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Comparative Evaluation of Four Phenotypic Methods for Detection of Class A and B Carbapenemase-Producing *Enterobacteriaceae* in China

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ABSTRACT The objective of this study was to evaluate the performance of four phenotypic methods in the detection of carbapenemase-producing *Enterobacteriaceae* (CPE) in China. We evaluated the performance of four carbapenemase detection methods, the modified Hodge test (MHT), the Carba NP test, the meropenem hydrolysis assay (MHA) with 1- and 2-h incubation, and the modified carbapenem inactivation method (mCIM) with meropenem, imipenem, and ertapenem, on 342 carbapenem-resistant *Enterobacteriaceae* isolates (CRE) in China. PCR was used as the gold standard. The 2-h-incubation MHA performed the best in carbapenemase detection (overall sensitivity, specificity, positive predictive value, and negative predictive value all 100%). Second was the Carba NP test, with a sensitivity of 99.6%. The 1-h-incubation MHA performed poorly in *Klebsiella pneumoniae* carbapenemase (KPC) detection (sensitivity, 71.3%). For mCIM, the best performance was observed with the meropenem disk. The MHT exhibited the worst performance, with a specificity of 88.8%. All assays except 1-h-incubation MHA, which failed to identify 68 KPC-2s, had a sensitivity of >98% in the detection of 172 KPCs. Likewise, all assays had a sensitivity of >95% in the detection of 70 class B carbapenemases, except for MHT (82.9%). The 2-h-incubation MHA significantly improved the accuracy in CPE detection compared with that for 1-h incubation and performed the best in the detection of class A and B carbapenemases. Our findings suggest that the MHA is the most practical assay for carbapenemase detection. For those who cannot afford the associated equipment, both the Carba NP test and mCIM are good alternatives with regard to the practical requirements of time and cost.

KEYWORDS modified Hodge test, Carba NP test, meropenem hydrolysis assay, modified carbapenem inactivation method, carbapenemase-producing *Enterobacteriaceae*

In 2013, the Centers for Disease Control and Prevention assigned the highest threat level to carbapenem-resistant *Enterobacteriaceae* (CRE) and declared it an urgent public health threat requiring attention (1). Infections caused by CRE, which are associated with high mortality rates, are now emerging worldwide, posing a formidable challenge to antimicrobial therapy (2, 3). The mechanisms underlying CRE are complex and include both the production of carbapenem-hydrolyzing β-lactamases (carbapenemase-producing CRE [CPE]) and resistance due to the presence of a combination of other factors (non-CPE), such as hyperproduction of AmpC-lactamases or extended-spectrum β-lactamases (ESBLs) combined with altered membrane permea-

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bility (4, 5). Previous studies have shown that CPE may be more virulent than non-CPE strains (6). The spread of CPE can be controlled through the implementation of supplemental infection prevention and control measures and prudent use of antibiotics (7). In this regard, the introduction of rapid and sensitive methodologies for the detection of CPE is of utmost importance.

For decades, genotypic assays (such as PCR and DNA microarray) have been considered the gold standard for the detection of carbapenemase genes. However, they are of limited practical use in most clinical laboratories due to drawbacks such as high cost, the need for significant expertise, and the inability to identify novel or unknown emerging resistance genes. Currently, several phenotypic methods are available for CPE screening, including growth-based assays (modified Hodge test [MHT]) (8), rapid colorimetric-based assays (manual and commercial versions of the Carba NP test) (9), matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS)-based meropenem hydrolysis assays (MHAs) (7), immunochromatographic lateral flow assays (10), and most recently, the modified carbapenem inactivation method (mCIM) (11).

There are limited studies on the performance of each of the above methods in the detection of CPE. Therefore, we evaluated the accuracy of four screening methods (MHT, Carba NP, MHA, and mCIM) in CPE detection using a genotypic assay as a gold standard.

MATERIALS AND METHODS

Bacterial isolates. The study was conducted at Peking Union Medical College Hospital (PUMCH), Beijing, China, between December 2016 and April 2017. A total of 342 prospectively collected, nonduplicate CRE isolates from midstream urine ($n = 70$), blood ($n = 70$), sputum ($n = 49$), ascitic fluid ($n = 43$), abscess ($n = 25$), drainage fluid ($n = 21$), bile ($n = 19$), tissue ($n = 12$), wound ($n = 9$), catheter ($n = 5$), and other sites ($n = 19$) referred by 34 teaching hospitals located in 23 provinces in China to PUMCH, from 2004 to 2014, were included (see Table S1 in the supplemental material). All the *Enterobacteriaceae* isolates were identified by MALDI-TOF MS (Bruker Biotyper; Bruker Daltonik, Bremen, Germany). CRE was defined as resistance to one of the carbapenems, imipenem (IPM), meropenem (MEM), or ertapenem (ETP), by the broth microdilution method (BMD) according to the latest Clinical and Laboratory Standards Institute (CLSI) 2017 breakpoints criteria at PUMCH (12).

