

# Direct Identification, Antimicrobial Susceptibility Testing, and Extended-Spectrum $\beta$ -Lactamase and Carbapenemase Detection in Gram-Negative Bacteria Isolated from Blood Cultures

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**Purpose:** To shorten the turnaround time for blood culture (BC) analyses, a rapid method was developed for the direct identification, antimicrobial susceptibility testing (AST), and multidrug resistance testing of bacteria-positive BCs.

**Materials and Methods:** The mixtures in BC bottles were treated with the multistep centrifugation method developed here and the conventional culture-based method. The bacterial sediment obtained after centrifugation was analyzed directly with MALDI-TOF MS and Vitek 2 Compact, and AST was performed directly with the Kirby–Bauer (K–B) disk diffusion, VITEK 2 Compact, and E-test methods. Extended spectrum lactamases (ESBLs) were detected with discs containing cefotaxime, cefotaxime/clavulanate, ceftazidime, and ceftazidime/clavulanate, and carbapenemase was detected with the modified carbapenem inactivation method (mCIM) and EDTA-mCIM (eCIM).

**Results:** All the results of direct testing were compared to those of the conventional methods, to evaluate the accuracy of the direct methods. The accuracies of the direct Vitek 2 Compact and MALDI-TOF MS methods were 95.5% (214/224) and 90.2% (202/224), respectively. Direct AST with K–B, Vitek 2, and E-test showed category agreement of 96.0% (2611/2721), 96.1% (2614/2721), and 97.4% (2650/2721), respectively, and the major errors and very major errors were < 2% for all three methods. In the direct determination of ESBLs, the results for cefotaxime combined with cefotaxime/clavulanate were completely consistent with those after the standard isolation method. The carbapenemase detection rate with direct mCIM and eCIM was exactly the same as that with the standard method.

**Conclusion:** These direct procedures based on multistep centrifugation are not only highly accurate but are appropriate for clinical laboratory use because the turnaround time is shorter.

**Keywords:** blood culture, direct identification, direct antimicrobial susceptibility testing, extended-spectrum  $\beta$ -lactamase, carbapenemase, multistep centrifugation

## Introduction

Bloodstream infections (BSIs) are life-threatening diseases that occur widely throughout the world.<sup>1,2</sup> Gram-negative bacteremia has attracted widespread attention because the antimicrobial resistance of the organisms involved is continuously increasing, with increased mortality in patients.<sup>3</sup> The earlier the relevant information on pathogenic bacteria is known, the easier it is to treat patients effectively. Clinical microbiology laboratories often play an important role in detecting the causative pathogens of BSIs. However, the information cannot be obtained rapidly with conventional blood culture (BC) procedures because time is required to grow colonies of bacteria from positive BCs, and then identify the

bacteria involved and determine their antimicrobial susceptibility. Clinicians cannot identify the etiological agent for 48–72 h after a BC is deemed positive. Therefore, the standard procedure does not allow the early development of precise therapeutic strategies for patients. The study by Kadri et al showed that approximately one in five patients with BSIs in US hospitals received discordant empiric antibiotic therapy, which was strongly associated with infections with drug-resistant pathogens. Furthermore, the receipt of discordant empiric antibiotic therapy was associated with increased overall mortality.<sup>4</sup> Consequently, a new method is required that allows laboratories to identify bacteria and their drug resistance as soon as possible after a BC is shown to be positive.

The rapid identification of causative bacteria of BSIs provides a preliminary basis for the clinical empirical treatment of patients with bacteremia. The prognoses of patients may improve if the microorganism is identified within 1–2 h after a BC is deemed positive.<sup>5</sup> To shorten the time required to identify pathogenic bacteria, some laboratories use molecular methods to identify those in BC bottles within 2 h. However, special equipment and skills are required for a molecular approach, and not all clinical laboratories can meet these requirements.<sup>6</sup> Therefore, other methods that can be used in most laboratories are under investigation, such as incubating bacteria for a short time to form colonies before their identification, or rapid identification after the direct treatment of the mixtures in a bacterium-positive BC bottle.<sup>7–11</sup> However, the results are not consistent when different processing methods are used.<sup>12–14</sup>

The classical method of detecting drug resistance in a positive BC requires overnight subculture of the organism on agar medium. Clinicians sometimes have to initiate empirical treatment before the results are available. This increases the likelihood of an unreasonable treatment. Furthermore, the increases in multidrug-resistant Gram-negative bacteria (MDR-GNB) have increased the failure of empirical treatments. This demonstrates the clinical importance of the early-stage diagnosis of drug resistance. Such early results can be obtained with various methods. PCR can be used to directly detect certain antibiotic resistance genes in positive BCs, but a major limitation is that negative amplification does not necessarily indicate susceptibility.<sup>15,16</sup> For instance, Gram-negative bacteria that lack carbapenemase can still be resistant to carbapenem.<sup>17–20</sup> Reducing the incubation time for AST improves the total time required, but some strains do not display antimicrobial resistance in the initial stage of culture.<sup>21,22</sup> Studies have shown that neither molecular nor phenotypic rapid antibiotic susceptibility testing has directly improved mortality relative to standard methods. However, phenotypic rapid methods may improve the time to appropriate antibiotic therapy compared with conventional methods, whereas molecular methods will not.<sup>23</sup> Therefore, the resistance phenotype of the bacteria in bacterium-positive BC bottles must be directly and accurately determined.

