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Carbapenem Inactivation Method: Accurate Detection and Easy Interpretation of Carbapenemase Production in *Enterobacteriaceae* and *Pseudomonas* spp.

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Background: We evaluated the carbapenem inactivation method (CIM) compared with the modified Hodge test (MHT) for the detection of carbapenemase-producing Gram-negative bacilli.

Methods: A total of 61 isolates of carbapenemase-producing *Enterobacteriaceae* (CPE: 14 KPC, 7 GES-5, 8 NDM-1, 9 VIM-2, 9 IMP-1, and 14 OXA-48-like), 34 isolates of metallo-β-lactamase (MBL)-producing *Pseudomonas* spp. (14 VIM-2 and 20 IMP-6), and 70 carbapenem-nonsusceptible carbapenemase-negative isolates were included. The CIM and MHT were performed for all of the isolates. To perform the CIM, a meropenem disk was incubated with a suspension of the isolate to be tested and then on Mueller-Hinton agar with the *Escherichia coli* ATCC 29522 strains. The absence of an inhibition zone indicates presence of a carbapenemase. The presence of a clearing zone indicates lack of a carbapenemase.

Results: The total sensitivity and specificity of CIM (96% sensitivity and 100% specificity) in carbapenem-nonsusceptible *Enterobacteriaceae* and *Pseudomonas* spp. were better than those of the MHT (77% sensitivity and 94% specificity). The interpretation of CIM results was easy, with no or ＜20 mm inhibition zones indicating positivity and ＞20 mm inhibition zones indicating negative carbapenemase activity.

Conclusion: The CIM had excellent sensitivity and specificity for detection of CPE and MBL-producing *Pseudomonas* spp., and a positive result was easily determined, unlike the MHT. **(Ann Clin Microbiol 2016;19:83-87)**

Key Words: Carbapenem inactivation method, Carbapenemase, *Enterobacteriaceae*, Modified Hodge test, *Pseudomonas* spp.

INTRODUCTION

The global spread of carbapenemase-producing gram-negative bacilli in the last decade is becoming a serious health threat, and limited treatment options are available for such infections [1]. Rapid and accurate detection of resistance mechanisms is essential for determining appropriate antimicrobial therapy and infection control measures.

Many phenotypic laboratory developed tests (LDTs) have been developed to detect carbapenemase activity [2-4]. The modified Hodge test (MHT) is inexpensive and feasible for practically all clinical laboratories. The MHT is a Clinical and Laboratory Standards Institute (CLSI)-recommended phenotypic carbapenemase detection method [5]. This recommendation includes *Enterobacteriaceae*, but not *Pseudomonas* spp. Although the MHT often presents high sensitivity, its interpretation is often difficult and subjective [6-9]. Moreover, the MHT have demonstrated false-positive results in the presence of extended-spectrum β-lactamases (ESBLs) and AmpC β-lactamases (AmpCs) [10,11]. Another new LDT, called carbapenem inactivation method (CIM), was developed to detect carbapenemase activity in gram-negative bacilli [12].

In this study, we evaluated the reliability of the CIM for the detection of carbapenemase-producing gram-negative bacilli.

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MATERIALS AND METHODS

1. Bacterial isolates

A total of 167 clinical isolates of *Enterobacteriaceae* (n=99) and *Pseudomonas* spp. (n=68) used in this study (Table 1, 2). A total of 61 isolates of carbapenemase-producing *Enterobacteriaceae* [CPE: KPC (n=14), GES-5 (n=7), NDM-1 (n=8), VIM-2 (n=9), IMP-1 (n=9), and OXA-48-like (n=14)] and 34 isolates of metallo-β-lactamase (MBL)-producing *Pseudomonas* spp. [VIM-2 (n=14) and IMP-6 (n=20)] were included. The remaining 70 carbapenem-resistant carbapenemase-negative isolates were AmpC-producing *Enterobacteriaceae* with porin loss (n=38) and *P. aeruginosa* overexpressed AmpC (n=32). All the isolates had been previously characterized by appropriate biochemical, phonotypic, and molecular procedures to determine their types of β-lactamase production [13,14].

