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Comparative Evaluation of Five Assays for Detection of Carbapenemases with a Proposed Scheme for Their Precise Application

Hazim O. Khalifa,*† Takashi Okanda,‡ Amer A. Abd El-Hafeez,§¶ Amera Abd El Latif,† Ahmed G.K. Habib,‖ Hisakazu Yano,** Yasuyuki Kato,* and Tetsuya Matsumoto*

From the Department of Infectious Diseases, Graduate School of Medicine, International University of Health and Welfare, Narita, Japan; the Department of Pharmacology,† Faculty of Veterinary Medicine, Kafrelsheikh University, Kafr El-Sheikh, Egypt; the Department of Microbiology,‡ St. Marianna University School of Medicine, Kawasaki, Japan; the Pharmacology and Experimental Oncology Unit,§ Cancer Biology Department, National Cancer Institute, Cairo University, Cairo, Egypt; the Department of Cellular and Molecular Medicine,¶ School of Medicine, University of California, San Diego, La Jolla, California; the Department of Biotechnology and Life Sciences, ‖ Faculty of Postgraduate Studies for Advanced Sciences, Beni-Suef University, Beni-Suef, Egypt; and the Department of Microbiology and Infectious Diseases,** Nara Medical University, Kashihara, Japan*

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Address correspondence to Hazim O. Khalifa, Ph.D., Department of Infectious Diseases, Graduate School of Medicine International University of Health and Welfare, Narita 286-0048, Japan. E-mail: hazem.khalifa1@vet.kfs.edu.eg or omarh_2007@yahoo.com.

The escalating problem of the dissemination of carbapenemase-producing bacteria (CPB) has gained worldwide attention. The prompt diagnosis of CPB and precise identification of carbapenemases are imperative to enable specific antibiotic therapy and control the spread of these bacteria. The present study was designed to assess the performance of five important assays for the detection of carbapenemases. The modified carbapenem inactivation method (mCIM), CARBA-5, GeneXpert Carba-R, BD MAX Check-Points CPO, and GeneFields CPE assays were evaluated with an international collection of 159 bacterial isolates, including 93 CPB and 66 non-CPB isolates. The overall accuracy/sensitivity/specificity for carbapenemase detection were 100% (95% CI, 97.7%—100%)/100% (95% CI, 96.1%—100%)/100% (95% CI, 94.6%—100%) for mCIM, 98.7% (95% CI, 95.5%—99.9%)/97.9% (95% CI, 92.5%—99.7%)/100% (95% CI, 94.6%—100%) for CARBA-5, 96.9% (95% CI, 92.8%—99%)/95.7% (95% CI, 89.4%—98.8%)/98.5% (95% CI, 91.8%—99.9%) for GeneXpert Carba-R, 94.3% (95% CI, 89.5%—97.4%)/90.3% (95% CI, 82.4%—95.5%)/100% (95% CI, 94.6%—100%) for BD MAX Check-Points CPO, and 86.2% (95% CI, 79.8%—91.1%)/77.4% (95% CI, 67.6%—85.5%)/98.5% (95% CI, 91.8%—100%) for GeneFields CPE. Interestingly, mCIM and CARBA-5 assays showed 100% accuracy/sensitivity/specificity for detection of the target genes. Furthermore, all the other assays showed comparable high accuracy (96.9% to 100%), sensitivity (100%), and specificity (96.4% to 100%) for the detection of the target genes. On the basis of these results, a new scheme was proposed for their efficient application. These results confirmed the high sensitivity of the evaluated assays, and the proposed scheme is reliable and improves the overall sensitivity and specificity of the assays. *(J Mol Diagn 2020, 22: 1129—1138; https://doi.org/10.1016/j.jmoldx.2020.05.012)*

Carbapenems are a group of life-saving antibiotics that represent the last resort for the treatment of infection caused by multidrug-resistant bacteria.[1,2] Carbapenem resistance is closely associated with increased hospitalization period and mortality rates with bloodstream-infected patients in low- and middle-income countries.[1] Therefore, the emergence and dispersal of carbapenem resistance have gained worldwide attention to mitigate such problems and prevent epidemic spread.[3] Carbapenem resistance is mediated by concomitant altered outer membrane permeability with the hyperproduction of AmpC or extended-spectrum β-lactamases or, most important, by carbapenem-hydrolyzing enzymes

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Current address of T.O., Department of Microbiology, St. Marianna University School of Medicine, Kawasaki, Japan.

