolates of the seven *Enterobacteriaceae* species (AUC 0.808; $p < 0.001$, 95% CI 0.719—0.897; Figures 1A—1C). In addition, we identified a total of 20 enteric GNB isolates (*C. freundii*, $n = 1$; *E. coli*, $n = 4$; *K. pneumoniae*, $n = 5$; and *E. cloacae*, $n = 10$) with a carbapenem-nonsusceptible phenotype. The performance of Vitek 2 system was much poorer than the disk diffusion and BD Phoenix methods in correctly classifying CFP—SUL susceptibility of these resistant GNB isolates (AUC 0.756; $p = 0.076$, 95% CI 0.513—0.999; figure not shown).

Discussion

This study highlights three important points. First, the Vitek 2 system performed poorly in recognizing the correct categories of CFP—SUL susceptibility among *E. coli* isolates. Second, the CFP—SUL susceptibility profiles obtained by the three comparator methods are in conflict with those from the agar dilution method for *E. cloacae* strains. Third, all three methods performed poorly in their ability to correctly categorize CFP—SUL susceptibility among the

Table 2 Comparisons of the capacity to detect nonsusceptibility to cefoperazone—sulbactam yielded from the three evaluation methods and the agar dilution method (reference) against Gram-negative bacteria (GNB).

Species, comparisons of the capacity of each method with the reference data in detecting the CFP—SUL nonsusceptible isolates	*p*	OR	95% CI
Escherichia coli ($n = 150$)			
Disk diffusion	<0.001	0.014	0.004—0.056
BD Phoenix system	<0.001	0.008	0.001—0.057
Vitek 2 system	0.027	0.287	0.123—0.671
Klebsiella pneumoniae ($n = 150$)			
Disk diffusion	<0.001	0.029	0.011—0.076
BD Phoenix system	<0.001	0.022	0.007—0.066
Vitek 2 system	<0.001	0.041	0.019—0.091
Enterobacter cloacae ($n = 77$)			
Disk diffusion	<0.001	0.018	0.003—0.126
BD Phoenix system	<0.001	0.018	0.003—0.126
Vitek 2 system	<0.001	0.041	0.010—0.161
Pseudomonas aeruginosa ($n = 110$)			
Disk diffusion	<0.001	0.033	0.011—0.100
BD Phoenix system	<0.001	0.035	0.011—0.106
Vitek 2 system	<0.001	0.023	0.006—0.092
Species of *Acinetobacter calcoaceticus—Acinetobacter baumannii* complex ($n = 120$)			
Disk diffusion	<0.001	0.033	0.011—0.101
BD Phoenix system	<0.001	0.180	0.111—0.293
Vitek 2 system	<0.001	0.121	0.073—0.202
Stenotrophomonas maltophilia ($n = 39$)			
Disk diffusion	0.013	0.600	0.293—1.227
BD Phoenix system	0.089	0.833	0.647—1.073
Vitek 2 system	>0.99	0.946	0.876—1.022
Other nonfermentative GNB ($n = 30$)[a]			
Disk diffusion	0.001	0.303	0.141—0.651
BD Phoenix system	0.417	0.635	0.243—1.657
Vitek 2 system	0.642	0.714	0.231—2.208

[a] Other nonfermentative GNB comprised *Chyrseobacterium indologenes* ($n = 10$), *Elizabethkingia meningoseptica* ($n = 12$), and *Burkholderia cepacia* complex ($n = 8$).
CFP—SUL = cefoperazone—sulbactam; CI = confidence interval; GNB = Gram-negative bacteria; OD = odds ratio.

Table 3 Comparisons of the cefoperazone—sulbactam susceptibility profiles (nonsusceptible vs. susceptible) obtained from the three evaluation methods and the reference (agar dilution) test for various kinds of Gram-negative bacteria.