Method comparison study. (i) Molecular detection of carbapenemase genes. bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{AIM}, bla_{SPM}, bla_{KPC}, bla_{DIM}, bla_{BIC}, bla_{GIM}, bla_{SIM}, and bla_{OXA-48} genes were detected by multiplex PCR as previously described (13). bla_{GES}, bla_{NMC}, bla_{SME}, and bla_{IMI} genes were each detected by a single primer set (14). Furthermore, all strains were analyzed for the presence of extended-spectrum or plasmid-mediated AmpC β-lactamase genes bla_{CTX-M}, bla_{SHV}, bla_{TEM}, bla_{MOX}, bla_{CMY}, bla_{LAT}, bla_{BIL}, bla_{DHA}, bla_{ACC}, bla_{MIR}, bla_{FOX}, and bla_{ACT} by PCR and subsequent DNA sequencing (15, 16). The obtained gene sequences were compared with those in the database located at NCBI blast server (http://blast.ncbi.nlm.nih.gov). A minimum of 99% sequence identity and 100% coverage threshold was deemed sufficient for the confirmation of each gene (17).

(ii) Multilocus sequence typing analysis. All the *K. pneumoniae* and *Escherichia coli* isolates positive for carbapenemase genes were further characterized by multilocus sequence typing analysis ([MLST] http://bigsdb.pasteur.fr/klebsiella/klebsiella.html and http://mlst.warwick.ac.uk/mlst/dbs/Ecoli).

(iii) Phenotypic detection of carbapenemase production. All the CRE isolates were subcultured from frozen stocks to 5% sheep blood agar at 35°C for an overnight incubation, followed by a second subculture prior to phenotypic testing, including MHT (8), Carba NP (9), mCIM (11), and MHA (7), as previously described or with some modifications. For mCIM, both IPM and ETP disks (Oxoid, France) were used in addition to MEM. For MHA, the hydrolysis setups (1 μl loopful of bacteria mixed with 50 μl reaction buffer containing MEM [Sigma-Aldrich, Munich, Germany] at 1 mg/ml in 10 mM NH_4HCO_3 and 10 μg/ml $ZnCl_2$ at pH 8.0 to 9.0) were incubated for 1 h and 2 h at 37°C under agitation (900 rpm) before centrifugation for 1 min at 14,000 $\times$ *g*. For measurement, 1 μl of the sample was applied on a stainless steel MALDI target plate (MSP 96 target; Bruker Daltonics) and allowed to dry at room temperature before 1 μl of matrix solution (α-cyano-4-hydroxycinnamic acid; Bruker Daltonics) was overlaid. Mass spectra were obtained and analyzed by employing an optimized automatic acquisition method in the MBT STAR-BL module in FlexControl 3.3 (Bruker Daltonik) within the *m/z* range 300 to 600. An isolate was considered CPE if at least one of the decarboxylated products of MEM (*m/z* 358.5/380.5) was detected, while a non-CPE was defined by the absence of both decarboxylated products of MEM and the presence of MEM and/or its sodium salt (*m/z* 384.5/406.5) (7). *Klebsiella pneumoniae* ATCC 1705 and ATCC 1706 were included as positive and negative quality controls (QCs), respectively.

Statistical analysis. The investigators performing phenotypic testing were blind to the identities of the isolates. Agreement and validity values were calculated with a 95% confidence interval (CI) based on an exact binomial distribution. Data were analyzed using SPSS, version 15.0 (SPSS Inc., Chicago, USA).

TABLE 1 Performance characteristics of four phenotypic tests for detection of carbapenemase-producing *Enterobacteriaceae*

			mCIM[c]			MHA[d]	
Accuracy[a]	MHT[b]	Carba NP	MEM	IMP	ETP	1 h	2 h
Sensitivity (%)	95.1	99.6	99.6	98.8	98.0	71.3	100
95% CI	91.6–97.4	97.7–100	97.7–100	96.5–99.8	95.3–99.3	65.2–76.9	98.5–100
Specificity (%)	88.8	100	99.0	96.9	99.0	100	100
95% CI	80.8–94.3	96.3–100	94.5–100	91.3–99.4	94.5–100	96.3–100	96.3–100
PPV (%)	95.5	100	99.6	98.8	99.6	100	100
95% CI	92.0–97.7	98.5–100	97.7–100	96.5–99.8	97.7–100	97.9–100	98.5–100
NPV (%)	87.9	99.0	99.0	96.9	95.1	58.3	100
95% CI	79.8–93.6	94.5–100	94.5–100	91.3–99.4	88.9–98.4	50.5–65.9	96.3–100

[a]PPV, positive predictive value; NPV, negative predictive value.
[b]MHT, modified Hodge test.
[c]mCIM, modified carbapenem inactivation method; MEM, meropenem; IMP, imipenem; ETP, ertapenem.
[d]MHA, merpenem hydrolysis assay.