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Table 1 Characterization of the Carbapenemase-Producing Bacterial Isolates Evaluated in this Study

Strain no.	Bacterial spp	Source	Sample	Country	Detected carbapenemases (PCR)
1	*Enterobacter cloacae*	Clinical	Sputum	Japan	IMP-6
2	*E. cloacae*	Clinical	Sputum	Japan	IMP-6
3	*E. cloacae*	Clinical	Sputum	Japan	IMP-6
4	*Klebsiella pneumoniae*	Animals	Nasal swab	Egypt	OXA-48
5	*Enterobacter hormaechei*	Animals	Nasal swab	Egypt	VIM-4
6	*E. hormaechei*	Animals	Nasal swab	Egypt	VIM-4
7	*E. hormaechei*	Animals	Nasal swab	Egypt	VIM-4
8	*E. hormaechei*	Animals	Nasal swab	Egypt	VIM-4
9	*Escherichia coli*	Animals	Nasal swab	Egypt	OXA-244
10	*E. hormaechei*	Animals	Nasal swab	Egypt	VIM-4
11	*Enterobacter kobei*	Clinical	Pus	Egypt	NDM-5
12	*K. pneumoniae*	Clinical	Urine	Egypt	OXA-48
13*	*K. pneumoniae*	Clinical	Blood	Egypt	NDM-5
14	*E. coli*	Clinical	Unknown	Japan	IMP-1
15	*E. coli*	Clinical	Unknown	Japan	IMP-1
16	*E. coli*	Clinical	Unknown	Japan	IMP-1
17	*E. coli*	Clinical	Unknown	Japan	IMP-1
18	*E. coli*	Clinical	Unknown	Japan	IMP-6
19	*E. coli*	Clinical	Unknown	Japan	IMP-6
20	*E. coli*	Clinical	Unknown	Japan	IMP-6
21	*E. coli*	Clinical	Unknown	Japan	IMP-6
22	*K. pneumoniae*	Clinical	Sputum	Bangladesh	NDM-1
23	*E. coli*	Clinical	Urine	Bangladesh	NDM-5
24	*E. coli*	Clinical	Pus	Bangladesh	NDM-5
25	*E. cloacae*	Clinical	Urine	Bangladesh	NDM-1
26	*K. pneumoniae*	Clinical	Unknown	Bangladesh	OXA-181 + NDM-5
27	*E. coli*	Clinical	Unknown	Bangladesh	OXA-181 + NDM-5
28	*Klebsiella aerogenes*	Clinical	Unknown	Bangladesh	OXA-181
29	*Proteus mirabilis*	Clinical	Unknown	Bangladesh	OXA-181
30	*Citrobacter freundii*	Clinical	Unknown	Japan	VIM-2
31	*E. coli*	Clinical	Unknown	Japan	KPC-2
32	*E. coli*	Clinical	Unknown	Japan	KPC-2
33	*E. coli*	Clinical	Unknown	Japan	KPC-2
34	*E. coli*	Clinical	Unknown	Japan	KPC-2
35	*Klebsiella oxytoca*	Clinical	Unknown	Japan	GES-20
36	*K. pneumoniae*	Clinical	Unknown	Japan	OXA-48
37	*K. pneumoniae*	Clinical	Unknown	Japan	OXA-244
38	*K. pneumoniae*	Clinical	Unknown	Japan	KPC-2
39	*K. pneumoniae*	Clinical	Unknown	Japan	KPC-2
40	*K. pneumoniae*	Clinical	Unknown	Japan	KPC-2
41	*E. cloacae*	Clinical	Unknown	Japan	KPC-2
42	*E. cloacae*	Clinical	Unknown	Japan	KPC-2
43	*K. pneumoniae*	Clinical	Unknown	Japan	NDM-7
44	*E. cloacae*	Clinical	Unknown	Japan	NDM-7
45	*E. cloacae*	Clinical	Unknown	Japan	NDM-7
46*	*K. pneumoniae*	Clinical	Endotracheal tube swab	Egypt	NDM-4
47	*K. pneumoniae*	Clinical	Unknown	Japan	NDM-1
48	*E. cloacae*	Clinical	Unknown	Japan	NDM-1
49	*K. pneumoniae*	Clinical	Unknown	Japan	IMP-1
50	*K. pneumoniae*	Clinical	Unknown	Japan	IMP-1
51	*E. cloacae*	Clinical	Unknown	Japan	IMP-1
52	*E. cloacae*	Clinical	Unknown	Japan	IMP-1
53	*K. pneumoniae*	Clinical	Unknown	Japan	IMP-6
54	*K. pneumoniae*	Clinical	Unknown	Japan	IMP-6
55	*K. pneumoniae*	Clinical	Unknown	Japan	IMP-6
56	*E. coli*	Clinical	Unknown	Japan	OXA-48
57	*E. coli*	Clinical	Unknown	Japan	OXA-181