The treatment of severe infections caused by MDR-GNB is one of the most important challenges for clinicians worldwide, partly because bacterial resistance may not be identified until it is determined with AST.<sup>24</sup> The therapy regimens differ for MDR bacteria with different resistance mechanisms.<sup>25,26</sup> Therefore, the rapid detection of resistance mechanisms allows clinicians to select the appropriate drugs to treat MDR bacteria. The production of carbapenemases by Gram-negative bacteria is the most important mechanism of carbapenem resistance. The modified carbapenem inactivation method (mCIM) is recommended by the Clinical Laboratory Standards Institute (CLSI) guidelines to detect the carbapenemases of Enterobacteriaceae and *Pseudomonas aeruginosa* with high sensitivity and specificity.<sup>27</sup> However, the test requires protracted culture, and little research has examined the direct testing of bacteria in BC bottles.

Recently, both the CLSI and the European Committee on Antimicrobial Susceptibility Testing have published guidelines for direct AST of positive BCs, which reduce the turnaround time of BC analyses to some extent. However, the rapid methods published in both guidelines target a limited number of bacteria, and only a subset of drugs are tested with AST, and carbapenems are not addressed by the CLSI guidelines.<sup>28,29</sup> The diameters of the zones of inhibition must be read at 4, 6, and 8 h during AST, which increases the workload of laboratory staff. Moreover, some results cannot be classified because there are areas of technical uncertainty.<sup>28</sup> Consequently, there is an urgent need for a method that allows the accurate, rapid and comprehensive AST of BCs, which can be readily used in most clinical microbiology laboratories.

## Materials and Methods

### Samples

This study was performed between July 2019 and January 2020 at two tertiary university hospitals in Hebei Province, China. Blood samples were collected from patients with suspected BSIs and injected into charcoal-based BC bottles (BacT/ALERT® FA and FN; bioMérieux, Paris, France) at the Affiliated Hospital of Chengde Medical University (Chengde, China) and the Second Hospital of Hebei Medical University (Shijiazhuang, China). The BC bottles were incubated in an automatic BacT/ALERT® 3D Microbial Detection System instrument (bioMérieux, Paris, France) until they were flagged positive or for up to 7 days. Once the bottles were designated positive, Gram staining of the fluid was performed immediately. The study was approved by the Institutional Review Board of the Affiliated Hospital of Chengde Medical University and the Second Hospital of Hebei Medical University. This study was performed in line with the principles of the Declaration of Helsinki. Before the commencement of study, the samples informed consent was obtained for the use of samples from all patients. The results obtained have only been used for scientific research, and the authors have no conflicts of interest.

### Standard Methods

Culture-based identification of bacteria using matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS; bioMérieux) was defined as the standard method of microorganism identification. Blood agar, MacConkey agar, and chocolate agar plates (Biokont, Wenzhou, China) were inoculated with the mixture from each positive BC bottle, and incubated at 36 °C for 18–24 h. The pure bacterial colonies were used for identification, according to the manufacturer's instructions (MALDI-TOF MS; bioMérieux).

Agar dilution is considered the standard method for determining the minimum inhibitory concentration (MIC) for AST. Specified solvents and diluents were used to prepare the dilutions of antimicrobial agents (National Institutes for Food and Drug Control, China) according to CLSI Table 6A.<sup>27</sup> The antibacterial agent was mixed with Mueller–Hinton (MH) agar (Oxoid; Thermo Fisher Scientific, Ely, UK) in a ratio of 1:10 and poured into an empty plate. AST was performed within 24 h of agar solidification. The bacterial colonies were picked from the plate and suspended in saline, adjusted to a turbidity of 0.5 McFarland and diluted 10-fold for AST using a multipoint inoculator (MIT-P, Sakuma, Japan). The plates were incubated at 36 °C for 16–20 h.

