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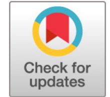

Blood-Modified Carbapenem Inactivation Method: a Phenotypic Method for Detecting Carbapenemase-Producing *Enterobacteriaceae* Directly from Positive Blood Culture Broths

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ABSTRACT A variant of the modified carbapenem inactivation method (mCIM) was developed to detect carbapenemase activity directly from positive blood culture broths. The method, termed "Blood-mCIM," was evaluated using Bactec blood culture bottles (Becton, Dickinson and Company, Franklin Lakes, NJ) inoculated with 27 different carbapenemase-producing *Enterobacteriaceae* (CPE) isolates and 34 different non-CPE isolates. The assay was positive for all blood culture broths inoculated with CPE isolates and negative for all blood culture broths inoculated with non-CPE isolates, corresponding to a diagnostic sensitivity and specificity of 100%, respectively. This assay is inexpensive using "off the shelf" reagents, does not require centrifugation or mechanical lysis, and can be readily implemented in any clinical microbiology laboratory. The Blood-mCIM should facilitate expedient administration of antimicrobial therapy targeted toward CPE bloodstream infections and assist infection control and public health surveillance.

KEYWORDS blood culture, bloodstream infection, carbapenemase, carbapenemase-producing *Enterobacteriaceae* (CPE), modified carbapenem inactivation method (mCIM), blood-modified carbapenem inactivation method (Blood-mCIM), phenotypic detection

Resistance to the carbapenems, potent broad-spectrum β-lactams, is one of the most concerning forms of antimicrobial resistance in members of the *Enterobacteriaceae*. Phenotypic resistance to carbapenems is often conferred by carbapenemases, enzymes that hydrolyze the carbapenem β-lactam ring (1). Bloodstream infections due to carbapenemase-producing *Enterobacteriaceae* (CPE) are accompanied by less favorable outcomes compared to non-carbapenemase-producing-carbapenem-resistant *Enterobacteriaceae* (2). Further, delayed treatment with effective antimicrobial agents in patients with septic shock is associated with a decrease in survival for every hour that therapy is delayed (3). Therefore, accurate and expedient methods to detect carbapenemase activity directly from positive blood culture broths are important for assisting physicians with antimicrobial therapy selection to treat bloodstream infections due to these organisms (4, 5).

Genotypic and immunologic assays for detecting carbapenemase-producing organisms directly from positive blood culture broths are described (6–8). These tests exhibit excellent diagnostic characteristics but their high cost often precludes their use in resource-limited settings. Additionally, they detect specific carbapenemase genes or proteins, and thus would not detect the emergence of new or previously uncommon carbapenemases. Phenotypic methods that detect carbapenemase activity are not limited thus, and have been employed for the detection of carbapenemase activity in

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TABLE 1 Carbapenemase-producing *Enterobacteriaceae* analyzed in this study

Clinical organism	Carbapenemase type[a]						
	Ambler class A			Ambler class B			Ambler class D
	NMC-A	KPC	SME	IMP	NDM	VIM	OXA-48-type
Citrobacter freundii	NA	**1**	NA	NA	**1**	NA	NA
Enterobacter cloacae complex	**1**	**2**	NA	NA	NA	**1**	NA
Escherichia coli	NA	**2**	NA	NA	**2**	NA	NA
Klebsiella aerogenes (formerly *Enterobacter aerogenes*)	NA	NA	NA	NA	NA	NA	**1**
Klebsiella ozaenae	NA	NA	NA	NA	NA	NA	**1**
Klebsiella pneumoniae	NA	NA	NA	**1**	NA	**1**	**4**
Morganella morganii	NA	**1**	NA	NA	**1**	NA	NA
Proteus mirabilis	NA	**1**	NA	NA	**1**	NA	NA
Providencia rettgeri	NA	NA	NA	NA	**1**	NA	NA
Raoultella ornithinolytica	NA	**1**	NA	NA	NA	NA	NA
Salmonella enterica serovar Senftenberg	NA	NA	NA	NA	**1**	NA	NA
Serratia marcescens	NA	NA	**2**	NA	NA	NA	NA
Total	**1**	**8**	**2**	**1**	**7**	**2**	**6**

[a]IMP, imipenemase; KPC, *Klebsiella pneumoniae* carbapenemase; NA, not applicable; NDM, New Delhi metallo-β-lactamase; NMC-A, nonmetallocarbapenemase-A; OXA, oxacillinase; SME, *Serratia marcescens* enzyme; VIM, Verona integron-encoded metallo-β-lactamase.

isolates recovered in culture (9–15). However, many of these assays have not been evaluated on positive blood culture broths, which are available for testing before the offending organism is recovered in culture on solid media.

