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LETTER TO THE EDITOR

Evaluation of a Modified Carbapenem Inactivation Method for Detection of Carbapenemases in *Pseudomonas aeruginosa*

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Detection of carbapenemases in *Pseudomonas aeruginosa* isolates is of utmost importance for preventing nosocomial transmission and detecting outbreaks. The availability of accurate and yet simple and affordable carbapenemase detection tests may offer an incentive for laboratories and hospitals to closely monitor this issue. One such assay is the modified carbapenem inactivation method (mCIM) which has recently been published by CLSI for use in detection of *Enterobacteriaceae* (1) and evaluated in a multicenter trial, with a resulting mean sensitivity of 97% and specificity of 99% across nine laboratories (2). Standardized guidelines for testing of glucose-nonfermenting Gram-negative bacilli by mCIM have not yet been published.

Here, we evaluated the performance of mCIM for detection of carbapenemases in 100 genotypically characterized, carbapenem-resistant *P. aeruginosa* clinical isolates. Resistance to carbapenems was confirmed by disk diffusion using CLSI M100-S27 breakpoints (1). Molecular detection of KPC, GES, IMP, VIM, NDM, OXA-48, and NMC/IMI carbapenemases was performed at the National Microbiology Laboratory (Winnipeg, Canada) (3). All carbapenemase PCR-positive organisms available in our collection ($n = 31$) plus an additional 69 carbapenemase PCR-negative organisms were included to complete a sample set of 100 isolates. mCIM was performed as described in CLSI M100-S27 (1) using a 10-μl inoculum based on preliminary CLSI results (CLSI January 2017 AST Subcommittee Meeting minutes [http://clsi.org/standards/micro/microbiology-files/]) and existing literature (4–10). Inocula were suspended in 2 ml of TSB broth (BD, Franklin Lakes, NJ), and a 10-μg meropenem disk (Oxoid, Hampshire, UK) was added prior to incubation at 35°C for 4 h. The disks were then transferred onto MH agar II (BD, Franklin Lakes, NJ), inoculated with a 0.5 McFarland suspension of *Escherichia coli* ATCC 25922, and incubated in ambient air at 35°C for 18 to 24 h. Interpretation of results was performed as follows. The presence of a carbapenemase was indicated by an inhibition zone ≤15 mm in diameter or the presence of colonies within the disk zone, and absence was indicated by zones ≥19 mm in diameter. Isolates requiring retesting (i.e., those giving discrepant and indeterminate results) were tested using broth volumes of 2 ml and 400 μl (4–8) and inocula obtained after subculture of isolates in the presence of a meropenem disk. An additional step of 10 to 15 s of vortex mixing was performed immediately after addition of the meropenem disk to facilitate suspension of the inocula (8). Tests using Carba NP and β Carba (Bio-Rad, Hercules, CA) were performed on isolates with persistently discrepant results after mCIM retesting.

A total of 100 *P. aeruginosa* isolates from unique local patients were included. The isolates were resistant to imipenem ($n = 99$) or meropenem ($n = 92$) or both ($n = 91$), with 31 containing a carbapenemase (GES-5 [$n = 18$], VIM [$n = 10$], or NDM [$n = 3$]).

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TABLE 1 Modified carbapenem inactivation method testing results from 100 carbapenem-resistant *Pseudomonas aeruginosa* clinical isolates (31 carbapenemase producers by PCR) upon initial testing and after retesting of 22 isolates with indeterminate or discrepant results[a]

	No. of isolates at indicated test broth vol		
	Upon testing	After retesting	
Result[b]	2 ml	2 ml	400 μl
mCIM			
Positive	27	27	29
Indeterminate	16		
Negative	57	73	71
Concordant			
mCIM$^+$/PCR$^+$	24	25	27
mCIM$^-$/PCR$^-$	54	67	67
Discrepant			
mCIM$^+$/PCR$^-$	3	2	2
mCIM$^-$/PCR$^+$	3	6	4

[a]PCR-based detection included the following carbapenemases: KPC, GES, IMP, VIM, NDM, OXA-48, and NMC/IMI.
[b]mCIM, modified carbapenem inactivation method; indeterminate, no carbapenemase detected ($n = 12$) or GES-5 detected ($n = 4$).