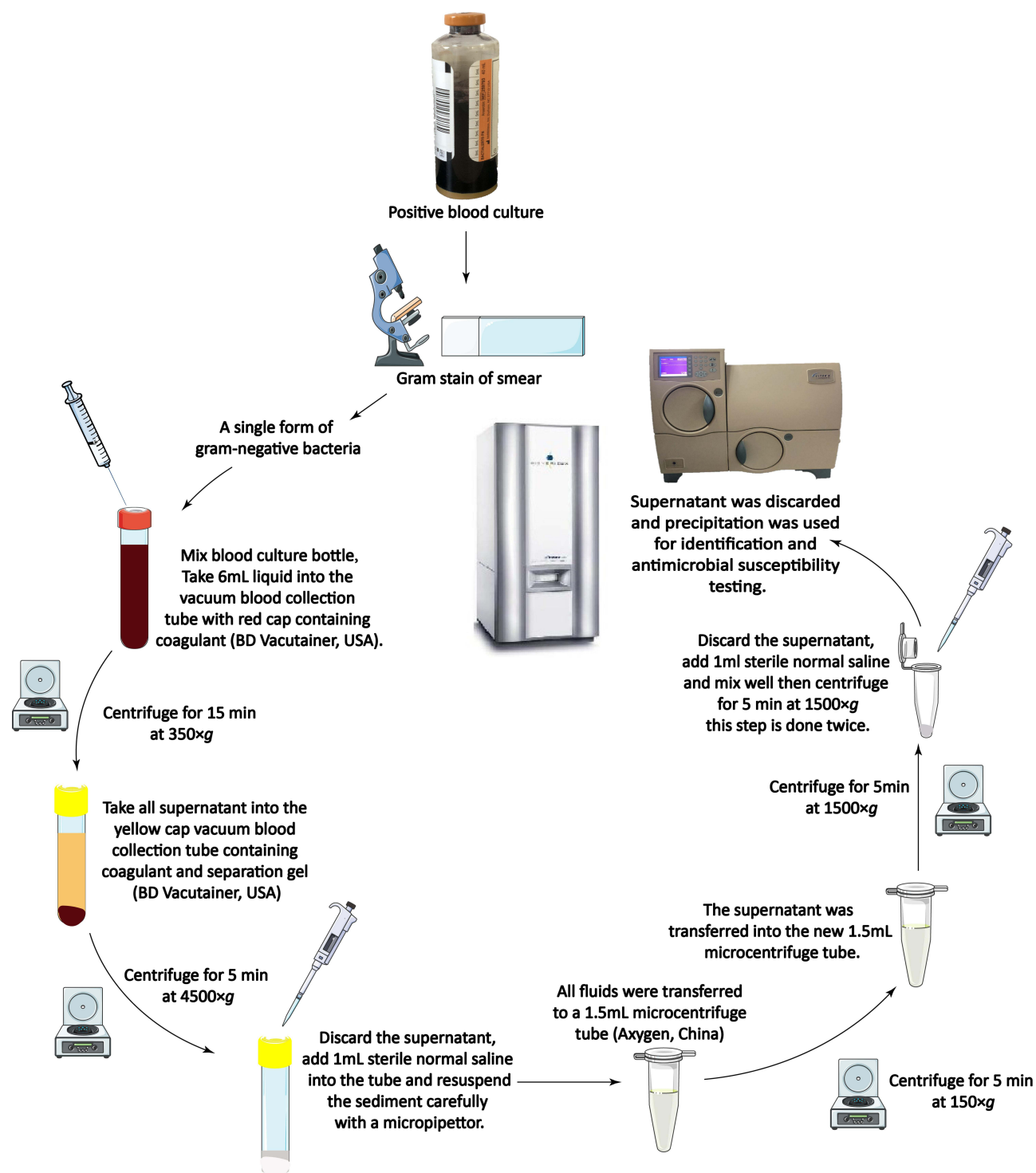
After bacterial culture, the standard method of detecting ESBLs was performed using cefotaxime (30 µg) and cefotaxime/clavulanate (CTL) (30/10 µg), and ceftazidime (30 µg) and ceftazidime/clavulanate (CAL) (30/10 µg) (Liofilchem S.R.L., Italy), according to CLSI Table 3A.<sup>27</sup> The medium was incubated in air at 36 °C for 16–18 h. Similarly, the pure bacterial colonies were used for standard mCIM and EDTA-mCIM (eCIM) detection. The procedures were strictly in accordance with CLSI Table 3C.<sup>27</sup>

Testing with all standard methods was repeated to resolve any inconsistency.

### Direct Methods

Bacteria were prepared for analysis with the direct methods as follows. The positive BCs were immediately subjected to Gram-staining. The BCs containing Gram-negative rods were included in the study, whereas Gram-positive organisms were excluded. However, Gram-negative rods with the appearance of “fine sand” were excluded as suspected *Brucella*. Gram-variable organisms were also treated them for testing with the direct methods. If colonies of these Gram-variable organisms were Gram-positive bacteria after overnight culture, when identified with MALDI-TOF MS, they were not be included in the study. The workflow is shown in Figure 1.

Direct identification: In this study, two instruments were used for direct bacterial identification. For MALDI-TOF MS, 1–2 µL of BC sediment prepared with multistep centrifugation was spotted onto a steel target plate, dried in air, and immediately mixed with 0.5 µL of formic acid solution (bioMérieux), combined with 1 µL of  $\alpha$ -cyano-4-hydroxycinnamic acid (CHCA) matrix solution (bioMérieux). Direct identification was performed with MALDI-TOF MS. For Vitek 2 Compact (bioMérieux) identification, the density of the bacterial sediment was adjusted to 0.6 McFarland by dilution with 0.45% saline. Vitek 2 Compact GN cards (bioMérieux) were used for the direct identification of the bacteria.



**Figure 1** Workflow of bacterial preparation from bacteria-positive blood cultures. After the liquid in the positive BC is mixed, a 6 mL aliquot is extracted with a syringe and transferred into a vacuum blood collection tube (with the red cap) containing coagulant (BD Vacutainer, USA). The centrifuge tube is gently mixed by inversion 5–6 times, and centrifuged at 350  $\times$  g for 15 min at room temperature. After centrifugation, the sediment is discarded. All the supernatant is transferred to a (yellow-capped) vacuum blood collection tube containing coagulant and separation gel (BD Vacutainer), and shaken gently to mix. It is then centrifuged for 5 min at 4500  $\times$  g at room temperature. After centrifugation, the supernatant is discarded and the sediment is carefully resuspended in 1 mL of saline solution with a micropipettor, and then all the liquid is transferred to a 1.5 mL microcentrifuge tube (Axygen, China). After centrifugation (150  $\times$  g, 5 min), the supernatant is transferred into a new 1.5 mL microcentrifuge tube and centrifuged for 5 min at 1500  $\times$  g. The supernatant is discarded and the pellet is resuspended in 1 mL of saline. The contents of the microcentrifuge tube are thoroughly mixed by vortexing for 5 s, and then centrifuged for 5 min at 1500  $\times$  g. The supernatant is discarded and the sediment is used for direct method tests.