Table 1. Results of the CIM and MHT in carbapenem-nonsusceptible *Enterobacteriaceae* isolates

Organism (n)	Carbapenemase (n)	No. of positive results	
		CIM	MHT
Carbapenemase producers (61)			
Citrobacter freundii (5)	NDM-1 (1)	1	0
	VIM-2 (4)	4	4
Enterobacter aerogenes (1)	IMP-1	1	1
Enterobacter cloacae (11)	KPC-2 (1)	1	1
	NDM-1 (2)	2	0
	IMP-1 (5)	5	5
	VIM-2 (3)	3	2
Escherichia coli (7)	KPC-2 (1)	1	1
	NDM-1 (1)	1	0
	OXA-232 (5)	4	4
Klebsiella oxytoca (3)	NDM-1 (2)	2	2
	VIM-2 (1)	1	1
Klebsiella pneumoniae (32)	KPC-2 (6)	6	6
	KPC-3 (6)	6	6
	GES-5 (7)	4	0
	NDM-1 (2)	2	0
	IMP-1 (1)	1	1
	VIM-2 (1)	1	1
	OXA-48 (1)*	1	1
	OXA-181 (1)*	1	1
	OXA-232 (7)*	7	7
Pantoea agglomerans (2)	IMP-1	2	2
Non-carbapenemase producers (38)			
Citrobacter freundii (1)	None	0	0
Enterobacter aerogenes (2)	None	0	0
Enterobacter cloacae (5)	None	0	2
Escherichia coli (6)	None	0	0
Klebsiella oxytoca (2)	None	0	0
Klebsiella pneumoniae (14)	None	0	1
Serratia marcescens (8)	None	0	1

*OXA-48, -181, and -232 belong to OXA-48-like.
Abbreviations: CIM, carbapenem inactivation method; MHT, modified Hodge test.

2. Carbapenem inactivation method

The CIM was performed as previously described the original protocol [12]. Briefly, a suspension was made by suspending a full of 10 μL loop, cultured colony of tested isolate, in 400 μL distilled water. Subsequently, 10 μg meropenem disk (Becton-Dickinson, Cockeysville, MD, USA) was immersed in the suspension and incubated for a two hours at 35°C. After incubation the disk was removed from the suspension and placed on a Mueller-Hinton agar (MHA) plate inoculated with *Escherichia coli* ATCC 29522 and subsequently incubated for overnight at 35°C. After this step, the absence of an inhibition zone indicates enzymatic hydrolysis of carbapenem (carbapenemase-positive), whereas a clear inhibition zone appears when the tested isolate does not express carbapenemase activity (carbapenemase-negative).

3. Modified Hodge test

The MHT was performed as previously described [14]. Ertapenem disk (Beckton-Dickinson) was placed on the MHA plate seeded with *E. coli* ATCC 25922. The length of a straight line from the enhanced growth obtained from the isolate to the end of inhibition zone (mm) was classified as negative ($<$3 mm) and positive ($\geq$3 mm).

Table 2. Characteristics of the CIM and MHT in carbapenem-nonsusceptible *Pseudomonas* spp. isolates

Organism (n)	Carbapenemase (n)	No. of positive results	
		CIM	MHT
Carbapenemase producers (34)			
Pseudomonas aeruginosa (31)	IMP-6 (20)	20	20
	VIM-2 (11)	11	5
Pseudomonas putida (3)	VIM-2	3	2
Non-carbapenemase producers (32)			
Pseudomonas aeruginosa	None	0	0

Abbreviations: CIM, carbapenem inactivation method; MHT, modified Hodge test.

RESULTS AND DISCUSSION

The CIM showed positive results for all the CPE and the MBL-producing *Pseudomonas* spp. except one of the 14 OXA-48-like *Enterobacteriaceae* and three of the 7 GES-5-producing *Klebsiella pneumoniae*. All the non-carbapenemase-producing *Enterobacteriaceae* and *P. aeruginosa* showed negative results (Table 1, 2). The MHT showed false-negative results for all of the seven GES-5-producing *K. pneumoniae*, six of the eight NDM-producing *Enterobacteriaceae*, one of the 14 OXA-48-like *Enterobacteriaceae*, and seven of the 14 VIM-producing *Pseudomonas* spp. The tests for all non-carbapenemase-producing isolates were negative except four *Enterobacteriaceae* (2 *Enterobacter cloacae*, 1 *K. pneumoniae*, and 1 *Serratia marcescens*) isolates (Table 1, 2). Like all isolates for which PCR and CIM yielded discrepant results, the PCR and CIM analysis was repeated.