Species (*n*)	No. of isolates:				Results (%) for:				
	+ by both tests	Method + and REF −	Method − and REF +	− by both tests	Sensitivity	Specificity	PPV	NPV	Agreement
Escherichia coli (150)									
Disk diffusion	9	2	0	139	100*	98.6	81.8*	100	98.7*
BD Phoenix	8	1	1	140	88.9*	99.3	88.9*	99.3	98.7*
Vitek 2	4	18	5	123	44.4*	87.2	18.2*	96.1	84.7*
Klebsiella pneumoniae (150)									
Disk diffusion	11	3	0	136	100*	97.8	78.6	100	98.0
BD Phoenix	11	3	0	136	100*	97.8	78.6	100	98.0
Vitek 2	5	0	6	139	45.5*	100	100	95.7	96.0
Enterobacter cloacae (77)									
Disk diffusion	17	1	1	58	94.4	98.3	94.4	98.3	97.4
BD Phoenix	17	1	1	58	94.4	98.3	94.4	98.3	97.4
Vitek 2	15	2	3	57	83.3	96.6	88.2	95.0	93.5
Citrobacter freundii (13)									
Disk diffusion	1	0	0	12	100	100	100	100	100
BD Phoenix	1	0	0	12	100	100	100	100	100
Vitek 2	1	0	0	12	100	100	100	100	100
Serratia marcescens (30)									
Disk diffusion	1	3	0	26	100	89.7	25	100	90
BD Phoenix	0	1	1	28	0	96.6	0	96.6	93.3
Vitek 2	1	0	0	29	100	100	100	100	100
Salmonella spp. (50)									
Disk diffusion	1	0	0	49	100	100	100	100	100
BD Phoenix	1	0	0	49	100	100	100	100	100
Vitek 2	1	0	0	49	100	100	100	100	100
Proteus mirabilis (30)									
Disk diffusion	0	0	0	30	ND	100	ND	100	100
BD Phoenix	0	2	0	28	ND	93.3	0	100	93.3
Vitek 2	0	0	0	30	ND	100	ND	100	100
Overall isolates of the 7 *Enterobacteriaceae* species (500)									
Disk diffusion	40	9	1	450	97.6*	98*	81.6*	99.8	98*
BD Phoenix	38	8	3	451	92.7*	98.3*	82.6*	99.2	99.3*
Vitek 2	27	20	14	439	65.9*	95.6*	57.4*	96.9	93.2*
Pseudomonas aeruginosa (110)									
Disk diffusion	19	3	0	88	100	96.7	86.4	100	97.3
BD Phoenix	18	3	1	88	94.7	96.7	85.7	98.9	96.4
Vitek 2	18	2	1	89	94.7	97.8	90	98.9	97.3
Species of *Acinetobacter calcoaceticus—Acinetobacter baumannii* complex (120)									
Disk diffusion	26	3	1	90	96.3*	96.8*	89.7*	98.9*	96.7*
BD Phoenix	23	15	3	79	88.5*	84*	60.5*	96.3*	85*
Vitek 2	13	0	13	94	50*	100*	100*	87.9*	89.2*
Stenotrophomonas maltophilia (39)									
Disk diffusion	34	3	0	2	100*	40	91.9	100*	92.3*
BD Phoenix	27	0	10	2	73*	100	100	16.7*	74.4*
Vitek 2	2	0	35	2	5.4*	100	100	5.7*	10.3*
Other nonfermentative GNB species (30)[a]									
Disk diffusion	11	1	5	13	68.8	92.9*	91.7*	72.2	80
BD Phoenix	13	9	3	5	81.3	35.7*	59.1*	62.5	60
Vitek 2	14	11	2	3	87.5	21.4*	56*	60	56.7
Overall isolates of the nonfermentative GNB species (299)									
Disk diffusion	90	10	6	193	93.8*	95.1*	90*	97*	94.6*
BD Phoenix	81	27	17	174	82.7*	86.6*	75*	91.1*	85.3*
Vitek 2	47	13	51	188	48*	93.5*	78.3*	78.7*	78.6*

[a] Other nonfermentative GNB (30) isolates comprised *Chyrseobacterium indologenes* (10), *Elizabethkingia meningoseptica* (12), and *Burkholderia cepacia* complex (8).