(*table continues*)

Table 1 (*continued*)

Strain no.	Bacterial spp	Source	Sample	Country	Detected carbapenemases (PCR)
58	*E. coli*	Clinical	Unknown	Japan	OXA-181
59	*K. pneumoniae*	Clinical	Unknown	Japan	OXA-48
60	*K. pneumoniae*	Clinical	Unknown	Japan	NDM-1
61	*K. pneumoniae*	Clinical	Unknown	Japan	NDM-1
62	*K. pneumoniae*	Clinical	Unknown	Japan	NDM-5
63	*E. cloacae*	Clinical	Urine	Japan	IMP-6
64	*E. cloacae*	Clinical	Unknown	Japan	IMP-6
65	*E. cloacae*	Clinical	Chest drain	Japan	IMP-6
66	*E. cloacae*	Clinical	Drain	Japan	IMP-6
67	*E. cloacae*	Clinical	Bile	Japan	IMP-6
68	*E. cloacae*	Clinical	Bile	Japan	IMP-6
69	*K. pneumoniae*	Clinical	Sputum	Bangaladish	OXA-48
70	*K. pneumoniae*	Clinical	Unknown	Bangaladish	OXA-232
71	*K. pneumoniae*	Clinical	Unknown	Bangaladish	OXA-232
72	*E. coli*	Clinical	Urine	Bangaladish	NDM-4
73	*E. coli*	Clinical	Urine	Bangaladish	NDM-4
74	*K. pneumoniae*	Clinical	Unknown	Bangaladish	OXA-232 + NDM-5
75	*E. coli*	Unknown	Unknown	Japan	KPC-2
76	*E. cloacae*	Unknown	Unknown	Japan	KPC-2
77	*K. pneumoniae*	Unknown	Unknown	Japan	KPC-2
78	*C. freundii*	Unknown	Unknown	Japan	KPC-2
79	*K. pneumoniae*	Unknown	Unknown	Japan	KPC-2
80	*K. pneumoniae*	Unknown	Unknown	Japan	KPC-19
81	*E. coli*	Unknown	Unknown	Japan	KPC-19
82	*K. pneumoniae*	Unknown	Unknown	Japan	KPC-2
83	*E. coli*	Unknown	Unknown	Japan	KPC-2
84	*K. pneumoniae*	Unknown	Unknown	Japan	KPC-2
85	*E. coli*	Unknown	Unknown	Japan	KPC-2
86	*Pseudomonas aeruginosa*	Unknown	Unknown	Japan	VIM-2
87[†]	*C. freundii*	Clinical	Retroperitoneal abscess	Japan	VIM-2
88	*Pseudomonas putida*	Environment	Water sample	Japan	VIM-2
89	*Pseudomonas mendocina*	Environment	Water sample	Japan	VIM-2
90	*Pseudomonas alcaligenes*	Environment	Water sample	Japan	VIM-2, GES-1
91	*P. mendocina*	Environment	Water sample	Japan	VIM-2
92	*P. aeruginosa*	Environment	Water sample	Japan	VIM-2
93	*P. putida*	Environment	Water sample	Japan	VIM-2