mCIM results are summarized in Table 1. Initial testing yielded 16 indeterminate results (4/16 isolates encoding GES-5; 12/16 carbapenemase PCR-negative isolates) and 6 mCIM/PCR discrepant results. Upon retesting of the isolates with indeterminate or discrepant results ($n = 22$) using 2 ml of broth, correct identification of 25/31 carbapenemase producers was achieved (sensitivity [Se], 81% [confidence interval {CI}, 67% to 95%]; specificity [Sp], 97% [CI, 93% to 100%]). In comparison, use of 400 μl of broth further improved mCIM performance (Se, 87% [CI, 75% to 99%]; Sp, 97% [CI, 93% to 100%]), correctly identifying 27/31 carbapenemase producers, including 2 additional GES-5-encoding isolates. Discrepant results persisting after retesting with reduced broth volume (Table 2) included 4 mCIM-negative/PCR-positive isolates (GES-5, $n = 2$; VIM, $n = 1$; NDM, $n = 1$) with no/low phenotypic carbapenemase activity and 2 mCIM-positive/PCR-negative isolates with strong carbapenemase activity upon additional phenotypic testing. The test performance remained unchanged (Se, 88% [CI, 72% to 97%]; Sp, 100% [CI, 95% to 100%]) after mCIM-positive/PCR-negative isolates were proven to encode a novel metallo-β-lactamase (unpublished data).

Our study was the second in the literature to evaluate mCIM for testing of *P. aeruginosa* and was unique in that it included a high proportion of isolates harboring the emerging Amber class A carbapenemase GES-5. Simner et al. evaluated mCIM alongside 10 other carbapenemase detection phenotypic tests in glucose-nonfermenting organisms, including 67 *P. aeruginosa* isolates, among which 21%

TABLE 2 Additional phenotypic carbapenemase test results for 6 carbapenem-resistant *Pseudomonas aeruginosa* clinical isolates with discrepant mCIM results after retesting in reduced broth volume[a]

Result	Carbapenemase	Carba NP result	β Carba result
mCIM$^+$/PCR$^-$	Novel MBL	+++	+++
	Novel MBL	+++	+++
mCIM$^-$/PCR$^+$	GES-5	+	+++
	GES-5	+	−
	NDM	−	−
	VIM	−	−

[a]Metallo-β-lactamase (MBL) phenotype as determined by KPC/MBL in *P. aeruginosa*/*Acinetobacter* Confirm kit 98020 (Rosco Diagnostica, Taastrup, Denmark). Carba NP was performed per CLSI M100-S27. Carba NP and β Carba results were graded according to the strength of colorimetric changes as follows: +++, strong/positive test result, <30 min; +, weak but clearly positive test result; −, no change/negative test result.

encoded a carbapenemase and only 2 carried a class A carbapenemase (KPC) (9). The *P. aeruginosa* collection in the current study contained 33% carbapenemase-encoding isolates and a near-50/50 split between class A and B carbapenemases, and the results showed slightly reduced mCIM sensitivity compared to that observed by Simner et al. for testing of *P. aeruginosa* (Se, 100% [CI, 73% to 100%]; Sp, 98% [CI, 89% to 100%]).

GES-5 has been documented in Canada since 2006 (11) and specifically in *P. aeruginosa* since 2009 to 2010, when it corresponded to the second-most-frequently detected carbapenemase in this organism species (12). Its presence in *P. aeruginosa* has been previously documented in the Americas, Africa, Europe, and Asia. Overall, mCIM as here described was able to detect almost 90% of GES-5-encoding isolates. GES-5 is known to have relatively lower carbapenem hydrolytic activity (13), perhaps helping to explain why a 5-fold inoculum concentration or different enzyme substrates utilized in Carba NP (imipenem) and β Carba (proprietary substrate) improved the detection results. Additional help for detection of GES-5 may have been offered by subculture in the presence of meropenem for isolates with indeterminate mCIM results, perhaps through selection of heteroresistant populations (14). Failure to detect a carbapenemase was also seen in Ambler class B isolates encoding VIM ($n = 1$) or NDM ($n = 1$), both of which are thought to have relatively higher hydrolytic activity and yet were not detectable by using different substrates. This observation suggests that other isolate-specific factors such as mutations could have affected carbapenemase hydrolytic activity and therefore might have been responsible for these mCIM-negative results.

Our contribution highlights that mCIM offers a viable alternative for carbapenemase detection in *P. aeruginosa*. While not all carbapenemases could be detected, that limitation was seen in similar proportions for the Ambler class A and B enzymes. Test volume reduction for retesting of isolates as presented may be an acceptable alternative to increase mCIM test sensitivity in regions where carbapenemases with lower hydrolytic activity are present.