RESULTS

Ten species were identified in 342 CRE isolates, including 187 *K. pneumoniae*, 67 *Enterobacter cloacae*, 49 *Escherichia coli*, 12 *Enterobacter aerogenes*, 9 *Klebsiella oxytoca*, 8 *Citrobacter freundii*, 6 *Serratia marcescens*, 2 *Klebsiella planticola*, and 1 each of *Proteus mirabilis* and *Providencia rettgeri*. A total of 244 isolates were positive for carbapenemase genes, among which 172 *Klebsiella pneumoniae* carbapenemase (KPC), 42 imipenem metallo-β-lactamase (IMP), 26 New Delhi metallo-β-lactamase (NDM), 2 each of IMP and Verona integron-encoded metallo-β-lactamase (VIM) and KPC and IMP carbapenemase genes, were identified (see Table S1 in the supplemental material).

MLST analysis. An MLST analysis revealed significant genetic diversity among carbapenemase-positive *K. pneumoniae* and *E. coli* isolates, with 10 sequence types (STs) identified in 29 *E. coli* isolates and 26 STs identified in 153 *K. pneumoniae* isolates. The most common STs for *E. coli* were ST354, ST131, ST167, and ST648, each accounting for 24.1% (7/29), 17.2% (5/29), 13.8% (4/29), and 13.8% (4/29) of the isolates, respectively, followed by ST2003 (10.3% [3/29]), ST410 (6.9% [2/29]), ST617 (3.4% [1/29]), ST1237 (3.4% [1/29]), ST6682 (3.4% [1/29]), and ST361 (3.4% [1/29]). For *K. pneumoniae*, ST11 (69.3% [106/153]) dominated among the isolates, followed by ST15 (3.9% [6/153]), ST17 (2.6% [4/153]), and ST290 (2.6% [4/153]) (Table S1).

Overall performance. The sensitivity, specificity, positive predictive values (PPVs), and negative predictive values (NPVs) of the various assays in detecting carbapenemase production among all CRE isolates are shown in Table 1. The 2-h-incubation MHA exhibited the highest overall sensitivity, specificity, PPV, and NPV of 100%, followed by the Carba NP and mCIM-MEM, with a sensitivity of 99.6% each. In contrast, the 1-h-incubation MHA performed poorly, with an overall sensitivity of 71.3%, mainly due to a failure in KPC detection. However, both the 1-h-incubation MHA and the Carba NP achieved a specificity of 100%. Among the three carbapenem disks using mCIM, MEM performed the best with the highest sensitivity (99.6%) and specificity (99.0%). The MHT performed the worst among all assays, with a specificity of 88.8%.

Accuracy of specific carbapenemase classes. All assays, except for the 1-h-incubation MHA, exhibited >98% sensitivity in detecting class A carbapenemase, with 172 KPCs correctly identified, failing to recognize 68 KPC-2 carbapenemase-producing isolates: 66 *K. pneumoniae* isolates, 1 *E. cloacae*, and 1 *S. marcescens* (Table 2). Sensitivities of more than 95% were shown by all the assays in detecting the 70 class B carbapenemases with the only exception of MHT, the sensitivity of which was 82.9% due to invalid results for 1 IMP and 11 NDM producers. The 1-h-incubation MHA also missed two IMP producers. Both the MHT (57.6%) and mCIM-ETP (88.5%) assays had a much lower sensitivity in identifying NDM enzymes. The mCIM-MEM and mCIM-IPM

TABLE 2 Performance characteristics of four phenotypic tests in the detection of specific carbapenemase classes