This present study describes the appropriation of one such phenotypic assay, the modified carbapenem inactivation method (mCIM), to generate the Blood-mCIM that allows detection of CPE directly from positive blood culture broths through detection of carbapenemase activity. Results are available between 22 to 28 h after the blood culture broth signals positive, and at least a day earlier than conventional antimicrobial susceptibility testing results.

MATERIALS AND METHODS

Bacterial isolates. A collection of 61 *Enterobacteriaceae* isolates (Table S1 in the supplemental material) from the Centers for Disease Control and Prevention and the United States Food and Drug Administration Antibiotic Resistance Isolate Bank (ARB; https://www.cdc.gov/drugresistance/resistance-bank/index.html) was analyzed in this study. The collection contained 34 non-CPE isolates as follows: *Escherichia coli* ($n = 12$), *Enterobacter cloacae* complex ($n = 5$), *Klebsiella aerogenes* (formerly *Enterobacter aerogenes*) ($n = 4$), *Klebsiella oxytoca* ($n = 1$), *Klebsiella pneumoniae* ($n = 11$), and *Proteus mirabilis* ($n = 1$). These isolates harbored a wide range of β-lactamases, cell wall permeability defects (such as truncated porins), or a combination of both. The meropenem MIC values of these non-CPE isolates were determined using broth microdilution and ranged from ≤0.12 μg/ml to >8 μg/ml, with 23/34 (67.7%) testing susceptible (meropenem MIC value, ≤1 μg/ml), 3/34 (8.8%) testing intermediate (meropenem MIC value, 2 μg/ml), and 8/34 (23.5%) testing resistant (meropenem MIC value, ≥4 μg/ml) according to Clinical and Laboratory Standards Institute breakpoints (16). Twenty-seven isolates were CPE (Table 1). The meropenem MIC values of these CPE isolates were also determined using broth microdilution and ranged from 1 μg/ml to >8 μg/ml, with 2/27 (7.4%) testing susceptible, 3/27 (11.1%) testing intermediate, and 22/27 (81.5%) testing resistant according to Clinical and Laboratory Standards Institute breakpoints (16).

Blood-mCIM. Prior to performing the Blood-mCIM, ARB bacterial isolates stored at −80°C were cultured onto tryptic soy agar with sheep blood (TSAB; Becton, Dickinson and Company [BD], Franklin Lakes, NJ) and incubated in 5% carbon dioxide (CO_2) at 35°C for 18 to 24 h to generate F1 cultures. The F1 cultures were subcultured onto TSAB and again incubated in 5% CO_2 at 35°C for 18 to 24 h to generate F2 cultures. These F2 cultures were used to generate a suspension of organism in sterile saline (MicroScan Inoculum Saline, Beckman Coulter, Brea, CA, USA) equivalent to a 0.5 McFarland standard. The bacterial suspension was serially diluted in sterile saline and ∼1,500 CFU/ml injected into a BD Bactec blood culture bottle (inoculated with human blood) that was negative for growth after 5 days of incubation in the BD Bactec FX blood culture system. The contrived blood culture bottles were returned to the blood culture system. Throughout the study a range of blood culture media were inoculated with test isolates as follows: BD Bactec Plus Aerobic/F, 33 bottles; BD Bactec Lytic/10 Anaerobic/F, 23 bottles; and BD Bactec Peds Plus/F, five bottles.