Carbapenemase nonproducing isolates included 52 isolates that were recovered from Egypt, 13 isolates that were recovered from Japan, and a single isolate that was recovered from Bangladesh. The isolates recovered from Egypt include 40 *Escherichia coli* isolates (36 clinical isolates from urine, 2 clinical isolates from pus, and 2 animal isolates from nasal swabs), 6 clinical *Klebsiella pneumoniae* isolates from urine, 5 clinical *Enterobacter cloacae* isolates from urine, and 1 clinical *Enterobacter kobei* isolate from urine. Furthermore, 13 isolates were recovered from Japan, including 6 *E. cloacae* isolates (4 clinical isolates from urine, 1 clinical isolate from bile, and 1 unknown isolate), 3 *E. coli* isolates (1 clinical isolate from bile and 2 unknown isolates), 3 *K. pneumoniae* isolates (unknown), and 1 clinical *Enterobacter aerogenes* isolate from an abdominal drain. Finally, a single community *Serratia plymuthica* isolate was recovered from Bangladesh. All remaining isolates are from the current study.

*Isolates from Khalifa et al.[4]

[†]Isolates from Ando et al.[12]

GES, Guiana extended-spectrum β-lactamase; IMP, Imipenemase-type metallo-β-lactamase; KPC, *K. pneumoniae* carbapenemase; NDM, New Delhi metallo-β-lactamase; OXA, oxacillinase group of β-lactamase; VIM, Verona integron-encoded metallo-β-lactamase.

(carbapenemases).[2–4] Carbapenemases are a unique group of β-lactamases that have interesting hydrolyzing activity against most β-lactams and are malleable against inhibition by nearly all β-lactamase inhibitors.[5] During the last decade, a wide variety of carbapenemases have been reported worldwide. However, the most common belong to class B metallo-β-lactamases [New Delhi metallo-β-lactamase (NDM), Verona integron-encoded metallo-β-lactamase (VIM), and Imipenemase-type metallo-β-lactamase (IMP)], Ambler class A carbapenemases [*K. pneumoniae* carbapenemase (KPC)], and class D oxacillinases [oxacillinase group of β-lactamase (OXA)−48 and OXA-48 like].[4,5]

The rapid and proper detection of carbapenemase-producing bacteria (CPB) is of considerable importance to overcome the escalation of carbapenem resistance.[6] The detection of carbapenemases will be helpful to guide

empirical and specific antibiotic therapy and to improve the therapeutic efficacy of antibiotics. Moreover, the rapid identification of carbapenemases could be of significant value to public health officials for infection control and epidemiological assays.[6,7] Although PCR combined with sequencing is still used as the gold standard for the detection of carbapenemases, its limitations, such as the expensive equipment, requirement of skilled staff, and long detection times, have motivated various scholars and companies to develop new diagnostic methods for the detection of CPB.[6] Recently, a variety of methods have been developed for the detection of carbapenemases that depend on their phenotypic, biochemical, electrochemical, colorimetric, and immunochromatographic charactereristics.[6−10] The developed techniques show considerable variation in their specificity and sensitivity, depending on the evaluated enzymes and bacterial species.[6,8] In addition, phenotypic tests can detect only carbapenem hydrolysis activity but not the specific gene involved, and most of the developed techniques are restricted to certain target enzymes, with a high possibility of missing nontarget or new enzymes.[8,10] Therefore, it is necessary to compare the different methods available for the detection of carbapenemases to identify the one that is most accurate and effective. Furthermore, the absence of a single ideal method for detection of carbapenemases[6,11] regenerates the interest to propose an approach for their precise use that could improve their potential application for carbapenemase detection.

The present study was conducted to evaluate and compare the effectiveness of five carbapenemase detection methods. In this study, the phenotypic modified carbapenem inactivation method (mCIM), the immunochromatographic CARBA-5 assay (CARBA-5; NG Biotech, Guipry, France), the automated real-time quantitative PCR (qPCR)−based GeneXpert Carba-R assay (Cepheid, Frankfurt, Germany), the nucleic acid chromatography GeneFields CPE assay (KURABO, Tokyo, Japan), and the real-time PCR BD MAX Check-Points CPO assay (Check-Points Health, Wageningen, the Netherlands) using the BD MAX System (Becton, Dickinson and Company, Franklin Lakes, NJ) were evaluated and compared for the identification of carbapenemases. On the basis of these results, a proposed approach was developed for their accurate use for carbapenemase identification.