Classification	% positive (no. of positive tests/no. of isolates) producing the specific carbapenemase[a]						
			mCIM			MHA	
	MHT	Carba NP	MEM	IMP	ETP	1 h	2 h
Class A							
KPC (n = 172)	100 (172/172)	99.4 (171/172)	98.2 (169/172)	99.4 (171/172)	98.8 (170/172)	60.4 (104/172)	100 (172/172)
Class B							
IMP (n = 42)	97.6 (41/42)	100 (42/42)	100 (42/42)	100 (42/42)	100 (42/42)	95.2 (40/42)	100 (42/42)
NDM (n = 26)	57.6 (15/26)	100 (26/26)	96.2 (25/26)	92.3 (24/26)	88.5 (23/26)	100 (26/26)	100 (26/26)
IMP & VIM (n = 2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)
All class B (n = 70)	82.9 (58/70)	100 (70/70)	98.6 (69/70)	97.1 (68/70)	95.7 (67/70)	97.1 (68/70)	100 (70/70)
Class A and B							
KPC & IMP (n = 2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)	100 (2/2)
Total (n = 244)	95.1 (232/244)	99.5 (243/244)	98.0 (239/244)	98.8 (241/244)	98.0 (239/244)	71.3 (174/244)	100 (244/244)

[a]MHT, modified Hodge test; MHA, meropenem hydrolysis assay; mCIM, modified carbapenem inactivation method; MEM, meropenem; IMP, imipenem; ETP, ertapenem.

missed one and two NDM producers, respectively. As for the two isolates producing both KPC and IMP enzymes, all the assays were able to identify them accurately.

Accuracy of specific species. Table 3 further breaks down the CRE isolates into different species. All the assays performed excellently (sensitivity 100%) in the identification of *E. aerogenes*, *K. oxytoca*, *K. planticola*, and *P. mirabilis*, with a specificity of 100% for *K. oxytoca*, *K. planticola*, *P. rettgeri*, and *S. marcescens*. The 1-h-incubation MHA showed sensitivities of 56.2%, 75.0%, and 85.7% for *K. pneumoniae*, *Serratia marcescens*, and *C. freundii*, respectively. The MHT, on the other hand, showed a sensitivity of 89.7% for *E. coli*. Apart from the above, all assays had sensitivities and specificities over 90% for the 10 species, with the notable exception of MHT in the detection of carbapenemases in *C. freundii* (0%), *E. aerogenes* (87.5%), and *E. cloacae* (86.7%). The 2-h-incubation MHA significantly improved the accuracy in CPE detection compared to that for the 1-h incubation. The sensitivity and specificity of the 2-h-incubation MHA for all the species tested and for all classes of carbapenemases were each 100%.

Discrepant results between phenotypic and molecular tests for carbapenemase detection. All discrepant results (after repeated trials) between phenotypic and molecular tests for carbapenemase detection are summarized in Table 4. The MHT assay had the highest number of false-positive (FP) results, which were all interpreted as weak positive, and false-negative (FN) results, accounting for 11 NDM-1 and 1 IMP-4 producers in various species. The Carba NP test showed an FN result for a KPC-2-producing *E. coli*. As for mCIM with MEM, one FP and FN (NDM-1 producing) each were observed in *E. cloacae*. The 2-h-incubation MHA did not give any FP or FN results as a whole.

DISCUSSION

Over the past years, CRE has emerged worldwide, with outbreaks reported in several hospital settings (18, 19). Therefore, the rapid and accurate detection of carbapenemase producers is critical to implementing timely contact isolation and antibiotic treatment decisions, as CPE can have devastating consequences in health care settings (20). We compared four phenotypic tests, including the recently described mCIM (11), to detect carbapenemase production among well-characterized *Enterobacteriaceae* isolates. Although several studies have evaluated the performance of mCIM in comparison with other phenotypic assays such as MHT and the Carba NP test in *Enterobacteriaceae* (11, 21), to the best of our knowledge, this is the first report on the performance of mCIM in comparison to MHA, MHT, and Carba NP assays.

Although molecular detection by PCR remains the gold standard for carbapenemase detection, practical clinical use is hindered by the associated high cost, handling complexity, and equipment requirements (Table 5). Thus, it has become customary for

TABLE 3 Accuracy of carbapenemase detection for specific species by the four phenotypic tests