Direct AST: For the direct K–B and E-test methods, the bacterial sediment was adjusted to 0.5–0.6 McFarland with normal saline. The bacterial suspension was spread on an MH plate with a sterile swab. The plate was placed at room temperature for 3 min and the disks (Oxoid; Thermo Fisher Scientific, Ely, UK) or E-test strips (Biokont) were placed on it. The plate was then incubated in air for 16–18 h. For the Vitek 2 Compact test, the density of the bacterial sediment was adjusted to 0.6–0.7 McFarland by dilution with 0.45% saline. Vitek 2 Compact GN-09 cards (bioMérieux) were used for the direct AST of the bacteria.

Direct detection of MDR-GNB: To detect ESBLs, the bacterial sediment was adjusted to 0.5–0.6 McFarland with normal saline. The bacterial suspension was spread on an MH plate with a sterile swab. The remaining steps were the same as those for the standard method of ESBL detection. For the mCIM and eCIM analyses, 2 µL Enterobacteriaceae sediment or 10 µL of *P. aeruginosa* sediment was emulsified in 2 mL of tryptic soy broth. The remaining operational steps were the same as those described for standard mCIM and eCIM detection.

## Data Analysis and Quality Control

All antimicrobial results were interpreted as indicating antimicrobial susceptible, intermediate, or resistant bacteria, according to the CLSI guidelines.<sup>27</sup> All the results obtained with the direct methods were compared with those obtained with the standard corresponding culture-based methods. Category agreement (CA) was calculated and discrepant results were categorized as minor errors (MinE), major errors (ME; false resistance), or very major errors (VME; false susceptibility). MinE was calculated by dividing the number of minor errors by the total number of isolates detected; ME was calculated by dividing the number of major errors by the number of susceptible isolates detected; and VME was calculated by dividing the number of VME by the number of resistant isolates detected. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *K. pneumoniae* ATCC BAA-1705 (American Type Culture Collection) were used as the quality control strains to ensure the quality of the medium.<sup>27</sup>

## Results

A total of 253 Gram-negative organisms were grown from positive BCs during the study period. Five positive BCs were found to be in fine shape when smears were Gram-stained, and were therefore, suspected of being *Brucella* and were not treated with the direct methods. Six Gram-variable organisms were detected with Gram staining, and two of them were excluded because they were identified as *Bacillus cereus*. The remaining four were included in this study as *Acinetobacter baumannii*. Twenty-two of the BCs analyzed with the direct methods were shown to contain anaerobes ( $n = 17$ ) or polymicrobials ( $n = 5$ ), so these 22 isolates were not included in subsequent experiments. Therefore, a total of 224 BCs containing mono-aerobic bacteria were included in this study (Table 1).

## Direct Identification of Microorganisms

The results of direct identification with VITEK 2 Compact and MALDI-TOF MS were compared to those acquired with standard laboratory culture-based methods. Overall, the accuracy of identification achieved with direct Vitek 2 Compact and MALDI-TOF MS was 95.5% (214/224) and 90.2% (202/224), respectively (Table 1).

Direct VITEK 2 Compact misidentified 2.7% of the isolates. In detail, *Acinetobacter baumannii* was incorrectly identified once as *P. luteola*; *Burkholderia cepacia* was incorrectly identified once as *B. pseudomallei*; *Stenotrophomonas maltophilia* was incorrectly identified once as *Sphingomonas paucimobilis*; *Ralstonia mannitolilytica* was incorrectly identified twice as *P. fluorescens*; *Pantoea agglomerans* was incorrectly identified once as *Escherichia coli*. Direct MALDI-TOF MS did not incorrectly identify any bacterium. However, MALDI-TOF MS failed to identify 22 strains (9.8%), and of these, half the *Enterobacter cloacae* (9/18) could not be identified (Table 1).

## Results of Direct AST

All 224 bacterial strains examined in this study were analyzed with direct AST. Each isolate was tested simultaneously with the K–B disk diffusion, Vitek 2 Compact, and E-test AST methods. For each AST method, a total of 2721 microorganism–antimicrobial combinations were detected, and compared with the results of culture-based AST with agar dilution. The K–B, Vitek 2 Compact, and E-test direct AST methods showed CAs of 96.0% (2611/2721), 96.1% (2614/