Materials and Methods

Bacterial Isolates

A wide range of bacterial strains from an international collection of 159 Gram-negative isolates from Egypt, Japan, and Bangladesh were evaluated in this study (Table 1).[4,12] The isolates were identified by matrix-assisted laser desorption/ionization−time-of-flight analysis and molecularly characterized by PCR and the sequencing of different carbapenemase-encoding genes (Table 2).[13−17] The isolate collection consisted of 66 carbapenemase-negative isolates and 93 CPB encoding a total of 97 carbapenemases, including isolates belonging to Ambler classes A ($n = 22$), B ($n = 58$), and D ($n = 17$) (Table 1). For the comparison and evaluation of the test results, a fresh overnight bacterial culture on Mueller-Hinton agar plates was used. All the evaluated assays were performed according to the manufacturer's instructions and recommendations, unless reported otherwise.

The Phenotypic Modified Carbapenem Inactivation Method

The phenotypic mCIM test was performed as described previosely.[18] Briefly, a 1-μL inoculation loop of the overnight cultured bacteria was added to a tube containing 2 mL of tryptic soy broth. A 10-μg meropenem disk was added to the bacterial suspension after vortex for 10 seconds, followed by incubation at 37°C for 4 hours. After the incubation time, the meropenem disk was placed on Mueller-Hinton agar previously inoculated with the 0.5 McFarland standard *Escherichia coli* 25922 (ATCC, Manassas, VA) as an indicator organism. The result was evaluated by measuring the inhibition zone around the meropenem disk after incubation at 37°C for 18 to 24 hours.

The Immunochromatographic CARBA-5 Assay

In this assay, a full 1-μL inoculation loop of overnight bacteria cultured on Mueller-Hinton agar was mixed with five drops of CARBA-5 extraction buffer. After gentle vertexing, 100 μL of the mixture was transferred into the CARBA-5 cassette, and the results were evaluated after incubation for 15 minutes at room temperature.

The Automated qPCR-Based GeneXpert Carba-R Assay

The qPCR-based GeneXpert Carba-R assay was performed as follows: a full 10-μL suspension of 0.5 McFarland standard harvested from bacteria and cultured overnight on Mueller-Hinton agar was mixed with the sample reagent in the GeneXpert Carba-R assay sample reagent vial. After 10 seconds of vortexing, the recommended volume was added to the GeneXpert Carba-R cartridge with a disposable transfer pipette and run on the GeneXpert IV system (Cepheid). All procedures, including sample preparation, DNA amplification, and gene detection, were automated, and the results were automatically explicated in <1 hour.

The Real-Time PCR BD MAX Check-Points CPO Assay

A full 50 μL of 1:400-diluted 0.5 McFarland bacterial cell suspension was added to BD MAX Check-Points CPO sample buffer tubes. After vortexing for 10 seconds, the sample buffer tubes and the unitized reagent strip were placed into the BD MAX system rack together with the extraction and master mix tubes, and the BD MAX system

Table 2 Oligonucleotides Used for Identification of the Acquired Carbapenemase Genes