Species	Accuracy	Percentage[a]						
				mCIM			MHA	
		MHT	Carba NP	MEM	IMP	ETP	1 h	2 h
Citrobacter freundii								
CP (n = 7)	Sensitivity	100	100	100	100	100	85.7	100
	95% CI	59.0–100	59.0–100	59.0–100	59.0–100	59.0–100	42.1–99.6	59.0–100
None (n = 1)	Specificity	0	100	100	100	100	100	100
	95% CI	IC[b]	IC	IC	IC	IC	IC	IC
All (n = 8)	PPV	87.5	100	100	100	100	100	100
	95% CI	47.4–99.7	59.0–100	59.0–100	59.0–100	59.0–100	54.1–100	59.0–100
	NPV	IC	100	100	100	100	50.0	100
	95% CI		2.5–100	2.5–100	2.5–100	2.5–100	1.3–98.7	2.5–100
Enterobacter aerogenes								
CP (n = 4)	Sensitivity	100	100	100	100	100	100	100
	95% CI	39.8–100	39.8–100	39.8–100	39.8–100	39.8–100	39.8–100	39.8–100
None (n = 8)	Specificity	87.5	100	100	100	100	100	100
	95% CI	47.4–99.7	63.1–100	63.1–100	63.1–100	63.1–100	63.1–100	63.1–100
All (n = 12)	PPV	80.0	100	100	100	100	100	100
	95% CI	28.4–99.5	39.8–100	39.8–100	39.8–100	39.8–100	39.8–100	39.8–100
	NPV	100	100	100	100	100	100	100
	95% CI	59.0–100	63.1–100	63.1–100	63.1–100	63.1–100	63.1–100	63.1–100
Enterobacter cloacae								
CP (n = 37)	Sensitivity	94.6	100	97.3	94.6	94.6	97.3	100
	95% CI	81.8–99.3	90.5–100	85.8–99.9	81.8–99.3	81.8–99.3	85.8–99.9	90.5–100
None (n = 30)	Specificity	86.7	100	96.7	96.7	96.7	100	100
	95% CI	69.3–96.2	88.4–100	82.8–99.9	82.8–99.9	82.8–99.9	88.4–100	88.4–100
All (n = 67)	PPV	89.7	100	97.3	97.2	97.2	100	100
	95% CI	75.8–97.1	90.5–100	85.8–99.9	85.5–99.9	85.5–99.9	90.3–100	90.5–100
	NPV	92.9	100	96.7	93.6	93.6	96.8	100
	95% CI	76.5–99.1	88.4–100	82.8–99.9	78.6–99.2	78.6–99.2	83.3–99.9	88.4–100
Escherichia coli								
CP (n = 29)	Sensitivity	89.7	96.6	100	100	100	100	100
	95% CI	72.7–97.8	82.2–99.9	88.1–100	88.1–100	88.1–100	88.1–100	88.1–100
None (n = 20)	Specificity	90.0	100	100	100	100	100	100
	95% CI	68.3–98.8	83.2–100	83.2–100	83.2–100	83.2–100	83.2–100	83.2–100
All (n = 49)	PPV	92.9	100	100	100	100	100	100
	95% CI	76.5–99.1	87.7–100	88.1–100	88.1–100	88.1–100	88.1–100	88.1–100
	NPV	85.7	95.2	100	100	100	100	100
	95% CI	63.7–97.0	76.2–99.9	83.2–100	83.2–100	83.2–100	83.2–100	83.2–100
Klebsiella oxytoca								
CP (n = 8)	Sensitivity	100	100	100	100	100	100	100
	95% CI	59.0–100	63.1–100	63.1–100	63.1–100	63.1–100	63.1–100	63.1–100
None (n = 1)	Specificity	100	100	100	100	100	100	100
	95% CI	IC	IC	IC	IC	IC	IC	IC
All (n = 9)	PPV	100	100	100	100	100	100	100
	95% CI	59.0–100	63.1–100	63.1–100	63.1–100	63.1–100	63.1–100	63.1–100
	NPV	100	100	100	100	100	100	100
	95% CI	2.5–100	2.5–100	2.5–100	2.5–100	2.5–100	2.5–100	2.5–100
Klebsiella pneumoniae								
CP (n = 153)	Sensitivity	96.1	100	100	98.0	98.0	56.2	100
	95% CI	91.7–98.6	97.6–100	97.6–100	94.4–99.6	94.4–99.6	48.0–64.2	97.6–100
None (n = 34)	Specificity	91.2	100	100	94.1	94.1	100	100
	95% CI	76.3–98.1	89.7–100	89.7–100	80.3–99.3	80.3–99.3	89.7–100	89.7–100
All (n = 187)	PPV	98.0	100	100	98.7	98.7	100	100
	95% CI	94.3–99.6	97.6–100	97.6–100	95.3–99.8	95.3–99.8	95.8–100	97.6–100
	NPV	83.8	100	100	91.4	91.4	33.7	100
	95% CI	68.0–93.8	89.7–100	89.7–100	76.9–98.2	76.9–98.2	24.6–43.8	89.7–100
Klebsiella planticola								
CP (n = 1)	Sensitivity	100	100	100	100	100	100	100
	95% CI	IC	IC	IC	IC	IC	IC	IC

(Continued on next page)

TABLE 3 (Continued)