**Table 1** Results of Direct Bacterial Identification with VITEK 2 Compact and MALDI-TOF MS Using the Developed Method

	Number of Isolates	VITEK 2 Compact Identification Result			MALDI-TOF MS Identification Result		
		Correctly Identified (n/%)	Misidentified (n/%)	Not Identified (n/%)	Correctly Identified (n/%)	Misidentified (n/%)	Not Identified (n/%)
<i>Escherichia coli</i>	117	116/99.2	0	1/0.8	115/98.3	0	2/1.7
<i>Klebsiella pneumoniae</i>	43	43/100.0	0	0	39/90.7	0	4/9.3
<i>Enterobacter cloacae</i>	18	18/100.0	0	0	9/50.0	0	9/50.0
<i>Pseudomonas aeruginosa</i>	11	11/100.0	0	0	9/81.8	0	2/18.2
<i>Klebsiella aerogenes</i>	5	5/100.0	0	0	5/100.0	0	0
<i>Salmonella spp</i>	5	5/100.0	0	0	5/100.0	0	0
<i>Acinetobacter baumannii</i>	4	3/75.0	1/25.0	0	3/75.0	0	1/25.0
<i>Burkholderia cepacia</i>	4	2/50.0	1/25.0	1/25.0	1/25.0	0	3/75.0
<i>Klebsiella oxytoca</i>	3	3/100.0	0	0	3/100.0	0	0
<i>Stenotrophomonas maltophilia</i>	2	1/50.0	1/50.0	0	1/50.0	0	1/50.0
<i>Enterobacter amnigenus</i>	2	2/100.0	0	0	2/100.0	0	0
<i>Ralstonia mannitolitica</i>	2	0	2/100.0	0	2/100.0	0	0
<i>Pantoea agglomerans</i>	2	1/50.0	1/50.0	0	2/100.0	0	0
<i>Aeromonas hydrophila/caviae</i>	2	2/100.0	0	0	2/100.0	0	0
<i>Raoultella planticola</i>	2	2/100.0	0	0	2/100.0	0	0
<i>Serratia marcescens</i>	1	1/100.0	0	0	1/100.0	0	0
<i>Aeromonas sobria</i>	1	1/100.0	0	0	1/100.0	0	0
<b>Total</b>	<b>224</b>	<b>216/96.4</b>	<b>6/2.7</b>	<b>2/0.9</b>	<b>202/90.2</b>	<b>0</b>	<b>22/9.8</b>



2721), and 97.4% (2650/2721), respectively. The lowest CA observed was for levofloxacin detected with direct Vitek 2 Compact t AST (87.5%). In contrast, the AST CA values for all other antibiotics were very high (> 90%). Most VMEs were detected for the antibiotics tobramycin and aztreonam. The CAs and errors for all bacterium–antibiotic combinations are listed in Table 2. No ME or VME occurred in the analysis of 11 microorganisms (Table 3).

## Comparison of ESBL Detection with Direct Methods and Standard Culture-Based Methods

During the study period, the *E. coli*, *Klebsiella pneumoniae*, and *K. oxytoca* isolates from positive BC bottles were directly tested for ESBLs. The results of the direct method based on cefotaxime combined with cefotaxime/clavulanate (CTX+CTL) were completely consistent with those of the standard method. There was a discordant result between the direct method that combined ceftazidime and ceftazidime/clavulanate (CAZ+CAL) and the standard culture-based method. One ESBL-producing *E. coli* strain was missed when the direct CAZ+CAL method was used; when detected with the direct method, the inhibition zone of ceftazidime for this strain was 28 mm, but 30 mm when clavulanate was added. The difference in the zone diameter between the two discs was less than 5 mm, and the strain was judged to be ESBL negative. However, with the standard method, the ceftazidime inhibition zone was 23 mm, but 28 mm with ceftazidime/clavulanate, so the strain was judged to be ESBL positive. With the exception of this isolate, the ESBL results for all organisms were consistent when either the direct CAZ+CAL method or the standard method was used (Table 4).

## Comparison of mCIM/eCIM Detection with Direct Methods and Standard Culture-Based Methods

In this study, the carbapenemases of Enterobacteriaceae and *P. aeruginosa* were detected with direct mCIM and eCIM. To reflect the effect of direct method, we conducted mCIM and eCIM simultaneously. The eCIM results were considered valid when the mCIM results were positive. In this study, one strain of *Enterobacter cloacae* was carbapenemase positive, but only according to eCIM, and this measurement was excluded from the analysis. A total of 18 mCIM results were positive when determined with the standard culture-based mCIM method, including *K. pneumoniae* (n = 12), *E. coli* (n = 4), *Enterobacter cloacae* (n = 1), and *Raoultella planticola* (n = 1). Four strains of *E. coli* had positive eCIM results. Overall, the carbapenemase detection rates with direct mCIM and eCIM were exactly the same with the detection rates of the standard methods (Table 5).

## Discussion

Immediate effective antimicrobial treatment is necessary to ensure the survival of patients with Gram-negative bacteremia. The rapid analysis of BCs can provide a strong basis for clinically accurate treatment. Clinical microbiology laboratories have made sustained efforts to develop ways to accelerate BC analysis.<sup>30,31</sup> In this study, a multistep centrifugation method was used to isolate bacteria from bacteria-positive BC bottles. These were then identified, subjected to AST with several methods, their expression of ESBLs confirmed directly, and their carbapenemase expression tested with direct mCIM/eCIM. We effectively removed the blood cells, charcoal, and impurities from the blood with the multistep centrifugation method to obtain pure bacterial isolates from the bacteria-positive BC bottles. Our results show that the method developed here is not only highly accurate, but reduced the time required for pathogen identification to 1 h and reduced the time of AST by about 24h relative to that required for the standard culture-based method. Therefore, microbiology laboratories can identify bacterial pathogens, determine their antimicrobial susceptibility, and detect MDR strains responsible for BSIs on the day that a BC is deemed positive.