Upon signaling positive, the blood culture bottle was vented using a subculturing/aerobic venting unit (BD) and four drops of the positive blood culture broth was added to 2 ml of tryptic soy broth (TSB) (BD) in a plastic assay tube and the mixture was vortexed briefly. A meropenem disk (10 μg) (BD) was added and fully immersed in the TSB/blood culture broth mixture. The tubes were incubated at 35˚C in ambient air without agitation for 4 h ± 15 min. Subsequently, the meropenem disks were removed using

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a 10 μl inoculation loop and applied to Mueller-Hinton agar plates (BD) freshly inoculated with a 0.5 McFarland suspension of a carbapenem-susceptible reporter strain (*Escherichia coli* ATCC 25922). The plates were incubated in ambient air at 35°C for 18 to 24 h. Results were interpreted as described by Pierce and colleagues for the mCIM, and as presented in the Clinical and Laboratory Institute M100 document (12, 16). The mCIM is considered negative (i.e., the test isolate does not produce a carbapenemase) if the *E. coli* ATCC 25922 zone size is ≥19 mm. In contrast, the mCIM is reported positive (i.e., the test isolate does produce a carbapenemase) if the *E. coli* ATCC 25922 zone size is 6 to 15 mm or pinpoint colonies are present within a 16 to 18 mm zone (for an example of pinpoint colonies, see Fig. 2 in reference 12). Finally, the mCIM result is considered indeterminate if the *E. coli* ATCC 25922 zone size is either 16 to 18 mm, ≥19 mm with pinpoint colonies present within the zone, or the absence or presence of a carbapenemase cannot be confirmed. All positive blood culture broths were subcultured to determine purity.

On each day of testing, a negative control (*K. pneumoniae* ATCC 700603, which expresses a SHV-18 enzyme) and a positive control (*K. pneumoniae* ATCC BAA-1705, which expresses a *Klebsiella pneumoniae* carbapenemase [KPC]) were prepared as described above and injected into a range of BD Bactec bottles (BD Bactec Plus Aerobic/F, 15 bottles; BD Bactec Lytic/10 Anaerobic/F, 13 bottles; and BD Bactec Peds Plus/F, six bottles) inoculated with human blood that were negative for growth after 5 days of incubation. Upon signaling positive, the positive blood culture broths were assayed as described above for the test isolates and results were interpreted as described above.

Data analysis. The reference method was genotypic detection of carbapenemase genes by whole-genome sequencing. A true positive result was defined as a positive Blood-mCIM result in the presence of any carbapenemase gene. A true negative result was defined as a negative Blood-mCIM result in the absence of any carbapenemase gene. A false-positive result was defined as a positive Blood-mCIM result in the absence of any carbapenemase gene, and a false-negative result was defined as a negative Blood-mCIM result in the presence of any carbapenemase gene. Diagnostic sensitivity and specificity, and associated confidence intervals, were calculated using an internet-based calculator (https://www.medcalc.org).

RESULTS

Performance of Blood-mCIM. To determine the diagnostic performance of Blood-mCIM, we assayed a collection of 61 *Enterobacteriaceae* comprising 27 CPE isolates (11 class A enzymes, 10 class B, and 6 class D; Table 1) and 34 non-CPE isolates (Table S1 in the supplemental material). The Blood-mCIM test was positive for all 27 CPE isolates and was negative for all 34 non-CPE isolates, corresponding to a diagnostic sensitivity of 100% (95% confidence interval [CI], 87.2% to 100%) and diagnostic specificity of 100% (95% CI, 89.7% to 100%). The zone sizes for the *E. coli* ATCC 25922 reporter strain were between 22 and 24 mm with an arithmetic mean of 22.9 mm (standard deviation of the mean [SD], 0.3 mm) for all non-CPE, and 6 mm (i.e., the *E. coli* ATCC 25922 reporter strain grew up to the edge of the meropenem disk) for all CPE.

Reproducibility of quality control testing. Quality control testing was performed each day of testing on a total of 17 different days. When examining the meropenem zone sizes of the *E. coli* ATCC 25922 reporter strain for the negative control (*K. pneumoniae* ATCC 700603), the range in zone sizes was between 19 and 23 mm with an arithmetic mean of 22.2 mm (SD, 1 mm), and 6 mm for all positive-control (*K. pneumoniae* ATCC BAA-1705) tests. These data support the use of these isolates as quality control isolates for the Blood-mCIM.