The sensitivity and specificity of CIM in carbapenem-nonsusceptible *Enterobacteriaceae* (93% sensitivity and 100% specificity) and carbapenem-nonsusceptible *Pseudomonas* spp. (100% sensitivity and 100% specificity) were excellent. The total of sensitivity and specificity of CIM were 95.8% and 100%, respectively. The total of sensitivity and specificity of MHT were 76.8% and 94.3%, respectively (Table 3).

Previously, CIM was proved to be very efficient in the detection of carbapenemase activity [12,15,16]. These studies had compared to Carba NP test. In comparison to the Carba NP test, CIM has similar high performance, significantly low cost, easy to interprete, and longer turnaround time for carbapenemase detection. The Carba NP test is fast and accurate phenotypic method and is now being recommended in the CLSI guidelines for carbapenemase detection [17,18]. However, recent studies have shown that this test has lower sensitivity particularly against isolates producing OXA-48-like or expressing mucoid colonies. Sometimes this test may be difficult to decide the result when the color changes became orange [19].

The MHT is suitable for the screening of carbapenemase production. However, its results are often difficult to interpret,

Table 3. Sensitivity and specificity of the CIM and MHT in carbapenem-nonsusceptible Gram-negative bacilli

Test	*Enterobacteriaceae*		*Pseudomonas* spp.		Total	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
CIM	93.4	100	100	100	95.8	100
MHT	75.4	89.5	79.4	100	76.8	94.3

Abbreviations: CIM, carbapenem inactivation method; MHT, modified Hodge test.

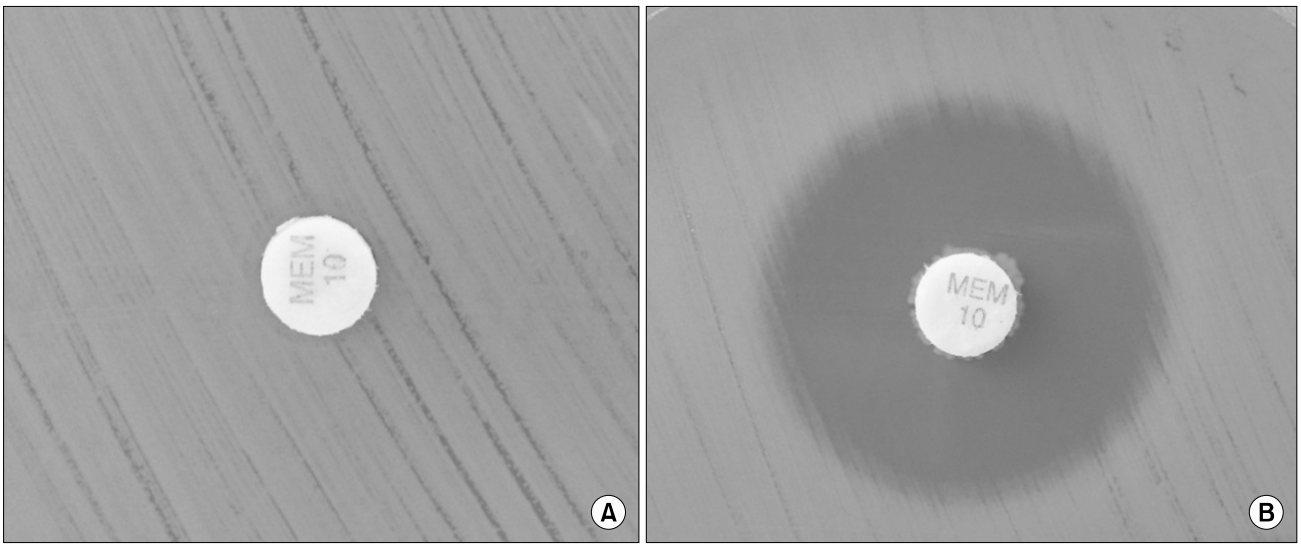

Fig. 1. Interpretation of carbapenem inactivation method (CIM). The positive results showed the absence of an inhibition zone (A) and the negative results appeared >20 mm of inhibition zone diameter (B).