Species	Accuracy	Percentage[a]						
				mCIM			MHA	
		MHT	Carba NP	MEM	IMP	ETP	1 h	2 h
None (n = 1)	Specificity	100	100	100	100	100	100	100
	95% CI	IC	IC	IC	IC	IC	IC	IC
All (n = 2)	PPV	100	100	100	100	100	100	100
	95% CI	2.5–100	2.5–100	2.5–100	2.5–100	2.5–100	2.5–100	2.5–100
	NPV	100	100	100	100	100	100	100
	95% CI	2.5–100	2.5–100	2.5–100	2.5–100	2.5–100	2.5–100	2.5–100
Proteus mirabilis								
CP (n = 1)	Sensitivity	100	100	100	100	100	100	100
	95% CI	IC	IC	IC	IC	IC	IC	IC
All (n = 1)	PPV	100	100	100	100	100	100	100
	95% CI	IC	IC	IC	IC	IC	IC	IC
Providencia rettgeri								
None (n = 1)	Specificity	100	100	100	100	100	100	100
	95% CI	IC	IC	IC	IC	IC	IC	IC
All (n = 1)	NPV	100	100	100	100	100	100	100
	95% CI	IC	IC	IC	IC	IC	IC	IC
Serratia marcescens								
CP (n = 4)	Sensitivity	100	100	100	100	100	75.0	100
	95% CI	39.8–100	39.8–100	39.8–100	39.8–100	39.8–100	19.4–99.4	39.8–100
None (n = 2)	Specificity	100	100	100	100	100	100	100
	95% CI	15.8–100	15.8–100	15.8–100	15.8–100	15.8–100	15.8–100	15.8–100
All (n = 6)	PPV	100	100	100	100	100	100	100
	95% CI	39.8–100	39.8–100	39.8–100	39.8–100	39.8–100	29.2–100	39.8–100
	NPV	100	100	100	100	100	66.7	100
	95% CI	15.8–100	15.8–100	15.8–100	15.8–100	15.8–100	9.4–99.2	15.8–100

[a]MHT, modified Hodge test; MHA, meropenem hydrolysis assay; mCIM, modified carbapenem inactivation method; MEM, meropenem; IMP, imipenem; ETP, ertapenem.
[b]IC, incalculable.

clinical laboratories to utilize some phenotypic tests to categorize CRE into CPE and non-CPE.

MHT was the first recognized carbapenemase screening method recommended by the CLSI in 2009 (22). The advantages of this method include the ease of performance, low cost, and nonrequirement of specialized reagents. However, the interpretation of the results is subjective, and the method yields both FP results, particularly with *Enterobacter* spp. that have AmpC enzymes and porin alterations, and FN results for NDM-producing isolates (23, 24), which is also confirmed in the present study. Isolates which exhibited weak positive reactions by the MHT assay possessed at least one extended-spectrum or an AmpC β-lactamase gene, except for one *E. cloacae* isolate (Table 4), suggesting the presence of other carbapenem resistance mechanisms in this strain. The 12 FN results were observed in 11 NDM-1 and 1 IMP-4 producer, a phenomenon previously described (25, 26). Thus, the sensitivity (95.1%) and specificity (88.8%) of MHT were lower than those of the other three. Furthermore, an overnight incubation was required for this test, since it is a growth-based assay (Table 5).

The Carba NP test was developed by Nordmann et al. in 2012 and was approved by the CLSI in 2015 (27). Within 2 to 3 h, the Carba NP test can detect not only the known carbapenemases but also newly emerging carbapenemases, with high sensitivity and specificity (28). However, it also has some disadvantages, including requirements for dedicated reagents (with associated costs and training needs) and subjective result interpretation based on color change, which may be difficult to appreciate for inexperienced personnel. All of these may contribute to the triviality and uncertainty of the procedure (Table 5). Previous studies have shown that this test has a low sensitivity in the detection of OXA-48-type carbapenemases (7, 11). In our setting, the Carba NP test had a high sensitivity and specificity of 99.6% and 100%, respectively, with only one FN

TABLE 4 Discrepant results between phenotypic and molecular tests for carbapenemase detection