Primer name	Sequence (5′ to 3′)	Target	Reference
IMP-F	5′-GGAATAGAGTGGCTTAAYTCTC-3′	*bla*$_{IMP}$	13
IMP-R	5′-GGTTTAAYAAAACAACCACC-3′		
SPM-F	5′-AAAATCTGGGTACGCAAACG-3′	*bla*$_{SPM}$	13
SPM-R	5′-ACATTATCCGCTGGAACAGG-3′		
VIM-F	5′-GATGGTGTTTGGTCGCATA-3′	*bla*$_{VIM}$	13
VIM-R	5′-CGAATGCGCAGCACCAG-3′		
OXA-F	5′-GCGTGGTTAAGGATGAACAC-3′	*bla*$_{OXA-48\ like}$	13
OXA-R	5′-CATCAAGTTCAACCCAACCG-3′		
OXA-48A	5′-TTGGTGGCATCGATTATCGG-3′	*bla*$_{OXA-48\ like}$	14
OXA-48B	5′-GAGCACTTCTTTTGTGATGGC-3′		
BIC-F	5′-TATGCAGCTCCTTTAAGGGC-3′	*bla*$_{BIC}$	13
BIC-R	5′-TCATTGGCGGTGCCGTACAC-3′		
NDM-F	5′-GGTTTGGCGATCTGGTTTTC-3′	*bla*$_{NDM}$	13
NDM-R	5′-CGGAATGGCTCATCACGATC-3′		
NDM-full F	5′-ATGGAATTGCCCAATATTATGCAC-3′	Whole *bla*$_{NDM}$	15
NDM-full R	5′-TCAGCGCAGCTTGTCGGC-3′		
KPC-F	5′-CGTCTAGTTCTGCTGTCTTG-3′	*bla*$_{KPC}$	13
KPC-R	5′-CTTGTCATCCTTGTTAGGCG-3′		
KPC forward	5′-ATGTCACTGTATCGCCGTCT-3′	*bla*$_{KPC}$	16
KPC reverse	5′-TTTTCAGAGCCTTACTGCCC-3′		
AIM-F	5′-CTGAAGGTGTACGGAAACAC-3′	*bla*$_{AIM}$	13
AIM-R	5′-GTTCGGCCACCTCGAATTG-3′		
GIM-F	5′-TCGACACACCTTGGTCTGAA-3′	*bla*$_{GIM}$	13
GIM-R	5′-AACTTCCAACTTTGCCATGC-3′		
SIM-F	5′-TACAAGGGATTCGGCATCG-3′	*bla*$_{SIM}$	13
SIM-R	5′-TAATGGCCTGTTCCCATGTG-3′		
DIM-F	5′-GCTTGTCTTCGCTTGCTAACG-3′	*bla*$_{DIM}$	13
DIM-R	5′-CGTTCGGCTGGATTGATTTG-3′		
GES-F	5′-GCTTCATTCACGCACTATT-3′	*bla*$_{GES}$	17
GES-MR	5′-CGATGCTAGAAACCGCTC-3′		

AIM, Adelaide imipenemase; BIC, Bicêtre carbapenemase; DIM, Dutch imipenemase; F, forward; GES, Guiana extended-spectrum β-lactamase; GIM, German imipenemase; IMP, Imipenemase-type metallo-β-lactamase; KPC, *K. pneumoniae* carbapenemase; NDM, New Delhi metallo-β-lactamase; MR, reverse; OXA, oxacillinase group of β-lactamase; R, reverse; SIM, Seoul imipenemase; SPM, Sao Paulo metallo-β-lactamase; VIM, Verona integron-encoded metallo-β-lactamase.

was run as recommended by the company. The sample preparation, lysis, DNA extraction, and multiplex RT-PCR were automatically performed with the BD MAX system, and the results were automatically interpreted after approximately 2 hours.

The Nucleic Acid Chromatography GeneFields CPE Assay

The bacterial DNA was extracted with DNA extraction kits (Cica Geneus Extraction Reagent, Tokyo, Japan), according to the manufacturer's instructions. The extracted DNA (2 μL) was used as a template for the nucleic acid chromatography assay in a 30-μL reaction mixture containing 15 μL of 2× MightyAmp Buffer Ver.3 (TAKARA BIO Inc, Shiga, Japan), 3 μL of 10× additive, 0.6 μL of MightyAmp Polymerase Ver.3 (TAKARA BIO Inc), and 5 μL of PCR Oligo Mix (KURABO). The thermal cycler was run at 98°C for 3 minutes and then for 35 cycles of 98°C for 30 seconds, 62°C for 30 seconds, and 72°C for 30 seconds, followed by a final step at 72°C for 7 minutes. For detection of the suspected carbapenemases, 5 μL of the DNA product was mixed with 11 μL of coloring buffer and 5 μL of sterilized nuclease-free water in a 1.5-mL tube. The DNA strip was immersed in the mixture, and the result was evaluated after 15 minutes by comparing the band obtained on the DNA strip with the judgment card provided by the company.

Statistical Analysis

The results of the molecular characterization of the CPB by PCR and sequencing served as a standard and were compared with the results of the different assays to calculate the accuracy, sensitivity, specificity, and 95% CIs, as previously described.[6,19] The accuracy is the proportion of true-positive and true-negative results of the evaluated assays and indicates the overall probability that a gene was correctly identified.[19]

Results

Performance of the Tested Assays for Detection of CPB

The mCIM and CARBA-5 were the most accurate assays for the detection of CPB, with 100% (95% CI, 97.7%–

Table 3 Performance of the Five Assays for the Detection of Carbapenemase Production and Identification of Target Genes