and false-positive results are observed for strains producing ESBL or AmpC with porin loss [6,7]. Furthermore, it may be difficult for laboratories lacking experience to interpret results because of the subjective nature of the MHT [8,9]. Yamada et al. [16] have reported the evaluation between the performance of MHT and CIM. The results indicated while MHT produced false-negative results for NDM-producing *Enterobacteriaceae*, the CIM showed positive results for these isolates. This study includes only *Enterobacteriaceae* and have no GES-5-producing isolates. In our study included the seven GES-5-producing *K. pneumoniae*, the CIM showed positive results in the four isolates but the MHT showed negative results in all of the isolates. Unfortunately, CIM also couldn't detect GES-5 class A carbapenemase well.

All of the positive results of CIM showed the absence of an inhibition zone and all of the negative results appeared >20 mm of inhibition zone diameter (Fig. 1). It means that the interpretation of the results was easy.

In conclusion, our results indicate that the CIM had excellent sensitivity and specificity for detection of CPE and MBL-producing *Pseudomonas* spp. And, the interpretation of the CIM was easy, unlikely with MHT.

REFERENCES

1. Patel JB, Rasheed JK, Kitchel B. Carbapenemases in *Enterobacteriaceae*: activity, epidemiology, and laboratory detection. Clin Microbiol Newsletter 2009;31:55-62.
2. Girlich D, Poirel L, Nordmann P. Value of the modified Hodge test for detection of emerging carbapenemases in *Enterobacteriaceae*. J Clin Microbiol 2012;50:477-9.
3. Jeong SH, Song W, Bae IK, Kim HS, Kim JS, Park MJ, et al. Broth microdilution methods using B-lactamase inhibitors for the identification of *Klebsiella pneumoniae* carbapenemases and metallo-β-lactamases in Gram-negative bacilli. Ann Clin Lab Sci 2014;44:49-55.
4. Papagiannitsis CC, Študentová V, Izdebski R, Oikonomou O, Pfeifer Y, Petinaki E, et al. Matrix-assisted laser desorption ionization-time of flight mass spectrometry meropenem hydrolysis assay with NH_4HCO_3, a reliable tool for direct detection of carbapenemase activity. J Clin Microbiol 2015;53:1731-5.
5. CLSI. Performance standards for antimicrobial susceptibility testing. CLSI document M100-S23. Wayne, PA: Clinical and Laboratory Standards Institute; 2013.
6. Anderson KF, Lonsway DR, Rasheed JK, Biddle J, Jensen B, McDougal LK, et al. Evaluation of methods to identify the *Klebsiella pneumoniae* carbapenemase in *Enterobacteriaceae*. J Clin Microbiol 2007;45:2723-5.
7. Pasteran F, Mendez T, Guerriero L, Rapoport M, Corso A. Sensitive screening tests for suspected class A carbapenemase production in species of *Enterobacteriaceae*. J Clin Microbiol 2009;47:1631-9.
8. Pasteran F, Mendez T, Rapoport M, Guerriero L, Corso A. Controlling false-positive results obtained with the Hodge and Masuda assays for detection of class a carbapenemase in species of *Enterobacteriaceae* by incorporating boronic acid. J Clin Microbiol 2010;48:1323-32.
9. Carvalhaes CG, Picão RC, Nicoletti AG, Xavier DE, Gales AC. Cloverleaf test (modified Hodge test) for detecting carbapenemase production in *Klebsiella pneumoniae*: be aware of false positive results. J Antimicrob Chemother 2010;65:249-51.
10. Doumith M, Ellington MJ, Livermore DM, Woodford N. Molecular mechanisms disrupting porin expression in ertapenem-resistant *Klebsiella* and *Enterobacter* spp. clinical isolates from the UK. J Antimicrob Chemother 2009;63:659-67.
11. Hirsch EB and Tam VH. Detection and treatment options for *Klebsiella pneumoniae* carbapenemases (KPCs): an emerging cause of multidrug-resistant infection. J Antimicrob Chemother 2010;65: 1119-25.
12. van der Zwaluw K, de Haan A, Pluister GN, Bootsma HJ, de Neeling AJ, Schouls LM. 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 2015;10:e0123690.
13. Jeong S, Kim JO, Jeong SH, Bae IK, Song W. Evaluation of peptide nucleic acid-mediated multiplex real-time PCR kits for rapid detection of carbapenemase genes in gram-negative clinical isolates. J Microbiol Methods 2015;113:4-9.
14. Song W, Hong SG, Yong D, Jeong SH, Kim HS, Kim HS, et al. Combined use of the modified Hodge test and carbapenemase inhibition test for detection of carbapenemase-producing *Enterobacteriaceae* and metallo-β-lactamase-producing *Pseudomonas* spp. Ann Lab Med 2015;35:212-9.
15. Tijet N, Patel SN, Melano RG. Detection of carbapenemase activity in *Enterobacteriaceae*: comparison of the carbapenem inactivation method versus the Carba NP test. J Antimicrob Chemother 2016;71:274-6.
16. Yamada K, Kashiwa M, Arai K, Nagano N, Saito R. Comparison of the modified-hodge test, Carba NP test, and carbapenem inactivation method as screening methods for carbapenemase-producing *Enterobacteriaceae*. J Microbiol Methods 2016;128: 48-51.
17. Nordmann P, Poirel L, Dortet L. Rapid detection of carbapenemase-producing *Enterobacteriaceae*. Emerg Infect Dis 2012;18:1503-7.
18. Dortet L, Poirel L, Nordmann P. Rapid detection of carbapenemase-producing *Pseudomonas* spp. J Clin Microbiol 2012;50:3773-6.
19. Tijet N, Boyd D, Patel SN, Mulvey MR, Melano RG. Evaluation of the Carba NP test for rapid detection of carbapenemase-producing *Enterobacteriaceae* and *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2013;57:4578-80.