The use of MALDI-TOF MS is a major innovation in microbial identification.<sup>32</sup> After BCs are treated with specific methods, pathogenic bacteria can be rapidly identified directly with MALDI-TOF MS.<sup>10,33,34</sup> Pan et al used a lysis–centrifugation–wash procedure to prepare microorganisms from positive BCs, and then directly identified them with MALDI-TOF MS, with 96.49% accuracy, consistent with our results.<sup>35</sup> However, not all microbiology laboratories have access to MALDI-TOF MS for bacterial identification, and must identify bacteria with conventional biochemical-reaction-based instruments. In this study, we not only identified the bacteria rapidly with MALDI-TOF MS, but also evaluated the

**Table 2** Agreement and Errors in Antimicrobial Susceptibility Testing When Different Direct Methods Were Used

Antimicrobial Agent	No.	S (n)	I (n)	R (n)	Kirby-Bauer (K-B) Disk Diffusion Method AST				VITEK 2 Compact AST				E-Test AST			
					Category Agreement n (%)	Minor Error n (%)	Major Error n (%)	Very Major Error n (%)	Category Agreement n (%)	Minor Error n (%)	Major Error n (%)	Very Major Error n (%)	Category Agreement n (%)	Minor Error n (%)	Major Error n (%)	Very Major Error n (%)
AK	213	199	1	13	212(99.5)	1(0.5)	0(0)	0(0)	213(100.0)	0(0)	0(0)	0(0)	213(100.0)	0(0)	0(0)	0(0)
CRO	206	92	2	112	200(97.1)	6(2.9)	0(0)	0(0)	204(99.0)	2(1.0)	0(0)	0(0)	205(99.5)	1(0.5)	0(0)	0(0)
CN	213	142	0	71	211(99.1)	1(0.5)	1(0.7)	0(0)	210(98.6)	2(0.9)	1(0.7)	0(0)	212(99.5)	0(0)	1(0.7)	0(0)
MEM	222	195	1	26	220(99.1)	1(0.5)	0(0)	1(3.8)	220(99.1)	2(0.9)	0(0)	0(0)	220(99.1)	1(0.5)	1(0.5)	0(0)
TOB	210	129	20	61	196(93.3)	11(5.3)	0(0)	3(4.9)	191(91.0)	18(8.6)	0(0)	1(1.6)	202(96.2)	7(3.3)	0(0)	1(1.6)
PRL	209	81	12	116	199(95.2)	10(4.8)	0(0)	0(0)	202(96.7)	5(2.4)	2(2.5)	0(0)	198(94.7)	10(4.8)	0(0)	1(0.9)
TZP	218	169	7	42	211(96.8)	6(2.8)	1(0.6)	0(0)	211(96.8)	1(0.5)	0(0)	6(14.3)	212(97.2)	4(1.8)	1(0.6)	1(2.4)
ATM	214	130	0	84	193(90.2)	11(5.1)	1(0.8)	9(10.7)	205(95.8)	2(0.9)	4(3.1)	3(3.6)	197(92.1)	10(4.7)	0(0)	7(8.3)
LEV	224	79	37	108	203(90.6)	21(9.4)	0(0)	0(0)	196(87.5)	27(12.1)	1(1.3)	0(0)	212(94.6)	11(4.9)	1(1.3)	0(0)
KZ	176	40	5	131	173(98.3)	3(1.7)	0(0)	0(0)	176(100.0)	0(0)	0(0)	0(0)	176(100.0)	0(0)	0(0)	0(0)
CXM	174	69	7	98	169(97.1)	5(2.9)	0(0)	0(0)	165(94.8)	9(5.2)	0(0)	0(0)	169(97.1)	5(2.9)	0(0)	0(0)
CAZ	224	141	2	81	213(95.1)	11(4.9)	0(0)	0(0)	220(98.2)	2(0.9)	1(0.7)	1(1.2)	219(97.8)	5(2.2)	0(0)	0(0)
FEP	218	149	13	56	211(96.8)	6(2.8)	1(0.7)	0(0)	201(92.2)	15(6.9)	2(1.3)	0(0)	215(98.6)	2(0.9)	1(0.7)	0(0)
<b>Total</b>	<b>2721</b>	<b>1615</b>	<b>107</b>	<b>999</b>	<b>2611(96.0)</b>	<b>93(3.4)</b>	<b>4(0.2)</b>	<b>13(1.3)</b>	<b>2614(96.1)</b>	<b>85(3.1)</b>	<b>11(0.7)</b>	<b>11(1.1)</b>	<b>2650(97.4)</b>	<b>56(2.1)</b>	<b>5(0.3)</b>	<b>10(1.0)</b>

**Abbreviations:** AK, amikacin; CRO, ceftriaxone; CN, gentamicin; MEM, meropenem; TOB, tobramycin; PRL, piperacillin; TZP, piperacillin–tazobactam; ATM, aztreonam; LEV, levofloxacin; KZ, cefazolin; CXM, cefuroxime; CAZ, ceftazidime; FEP, cefepime; S, susceptible; I, intermediate; R, resistant; AST, antimicrobial susceptibility test.