DISCUSSION

In this study, we describe the diagnostic performance of the Blood-mCIM, a variant of the mCIM, which permits the phenotypic detection of CPE directly from positive blood culture broths. The assay has a diagnostic sensitivity and specificity of 100%, respectively, and quality control testing was highly reproducible. When taking into account the 18 to 24 h of incubation time required to evaluate the meropenem zone sizes, results can be obtained between 22 to 28 h after the blood culture signals positive. The test requires inexpensive "off the shelf" materials that are accessible to all clinical microbiology laboratories, including those in austere settings. Importantly, upon signaling positive, the Blood-mCIM does not require processing of the blood culture using centrifugation or mechanical lysis methodology, therefore simplifying its implementation and use.

Notwithstanding the favorable performance of the Blood-mCIM, our study has limitations. First, only contrived blood culture broths were assessed, although this appears to be a limitation of most studies assessing the performance of phenotypic

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methods for detecting carbapenemase activity directly from positive blood culture broths (17, 18). Second, we only assessed this method using a single blood culture system at one center. Again, this appears to be a limitation of previous studies (17, 18). Finally, an 18 to 24 h of incubation step is required to evaluate meropenem zone sizes. However, Caméléna and colleagues showed using the CIM that carbapenemase-producing isolates could be detected 8 h after setting up the assay (i.e., 2 h of incubation time for the CIM followed by evaluation of the meropenem zone sizes after 6 h of incubation on inoculated Mueller-Hinton agar plates). Therefore, it is conceivable that the Blood-mCIM could be evaluated in a similar time frame and additional investigations using shorter incubation times are warranted.

Several phenotypic methods for the detection of carbapenemase-producing isolates directly from positive blood culture broths have been reported. These include modifications of the Carba NP, β-CARBA, CIM, and NeoRapid CARB tests (17, 18). The reported diagnostic sensitivity of these assays varied between 97.9 and 100%, and the diagnostic specificity between 91.4 and 100%. However, these methods (with the exception of the bcCIM, which is a variant of the CIM used to detect CPE directly from blood culture broths) (18) require additional processing such as centrifugation or mechanical lysis, which may be prohibitive in some settings, and all of the aforementioned methods, including bcCIM, require supplementary reagents such as sodium chloride, sodium dodecyl sulfate, or zinc sulfate. This is in contrast to the Blood-mCIM, which requires neither additional processing nor reagents.

To improve the detection of metallo-β-lactamases (MBLs) using the bcCIM, supplementation with zinc sulfate was necessary (18). This is in contrast to our findings, where Blood-mCIM readily detected all MBLs without the need for zinc ion supplementation. Nonetheless, it is important to note that our study included a relatively small number of MBLs ($n = 10$) compared to the number of MBLs assayed using the bcCIM ($n = 50$). The apparent requirement for zinc sulfate implies that, unlike the Blood-mCIM, the bcCIM and the Carba NP, which are dependent on zinc sulfate, cannot be further modified by the addition of metal chelating agents, such as EDTA, to allow differentiation between serine carbapenemases (class A and D enzymes, e.g., KPC and OXA-48-type) and MBLs (class B enzymes, e.g., IMP, NDM, VIM) as described for bacterial isolates recovered on solid media (13–15).

In conclusion, we describe the characterization of the Blood-mCIM that permits the phenotypic detection of carbapenemase activity directly from positive blood culture broths. The assay displayed excellent diagnostic characteristics and was easy to perform and interpret, and only required equipment used in all clinical microbiology laboratories regardless of the setting. As part of a multicenter study, future studies will focus on determining the performance of the Blood-mCIM using patient-collected and contrived blood cultures incubated on a range of blood culture systems.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, XLSX file, 0.02 MB.

ACKNOWLEDGMENTS

Isolates in this study were acquired from the CDC and FDA antibiotic resistance bank (https://www.cdc.gov/drugresistance/resistance-bank/index.html).

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