GNB = Gram-negative bacteria; ND = not done; NPV = negative predictive value; PPV = positive predictive value; REF = reference (indicates the results of agar dilution); + = positive result for predicting nonsusceptibility; − = negative result for excluding nonsusceptibility.

*The difference of performance in parameters between the method(s) under evaluation and the agar dilution method (reference test) is significant ($p < 0.05$) by statistical analysis.

nonfermentative GNB isolates with the exception of *P. aeruginosa*.

For susceptibility testing by the broth microdilution method (the results equivalent to those of the agar dilution method recommended in CLSI), the CFP—SUL combination regimen consisting of a fixed 2:1 concentration ratio had been shown to maximize the spectrum of activity against many GNB species, and best simulate the parenteral formulation as well as the pharmacokinetics of this combination regimen.[5] Furthermore, Barry and Jones[5] clearly proved that the *in vitro* performance of these disks correlated well with that of the broth microdilution CFP—SUL (also at the 2:1 concentration ratio) susceptibility test that was recommended and outlined elsewhere.[6] Consequently, despite the use of disks containing 75 μg CFP and 30 μg SUL (close to 2:1 ratio) in susceptibility testing not being justified by the CLSI,[12,13] we adopted these disks to determine the CFP—SUL susceptibility test against the clinically important GNB isolates in this study.

Prior to this investigation, only one study had investigated the abilities of various AST evaluation methods against specific antibiotic agents for clinical heteroresistant *E. cloacae* and *A. baumannii* isolates.[21] To the best of our knowledge, no other study has compared the performance of the disk diffusion method with that of two commonly used, commercially available automated susceptibility