**Table 3** Microorganism–Antimicrobial Combinations That Differed from Those Identified with the Standard Method

Microorganism	Kirby-Bauer (K-B) AST		VITEK 2 Compact AST		E-test AST	
	Major error	Very major error	Major error	Very major error	Major error	Very major error
<i>Escherichia coli</i>	CN, ATM, FEP	TOB, ATM	CN,PRL,ATM,CAZ, FEP	TOB, TZP	CN, MEM, FEP	TOB, PRL, ATM
<i>Klebsiella pneumoniae</i>	TZP	ATM	ATM	TZP, ATM, CAZ	TZP	ATM
<i>Enterobacter cloacae</i>	–	–	–	TZP	–	TZP
<i>Pseudomonas aeruginosa</i>	–	–	–	–	–	–
<i>Klebsiella aerogenes</i>	–	ATM	–	–	–	ATM
<i>Salmonella spp</i>	–	–	–	–	–	–
<i>Acinetobacter baumannii</i>	–	–	–	–	–	–
<i>Burkholderia cepacia</i>	–	MEM	–	–	–	–
<i>Klebsiella oxytoca</i>	–	–	–	–	–	–
<i>Stenotrophomonas maltophilia</i>	–	–	LEV	–	LEV	–
<i>Enterobacter amnigenus</i>	–	–	–	–	–	–
<i>Ralstonia mannitolilytica</i>	–	–	–	–	–	–
<i>Pantoea agglomerans</i>	–	–	–	–	–	–
<i>Aeromonas hydrophila/caviae</i>	–	–	–	–	–	–
<i>Raoultella planticola</i>	–	–	–	–	–	–
<i>Serratia marcescens</i>	–	–	–	–	–	–
<i>Aeromonas sobria</i>	–	–	–	–	–	–

**Abbreviations:** CN, gentamicin; MEM, meropenem; TOB, tobramycin; PRL, piperacillin; TZP, piperacillin–tazobactam; ATM, aztreonam; LEV, levofloxacin; CAZ, ceftazidime; FEP, cefepime; AST, antimicrobial susceptibility test.

**Table 4** Results of Extended-Spectrum  $\beta$ -Lactamase (ESBL) Testing with the Direct Method and the Standard Culture-Based Method

Microorganism	No.	Direct method		Standard method	
		CTX+CTL(+) <sup>a</sup>	CAZ+CAL(+) <sup>a</sup>	CTX+CTL(+) <sup>a</sup>	CAZ+CAL(+) <sup>a</sup>
<i>Escherichia coli</i>	118	62	59	62	60
<i>Klebsiella pneumoniae</i>	43	8	8	8	8
<i>Klebsiella oxytoca</i>	3	0	0	0	0

**Note:** <sup>a</sup>The results of ESBL tests were positive, and the strains were confirmed as ESBL-producing isolates.

**Abbreviations:** CTL, cefotaxime/clavulanate; CAL, ceftazidime/clavulanate.

accuracy of their direct identification using VITEK 2 Compact. Both instruments highly accurately identified Gram-negative bacteria in BSIs. However, the accuracy of MALDI-TOF MS was slightly lower than that of VITEK 2 Compact (90.2% and 96.4%, respectively). This may have been because 22 strains (22/224, 9.8%) produced peaks too small to be identified with MALDI-TOF MS because too little sample was spotted onto the target plate. Among these, half of the *Enterobacter cloacae* (9/18) strains were not identified with direct MALDI-TOF MS, although the reason is unclear. We will examine possible reasons in further research. However, there was no misidentification, and the results detected with MALDI-TOF MS were all correct. Wu et al reported that five of 93 strains could not be identified directly with MALDI-TOF MS, whereas 88 strains were identified correctly.<sup>36</sup> These results suggest that rapid MALDI-TOF MS is reliable. However, exceptions have been observed with rapid MALDI-TOF MS. Gu et al identified to pathogens from bacteria-positive BC bottles with a separation gel–adsorption method.<sup>37</sup> One strain was incorrectly identified with MALDI-TOF MS, although the specific reason was not determined in that article. In our study, some erroneous identification results occurred when nonfermentative bacteria from

**Table 5** Comparison of Direct and Standard Methods in Detecting Carbapenemases in Bacterial Strains

Isolate no.	Microorganism	Direct Method		Standard Method	
		mCIM	eCIM	mCIM	eCIM
18	<i>Klebsiella pneumoniae</i>	+	–	+	–
22	<i>Klebsiella pneumoniae</i>	+	–	+	–
23	<i>Klebsiella pneumoniae</i>	+	–	+	–
24	<i>Raoultella planticola</i>	+	–	+	–
25	<i>Klebsiella pneumoniae</i>	+	–	+	–
30	<i>Enterobacter cloacae</i>	+	–	+	–
36	<i>Klebsiella pneumoniae</i>	+	–	+	–
53	<i>Klebsiella pneumoniae</i>	+	–	+	–
54	<i>Klebsiella pneumoniae</i>	+	–	+	–
57	<i>Klebsiella pneumoniae</i>	+	–	+	–
73	<i>Escherichia coli</i>	+	+	+	+
90	<i>Escherichia coli</i>	+	+	+	+
97	<i>Escherichia coli</i>	+	+	+	+
104	<i>Escherichia coli</i>	+	+	+	+
184	<i>Klebsiella pneumoniae</i>	+	–	+	–
185	<i>Klebsiella pneumoniae</i>	+	–	+	–
188	<i>Klebsiella pneumoniae</i>	+	–	+	–
189	<i>Klebsiella pneumoniae</i>	+	–	+	–