No.	Species	Specimen	Carbapenemase	Ambler class A			Plasmid-mediated AmpC	MHT[a]	Carba NP	MHA[b] (2h)	mCIM (MEM)[c]	
				TEM	SHV	CTX-M					diam (mm)	IP[d]
D01	*Enterobacter cloacae*	Blood						w+[e]			17[f]	+
D02	*Enterobacter cloacae*	Blood		TEM-1				w+			29	
D21	*Escherichia coli*	Blood	KPC-2	TEM-1		CTX-M-3, CTX-M-14		+		+	29	+
L001	*Klebsiella pneumoniae*	Sputum	NDM-1	TEM-1	SHV-11				+	+	15	+
L003	*Klebsiella pneumoniae*	Sputum	NDM-1		SHV-11				+	+	21	+
L004	*Klebsiella pneumoniae*	Sputum	NDM-1		SHV-11				+	+	24	+
L009	*Enterobacter cloacae*	Sputum	NDM-1	TEM-1	SHV-11		DHA-1		w+	+	26	+
L012	*Klebsiella oxytoca*	Blood	NDM-1		SHV-11	CTX-M-14			+	+	6	+
L013	*Klebsiella pneumoniae*	Bile	NDM-1	TEM-1	SHV-11				+	+	6	+
L014	*Klebsiella pneumoniae*	Sputum	NDM-1	TEM-1	SHV-11				+	+	6	+
L015	*Enterobacter cloacae*	Blood	NDM-1		SHV-11		DHA-1		+	+	6	+
L087	*Enterobacter cloacae*	Blood	NDM-1	TEM-1	SHV-11	CTX-M-3		+	+	+	20	
L104	*Escherichia coli*	Blood	NDM-1				CMY-2		+	+	6	+
L151	*Escherichia coli*	Drainage fluids	NDM-1						+	+	6	+
L153	*Escherichia coli*	Blood	NDM-1			CTX-M-3			+	+	6	+
R27	*Enterobacter cloacae*	Ascitic fluids		TEM-1				w+			24	
R35	*Klebsiella pneumoniae*	Ascitic fluids			SHV-11	CTX-M-55	CMY-2	w+			28	
Y024	*Enterobacter cloacae*	Urine		TEM-1			DHA-1	w+			29	
Y041	*Enterobacter aerogenes*	Urine				CTX-M-14	DHA-1	w+			28	
Y060	*Escherichia coli*	Sputum		TEM-1			CMY-2	w+			25	
Y067	*Klebsiella pneumoniae*	Sputum			SHV-2	CTX-M-14	DHA-1	w+			29	
Y078	*Citrobacter freundii*	Blood					CMY-2	w+			30	
Y081	*Klebsiella pneumoniae*	Blood		TEM-1	SHV-12		DHA-1	w+			30	
Y096	*Escherichia coli*	Drainage fluids		TEM-1		CTX-M-14	CMY-2	w+			28	
Y097	*Klebsiella pneumoniae*	Blood	IMP-4		SHV-33				+	+	6	+

[a]MHT, modified Hodge test.
[b]MHA, meropenem hydrolysis assay.
[c]mCIM, modified carbapenem inactivation method; MEM, meropenem.
[d]IP, interpretation.
[e]w+, results that were interpreted as weak positive and were classified as positive for analysis.
[f]Colonies grow within the diameter.

TABLE 5 Main characteristics of the most frequently used methods for carbapenemase detection

Parameter	MHT[a]	Carba NP	MHA (2 h)[b]	mCIM (MEM)[c]	PCR
Time (h)	18–24	2–3	2–3	18–24	4
Accuracy (%)	95.1	99.6	100	99.6	100
Cost	Low	Moderate	Low	Low	High
Equipment[d]	N	N	Y	N	Y
Experiment simplicity	Simple	Moderate	Simple	Simple	Complicated
Result interpretation	Simple	Moderate	Simple	Simple	Simple

[a]MHT, modified Hodge test.
[b]MHA, meropenem hydrolysis assay.
[c]mCIM, modified carbapenem inactivation method.
[d]Y, yes; N, no.

result for a KPC-2-producing *E. coli* encountered. Our results are in disagreement with some studies in which rapid colorimetric assays exhibited 100% sensitivity for KPC producers (5, 29). However, the overall high accuracy of this test may be due to the lack of OXA-48-type enzyme producers in our study isolates.

Recently, a new phenotypic test referred to as mCIM was introduced to detect carbapenemase activity in *Enterobacteriaceae* as recommended by the CLSI in 2017 (12). We evaluated the performance of IPM and ETP in addition to MEM, although they were not recommended by the CLSI in 2017. In this study, mCIM showed an overall sensitivity ranging from 98.0% to 99.2% and a specificity ranging from 96.9% to 99.0% for the three carbapenems. Furthermore, mCIM with MEM performed better than IPM and ETP, with higher sensitivity (99.6% versus 98.8% versus 98.0%, respectively) and specificity (99.0% versus 96.9% versus 99.0%, respectively). Both IPM and ETP gave a total of six FP/FN results.