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REFERENCES

1. CLSI. 2017. Performance standards for antimicrobial susceptibility testing. CLSI supplement M100, 27th ed. Clinical and Laboratory Standards Institute, Wayne, PA.
2. Pierce VM, Simner PJ, Lonsway DR, Roe-Carpenter DE, Johnson JK, Brasso WB, Bobenchik AM, Lockett ZC, Charnot-Katsikas A, Ferraro MJ, Thomson RB, Jr, Jenkins SG, Limbago BM, Das S. 2017. Modified carbapenem inactivation method for phenotypic detection of carbapenemase production among Enterobacteriaceae. J Clin Microbiol 55:2321–2333. https://doi.org/10.1128/JCM.00193-17.
3. Mataseje LF, Abdesselam K, Vachon J, Mitchel R, Bryce E, Roscoe D, Boyd DA, Embree J, Katz K, Kibsey P, Simor AE, Taylor G, Turgeon N, Langley J, Gravel D, Amaratunga K, Mulvey MR. 2016. Results from the Canadian Nosocomial Infection Surveillance Program on Carbapenemase-Producing Enterobacteriaceae, 2010 to 2014. Antimicrob Agents Chemother 60:6787–6794. https://doi.org/10.1128/AAC.01359-16.
4. Akhi MT, Khalili Y, Ghotaslou R, Kafil HS, Yousefi S, Nagili B, Goli HR. 2017. Carbapenem inactivation: a very affordable and highly specific method for phenotypic detection of carbapenemase-producing Pseudomonas aeruginosa isolates compared with other methods. J Chemother 29:144–149. https://doi.org/10.1080/1120009X.2016.1199506.
5. Aktaş E, Malkoçoğlu G, Otlu B, Çopur Çiçek A, Külah C, Cömert F, Sandallı C, Gürsoy NC, Erdemir D, Bulut ME. 2017. Evaluation of the carbapenem inactivation method for detection of carbapenemase-producing Gram-negative bacteria in comparison with the RAPIDEC CARBA NP. Microb Drug Resist 23:457–461. https://doi.org/10.1089/mdr.2016.0092.
6. Madkour LA, Soliman MS, Hassan DM, Soliman NS, ElMahdy YA. 2017. Detection of carbapenemase-producers: evaluating the performance of the carbapenem inactivation method and Carba NP test versus multiplex PCR. J Glob Antimicrob Resist 9:10–14. https://doi.org/10.1016/j.jgar.2016.12.015.
7. Malkoçoğlu G, Aktaş E, Bayraktar B, Otlu B, Bulut ME. 2017. VIM-1, VIM-2, and GES-5 carbapenemases among Pseudomonas aeruginosa isolates at a tertiary hospital in Istanbul, Turkey. Microb Drug Resist 23:328–334. https://doi.org/10.1089/mdr.2016.0012.
8. McMullen AR, Yarbrough ML, Wallace MA, Shupe A, Burnham CD. 2017. Evaluation of genotypic and phenotypic methods to detect carbapenemase production in Gram-negative bacilli. Clin Chem 63:723–730. https://doi.org/10.1373/clinchem.2016.264804.
9. Simner PJ, Opene BNA, Chambers KK, Naumann ME, Carroll KC, Tamma PD. 2017. Carbapenemase detection among carbapenem-resistant glucose-nonfermenting Gram-negative bacilli. J Clin Microbiol 55:2858–2864. https://doi.org/10.1128/JCM.00775-17.
10. van der Zwaluw K, de Haan A, Pluister GN, Bootsma HJ, de Neeling AJ, Schouls LM. 2015. The carbapenem inactivation method (CIM), a simple and low-cost alternative for the Carba NP test to assess phenotypic carbapenemase activity in Gram-negative rods. PLoS One 10:e0123690. https://doi.org/10.1371/journal.pone.0123690.
11. Poirel L, Carrer A, Pitout JD, Nordmann P. 2009. Integron mobilization unit as a source of mobility of antibiotic resistance genes. Antimicrob Agents Chemother 53:2492–2498. https://doi.org/10.1128/AAC.00033-09.

12. Mataseje LF, Bryce E, Roscoe D, Boyd DA, Embree J, Gravel D, Katz K, Kibsey P, Kuhn M, Mounchili A, Simor A, Taylor G, Thomas E, Turgeon N, Mulvey MR; Canadian Nosocomial Infection Surveillance Program. 2012. Carbapenem-resistant Gram-negative bacilli in Canada 2009–10: results from the Canadian Nosocomial Infection Surveillance Program (CNISP). J Antimicrob Chemother 67:1359–1367. https://doi.org/10.1093/jac/dks046.
13. Frase H, Shi Q, Testero SA, Mobashery S, Vakulenko SB. 2009. Mechanistic basis for the emergence of catalytic competence against carbapenem antibiotics by the GES family of beta-lactamases. J Biol Chem 284: 29509–29513. https://doi.org/10.1074/jbc.M109.011262.
14. Pournaras S, Ikonomidis A, Markogiannakis A, Spanakis N, Maniatis AN, Tsakris A. 2007. Characterization of clinical isolates of Pseudomonas aeruginosa heterogeneously resistant to carbapenems. J Med Microbiol 56:66–70. https://doi.org/10.1099/jmm.0.46816-0.