**Notes:** +, test result was positive; –, test result was negative.

BSIs were identified with the VITEK 2 Compact instrument. Therefore, microbiology laboratory staff should carefully consider the results of direct Vitek 2 Compact for the identification of these bacteria.

The multistep centrifugation method we used to prepare the strains from bacteria-positive BCs is suitable for direct AST. This bacterial preparation process takes about only 45 min, 10 h less than the conventional culture-based method, and is more likely to generate early AST results. An advantage of our method is that direct AST of the bacteria can be conducted simultaneously with three different methods (direct K–B AST, direct VITEK 2 Compact AST, and direct E-test AST), which better evaluates the feasibility of the direct AST methods, but has seldom been analyzed in previous studies. Our results show that the CAs of the three direct AST methods were > 90%. However, there were discrepancies between the different direct AST methods. Direct K–B AST and direct E-test AST had similar MEs and VMEs (both < 2%), whereas the ME of direct VITEK 2 Compact AST was slightly higher than those of the other two methods. Kavipriya et al showed direct susceptibility test by VITEK-2 from positively flagged BC broth for Gram-negative bacilli had a better CA of 97%.<sup>38</sup> MEs or VMEs were detected for gentamicin, piperacillin–tazobactam, aztreonam, and cefepime with all three direct methods, suggesting that the results for these antimicrobial agents may not be credible when determined with direct methods. Errors for meropenem were also detected with direct K–B AST and direct E-test AST. Gabriele et al also found VMEs for meropenem when bacteria-positive BCs were analyzed with the direct rapid E-test.<sup>39</sup> However, the analysis of meropenem with direct VITEK 2 Compact AST showed a high degree of concordance with the standard method. Hogan found 4.8% VME for piperacillin–tazobactam when it was analyzed directly from BCs with VITEK 2, which is consistent with our results.<sup>3</sup> However, they also detected 3.8% ME for meropenem, which is not in agreement with our results. This may be attributable to the different treatments applied to the bacteria-positive BCs. Our results indicate that when clinicians require rapid results for meropenem in BC analyses, the direct VITEK 2 Compact method is more likely to produce accurate results.

Confirmatory ESBL testing and mCIM/eCIM testing to determine whether a strain is multidrug resistant are recommended by the CLSI guideline.<sup>27</sup> The strains must be cultured overnight, so it takes at least 48 h for clinicians to obtain these results after a BC is deemed positive. In this study, we developed a multistep centrifugation method to prepare the organisms from positive BCs for confirmation with direct ESBL testing and mCIM/eCIM testing within

24 h. As far as we know, this is the first study to apply direct mCIM/eCIM testing to the bacteria in BCs. Although only 18 strains were tested for carbapenemase in this study, it is heartening to note that the results of the direct mCIM and eCIM tests were 100% accurate. This suggests that Enterobacteriaceae and *P. aeruginosa* causing BSIs can be directly tested for carbapenemase without waiting for the AST results on the second or third day. A positive ESBL test indicates that the isolate is resistant to moxalactam, cefonicid, cefamandole, and cefoperazone.<sup>27</sup> CAZ combined with clavulanate and CTX combined with clavulanate were used for the direct detection of ESBLs in the present study. The results of the direct CTX+CTL method were completely consistent with the results of the standard method. De Gheldre et al also reported that CTX+CTL discs showed the greatest reliability in detecting ESBL-producing *E. coli*, *Proteus* spp., *Klebsiella* spp., and *Enterobacter* spp.<sup>40</sup> However, among the 164 results, we found one discrepancy between the results of the direct CAZ+CAL method and the standard culture-based method. This may be attributable to specific enzymes produced by that isolate, but further research is required to clarify this issue. Consequently, highly accurate direct ESBL tests can be performed in combination with direct AST, which should provide more accurate assessments of bacterial resistance.

This study had several limitations. First, our direct methods were only tested on monomicrobial infections in BCs, and we did not evaluate the applicability of these methods to polymicrobial infections. Second, the sample size was relatively small, and a larger number of samples is required in future research.

## Conclusion

In summary, we have developed a new method for preparing Gram-negative bacilli from bacteria-positive BCs, based on a multistep centrifugation strategy. The accuracy of direct pathogen identification with two instruments, direct AST with three methods, and the direct detection of MDR bacteria was high. These direct methods not only effectively reduce the time required for clinicians to obtain BC results, but no additional reagents or staff are required by the laboratory. These methods are appropriate for clinical laboratories because they are inexpensive and simple, and can be used as the routine workflow for daily BC analysis in any microbiology laboratory.

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## Disclosure

The authors report no conflicts of interest associated with this work.

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