=국문초록=

Carbapenem Inactivation Method: 정확하고 판독이 쉬운 Carbapenemase 생성 그람음성막대균 검출법

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배경: Carbapenem inactivation method (CIM)의 carbapenemase 생성 그람음성막대균에 대한 검출력을 평가하기 위하여 modified Hodge test (MHT)와 비교하였다.

방법: 총 61주의 carbapenemase 생성 장내세균(CPE: KPC 14주, GES-5 7주, NDM-1 8주, VIM-2 9주, IMP-1 9주, OXA-48-like 14주), 34주의 metallo-β-lactamase (MBL) 생성 *Pseudomonas* spp. (VIM-2 14주, IMP-6 20주), 70주의 carbapenem 비감수성 carbapenemase 음성균주를 대상으로 CIM과 MHT를 시행하였다. CIM은 meropenem 디스크를 대상균주 혼합액에 넣고 2시간 배양한 후, 이 meropenem 디스크를 *Escherichia coli* ATCC 29522를 접종한 Mueller-Hinton 배지에 놓고 하룻밤 배양한다. 억제대가 없으면 carbapenemase 양성, 억제대가 생기면 carbapenemase 음성으로 판독하였다.

결과: CIM의 carbapenem 비감수성 장내세균 *Enterobacteriaceae*과 carbapenem 비감수성 *Pseudomonas* spp. (예민도 96%, 특이도 100%)의 예민도와 특이도는 MHT (예민도 77%, 특이도 94%)보다 우수하였다. CIM 결과, 양성인 경우는 모두 억제대가 없었고, 음성인 경우는 모두 억제대가 20 mm 이상이었다.

결론: CIM은 CPE와 MBL 생성 *Pseudomonas* spp. 검출에 매우 우수한 예민도와 특이도를 보였고 MHT와는 달리 결과 판독이 매우 용이하고 분명하였다. [Ann Clin Microbiol 2016;19:83-87]

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