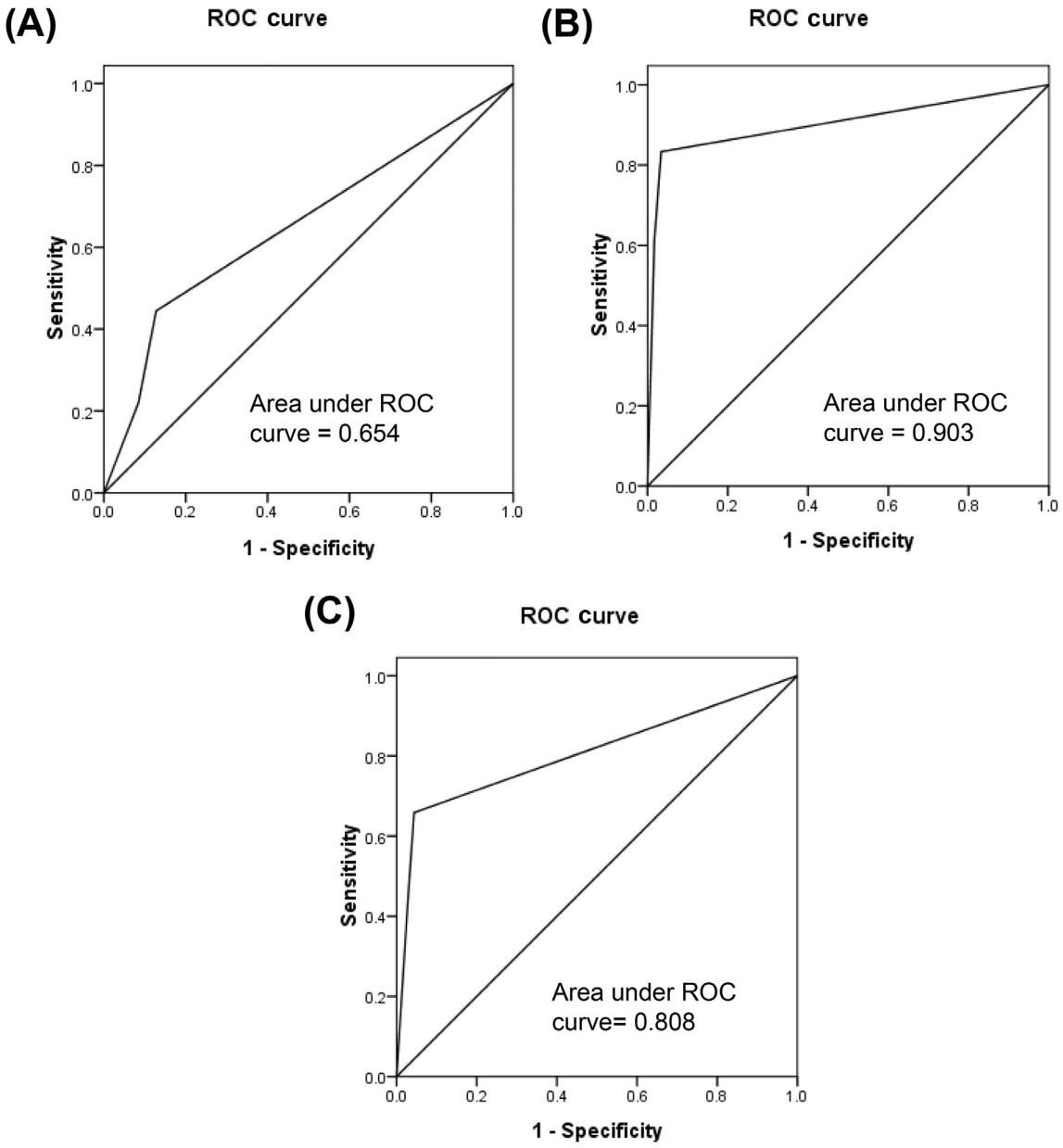

Figure 1. The discriminatory power [area under the receiver-operating-characteristic (ROC) curve] of the Vitek 2 system to correctly identify categories of susceptibility (susceptible, intermediate, or resistant) to cefoperazone—sulbactam among the isolates of (A) *Escherichia coli*, (B) *Enterobacter cloacae*, and (C) overall *Enterobacteriaceae* species. ROC = receiver-operating-characteristic.

systems in evaluating the CFP–SUL susceptibility profiles of such a large collection of the clinically important GNB isolates. Although the automated tests are relatively less labor-intensive for yielding AST data than the disk diffusion method, disk diffusion is still a convenient method for obtaining AST results against antibiotic(s). As shown by Hardy et al,[19] resistance among *Enterobacteriaceae* species to CFP–SUL, as also defined by the broth microdilution method, was highly concordant (85%, 17/20) with that obtained by disk diffusion testing.[19] This concordant rate in fact shows an insignificant contrast with the proportion of isolates determined by the disk diffusion method to be nonsusceptible to CFP–SUL in our study (97.6%, $p = 0.063$).

We found that all three comparator methods performed poorly in detecting nonsusceptible strains of nonfermentative GNB species with the exception of *P. aeruginosa*. In addition to the high ME rate (6.7%) yielded from the BD Phoenix system in the study conducted by Juretschko et al,[22] Donay et al[17] also found a strikingly lower CA rate (70.6%) by the BD Phoenix system for *P. aeruginosa* isolates against piperacillin–tazobactam. By contrast, the CA rate detected by the BD Phoenix system for *P. aeruginosa* isolates against CFP–SUL (94.5%) in our survey was similar to that also obtained from the BD Phoenix system against CFP (96.6%) in the survey of Menozzi et al.[18] Therefore, the BD Phoenix system appears to be an accurate method for testing susceptibility of *P. aeruginosa* isolates to CFP–SUL.