The mCIM method has several advantages over other phenotypic tests, including a low cost, no requirement for specialized equipment, and the ease of performance and interpretation (Table 5). The only one FN result with MEM was observed in an NDM-1-producing *E. cloacae* isolate. Previously, FN results were obtained with NDM-1-producing strains, which have been reported as a disadvantage for the CIM test (9). However, recent studies with mCIM have all reported an overall sensitivity of 100% for NDM producers, although no NDM-producing *E. cloacae* were included (11, 17, 30). Similar to MHT, the only one FP result with MEM was also observed in one *E. cloacae* isolate with the presence of colonies within the 17-mm zone (Table 4). To date, only one study has compared the performance of MEM and ETP, with a similar conclusion to our study that MEM is more suitable than ETP for carbapenemase detection (30), but none has been done with IPM.

As for the MALDI-TOF MS-based method, it is plausible that the modifications (buffer used and meropenem concentration) made to the MHA procedure could have affected the performance of the test in one way or another. On the basis of our literature review, we note that previous studies have used different methodologies and different sets of bacteria/antibiotic combinations, buffers, and incubation times for hydrolysis assays, and there is not a universal method for antibiotic hydrolysis assays on MALDI-TOF MS (31). Nevertheless, the addition of NH_4HCO_3 and $ZnCl_2$ have been reported to increase the sensitivity for the detection of carbapenemase, especially for OXA (although we did not have this type in our study) and VIM types (2, 7, 32). As for the concentration of meropenem and the amount of organism used, previous studies had used different combinations: final quantities of meropenem ranging from 0.1 mM (almost the same quantity as our study) to 10 mM (1, 7) and bacterial loads ranging from 1 (the same as our study) to 10 μl loopfuls (31, 32) or 3 to 8 McFarland (1, 2, 33), but none of the studies had evaluated the effect of both factors at the same time. We chose MEM as the substrate to incubate with bacteria based on previous suggestions that MEM is more efficient against *Enterobacteriaceae* (1, 2). We also compared the efficiencies of 1 h and 2 h of incubation in the detection of carbapenemase activity. Seventy FN results (68 for KPC and 2 for IMP producers) were obtained by 1-h-incubation MHA. It is highly

possible that these strains in this geographic region have a significantly different genetic makeup compared to that of other regions of the world and have not developed a more efficient meropenem-hydrolyzing system. However, this situation was significantly improved through an extension of the 1 h of incubation, with 100% sensitivity and specificity. These findings both accorded and contradicted those of the study by Calderaro et al., in which a complete hydrolysis of MEM was observed after 30 min for the KPC-producing strains and after 1 h for the class B carbapenemase-producing strains (1). Nevertheless, our results showed that 2 h of incubation is sufficient for the detection of all class A and class B carbapenemases. Furthermore, we utilized the MBT STAR-BL module for automatic spectrum compilation in our study. Although specific equipment was required for this method (Table 5), the MBT Compass software enables the identification of bacterial species and the carbapenem resistance assay in the same run, programming one spot for identification and another for testing carbapenem resistance simultaneously (32). This is an advantage not provided by any other molecular, biochemical, or phenotypic method.

Overall, our findings suggest that existing phenotypic assays generally have excellent sensitivity and specificity for detecting CPE isolates. One common disadvantage is the inability to provide information about the specific carbapenemase genes present in an isolate, which might be gleaned by utilizing a molecular test; nonetheless, the distinction between CPE and non-CPE may be sufficient to guide the stratification of infection control and antimicrobial stewardship interventions. Taking all parameters into account, including the local epidemiology of CPE genotypes, the costs of the assays, the ease of use, equipment requirements, the time to perform the tests, the turnaround time, and the testing volumes, we propose that the MHA is the most practical assay to select for carbapenemase detection, as MALDI-TOF MS is now an integral part of many clinical microbiology laboratories. For those who cannot afford the equipment, both the Carba NP and mCIM appear to be good alternatives taking into consideration the practical requirements of speed and cost.

A notable limitation of this study was the absence of isolates producing class D carbapenemases, mainly due to the fact that this class of carbapenemase is very rare in China. For decades, KPC, IMP, and NDM carbapenemases have predominated the resistance mechanisms in CRE strains from China, while VIM and OXA carbapenemases have scarcely been reported, especially for OXA (34–36). Currently, fewer than 50 isolates of OXA-positive CREs have been reported in published studies from China (37–39). Besides, we also performed OXA gene screening tests on more than 4,000 *Enterobacteriaceae* isolates from 20 hospitals all over China in 2016 but found no OXA-positive strains. In addition, the paucity of certain species (e.g., *K. planticola*, *P. mirabilis*, and *P. rettgeri*) precludes a real reflection of accuracy estimates for these organisms. The detection of CPEs by phenotypic assays needs to be repeated in a larger, more diverse prospective cohort of isolates to provide more accurate sensitivity and specificity estimates.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JCM.00395-18.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

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