Assay	Carbapenemases	True positive	False negative	False positive	True negative	Total	Accuracy, (95% CI), %	Sensitivity (95% CI), %	Specificity (95% CI), %
mCIM	All carbapenemases*	93	0	0	66	159	100 (97.7–100)	100 (96.1–100)	100 (94.6–100)
	KPC	20	0	0	139	159	100 (97.1–100)	100 (83.2–100)	100(97.4–100)
	OXA-48 like	17	0	0	142	159	100 (97.7–100)	100 (80.5–100)	100 (97.5–100)
	NDM	20	0	0	139	159	100 (97.1–100)	100 (83.2–100)	100 (97.4–100)
	IMP	24	0	0	135	159	100 (97.7–100)	100 (85.8–100)	100 (97.3–100)
	VIM	14	0	0	145	159	100 (97.7–100)	100 (76.8–100)	100 (97.5–100)
	GES	2	0	0	157	159	100 (97.7– 100)	100 (15.8–100)	100 (97.7–100)
CARBA-5	All carbapenemases*	91	2†	0	66	159	98.7 (95.5–99.9)	97.9 (92.5–99.7)	100 (94.6–100)
	KPC	20	0	0	139	159	100 (97.1–100)	100 (83.2–100)	100 (97.4–100)
	OXA-48 like	17	0	0	142	159	100 (97.7–100)	100 (80.5–100)	100 (97.5–100)
	NDM	20	0	0	139	159	100 (97.1–100)	100 (83.2–100)	100 (97.4–100)
	IMP	24	0	0	135	159	100 (97.7–100)	100 (85.8–100)	100 (97.3–100)
	VIM	14	0	0	145	159	100 (97.7–100)	100 (76.8–100)	100 (97.5–100)
	GES	0	2†	0	157	159	0 (0–84.2)		
GeneXpert Carba-R	All carbapenemases*	89	4†,‡,§	1¶	65	159	96.9 (92.8–99)	95.7 (89.4–98.8)	98.5 (91.8–99.9)
	KPC	20	0	1‡	138	159	99.4 (96.6–100)	100 (83.2–100)	99.3 (96–100)
	OXA-48 like	17	0	0	142	159	100 (97.7–100)	100 (80.5–100)	100 (97.5–100)
	NDM	20	0	1§	138	159	99.4 (96.6–100)	100 (83.2–100)	99.3 (96–100)
	IMP	24	0	1¶	134	159	99.4 (96.6–100)	100 (85.8–100)	99.3 (96–100)
	VIM	14	0	0	145	159	100 (97.7–100)	100 (76.8–100)	100 (97.5–100)
	GES	0	2†	0	157	159	0 (0–84.2)		
BD MAX Check-Points CPO	All carbapenemases*	84	9†,‖,**	0	66	159	94.3 (89.5–97.4)	90.3 (82.4–95.5)	100 (94.6–100)
	KPC	20	0	3‖	136	159	98.1 (94.6–99.6)	100 (83.2–100)	97.8 (93.8–99.5)
	OXA-48 like	17	0	0	142	159	100 (97.7–100)	100 (80.5–100)	100 (97.5–100)
	NDM	20	0	4**	135	159	97.5 (93.7–99.3)	100 (83.2–100)	97.1 (92.5–99.2)
	IMP and/or VIM††	38	0	0	121	159	100 (90.7–100)	100 (90.8–100)	100 (97–100)
	GES	0	2†	0	157	159	0 (0– 84.2)		
GeneFields CPE	All carbapenemases*	72	21†,‡‡,§§	1¶¶	65	159	86.2 (79.8–91.1)	77.4 (67.6–85.5)	98.5 (91.8–100)
	KPC	20	0	0	139	159	100 (97.1–100)	100 (83.2–100)	100 (97.4–100)
	OXA-48 like	17	0	1¶¶	141	159	99.4 (96.6–100)	100 (80.5–100)	99.3 (96–100)
	NDM	20	0	5§§	134	159	96.9 (92.8–99)	100 (83.2–100)	96.4 (91.8–98.8)
	IMP	24	0	0	135	159	100 (97.7–100)	100 (85.8–100)	100 (97.3–100)
	VIM	0	14‡‡	0	145	159	0 (0–23.2)		
	GES	0	2†	0	157	159	0 (0–84.2)		

*The isolates that differ from positive PCR results (by either giving negative results or showing multiple genes, even including target genes) are considered false negatives. With the exception of nontarget genes, all the false-positive and false-negative results were evaluated at least two times.