By comparison with the minimal performance requirements recommended by the National Committee for Clinical Laboratory Standards in 1994 and International Organization for Standardization,[20,23] we found that use of the formula suggested by Menozzi et al[18] for the Vitek 2 system resulted in significantly low CA rates (< 90%) as well as disproportionately high error rates (mostly much higher than 3.0%) for *E. coli* and *E. cloacae* isolates. Among the tested organisms of those two species of the *Enterobacteriaceae* family, we found that the Vitek 2 system showed a high number of minor errors among *E. coli* ($n = 10$), *K. pneumoniae* ($n = 6$), and *E. cloacae* ($n = 9$) isolates and a high number of major errors among *E. coli* ($n = 12$) isolates. In addition, despite the *E. coli* isolates accounting for only 30% of all *Enterobacteriaceae* isolates in our survey, the high number ($n = 18$) of false nonsusceptible *E. coli* strains detected by the Vitek 2 system has a great impact on calculating the PPV for *E. coli* isolates {< 20% [4/(4 + 18)]} as well as for *Enterobacteriaceae* isolates {< 60% [27/(27 + 18+2)]}, as seen in Table 3. By contrast, although higher mE rates were also observed by the disk diffusion method for *E. cloacae* and *S. marcescens* (15.6% and 10%, respectively) than for other *Enterobacteriaceae* species, no VME or ME strain was found for these two species.

The main drawback of this *in vitro* study is that the resistance mechanisms of the GNB isolates analyzed against β-lactam agents (especially β-lactamases) were not well characterized. Of 220 *E. coli* and *K. pneumoniae* isolates proven to be ESBL producers, Jang et al[24] found that the Vitek 2 system produced high rates of VME (27.4%) when testing susceptibility to cefepime and an error rate of 4.5% for ceftazidime compared to the reference data of broth microdilution test. Lat et al[25] also found that the Vitek 2 system resulted in high VME rates on the susceptibility of *K. pneumoniae* carbapenemase-producing *K. pneumoniae* strains to meropenem (27%) and cefepime (67%) when CLSI 2010-determined MIC breakpoints were applied.[12,25] In addition, a survey conducted by Pailhoriès et al[26], who recently investigated 14 ertapenem-nonsusceptible *E. cloacae* strains with potential ESBL production, observed that nine (64.3%) strains with mE in categorical discordance of susceptibility were detected from the Vitek 2 system when compared to MIC data from the agar dilution test. In our survey, the Vitek 2 method correspondingly showed the poorest discriminatory ability in identifying the correct CFP–SUL susceptibilities for carbapenem-nonsusceptible enterobacterial strains among three comparator testing methods. It remains to be investigated if the results of susceptibility testing for MDR strains of some members of the *Enterobacteriaceae* family against many β-lactam drugs obtained from the Vitek 2 system agree well with those obtained by the reference method. Additionally, the high VME rates of the Vitek 2 system in our survey referred to the very limited VME numbers (2 VMEs for *E. coli* and *E. cloacae* and 1 for *K. pneumoniae*) that were divided by small isolate numbers exhibiting a true CFP–SUL resistant phenotype (5 for *E. coli*, 6 for *K. pneumoniae*, and 10 for *E. cloacae*, respectively) for these three species. If the number of total isolates of respective species was used as a new denominator, significantly lower VME rates will be yielded (1.3% for *E. coli*, 0.7% for *K. pneumoniae*, and 2.6% for *E. cloacae*, respectively) than those in Table 1 of our survey. To avoid considerable deviations in reestimating the VME rates, more *E. coli* and *E. cloacae* strains proven with CFP–SUL resistant susceptibility by the reference testing might be needed. Finally, because the half-life of CFP differs considerably from that of SUL,[5] this impact regarding *in vitro* CFP–SUL susceptibilities obtained by disk or any automated method on its application for clinical management is worth being investigated.

In conclusion, although the Vitek 2 system still has acceptable accuracy in detecting the correct CFP–SUL susceptibility categories for isolates of some *Enterobacteriaceae* species, we found it to be an unreliable method for *E. coli* and *E. cloacae*. Furthermore, using the interpretive criteria applied in our study, none of the three tested methods are appropriate alternative means for yielding data on susceptibility of nonfermentative GNB to CFP–SUL with the exception of the disk diffusion method for *P. aeruginosa* and species of Acb complex. As the database of automated systems is updated, future studies should be undertaken to explore the changes in AST results obtained by these systems against CFP–SUL for important GNB species.

Conflicts of interest

None declared.

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