†One isolate producing GES-20 and another producing VIM-2 + GES-1 could not be identified as the assays were not developed for GES identification.

‡One isolate was falsely identified to produce both KPC and OXA-48 like, whereas it only produces OXA-48 according to PCR.

§One isolate was falsely identified to produce both NDM and KPC, whereas it only produces KPC-2 according to PCR.

¶Three isolates were falsely identified to produce both KPC and IMP, whereas they only produce IMP according to PCR.

‖One isolate was falsely identified to produce IMP, whereas it was carbapenemase negative according to PCR.

**Four isolates were falsely identified to produce both NDM and OXA, whereas according to PCR, two isolates were found to produce OXA-48 and two isolates were found to produce OXA-244.

††This assay was not developed to differentiate between VIM and/or IMP producers.

‡‡Fourteen VIM producers could not be identified, as the GeneFields CPE assay was not developed for VIM identification.

§§Five isolates were falsely identified to produce NDM, whereas one isolate was negative according to PCR, and the four isolates were found to produce GES-20 ($n = 1$) or VIM-4 ($n = 3$).

¶¶One isolate was falsely identified to produce OXA, whereas it was carbapenemase negative according to PCR.

GES, Guiana extended-spectrum β-lactamase; IMP, Imipenemase-type metallo-β-lactamase; KPC, *K. pneumoniae* carbapenemase; mCIM, modified carbapenem inactivation method; NDM, New Delhi metallo-β-lactamase; OXA, oxacillinase group of β-lactamase; VIM, Verona integron-encoded metallo-β-lactamase.

100%) and 98.7% (95% CI, 95.5%–99.9%) accuracy, respectively (Table 3). The accuracies of the GeneXpert Carba-R and BD MAX assays were 96.9% (95% CI, 92.8%–99%) and 94.3% (95% CI, 89.5%–97.4%), respectively. Results showed that four and nine isolates were false negative for GeneXpert Carba-R and BD MAX assays, respectively, with only a single isolate that was false positive for the GeneXpert Carba-R assay (Table 3). The GeneFields CPE showed the lowest overall accuracy (86.2%; 95% CI, 79.8%–91.1%) for the detection of CPB, with 21 false-negative isolates and a single false-positive isolate (Table 3).

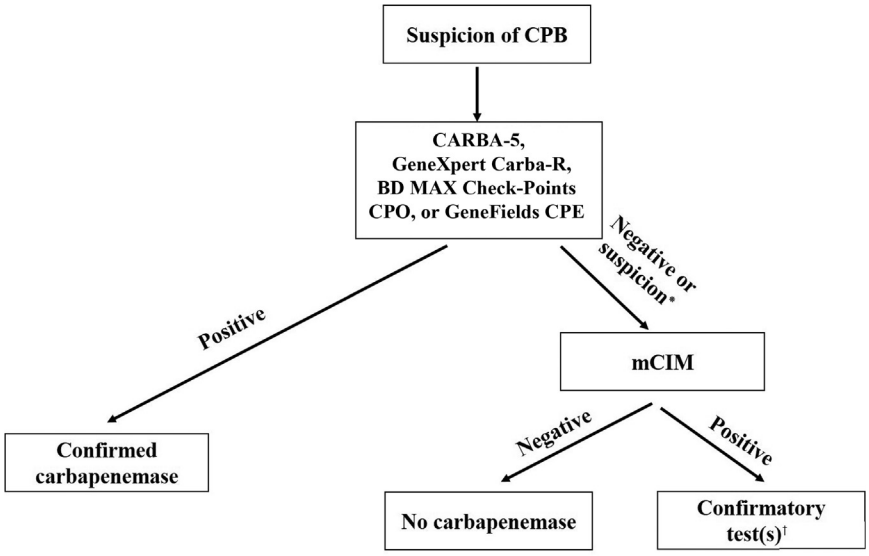

Figure 1 A proposed scheme for efficient and accurate identification of carbapenemases using the evaluated assays and improving the overall performance for not only detection of the target genes, but also for detection of other carbapenemases. The **asterisk** indicates that suspicion results might include double carbapenemase producers with the BD MAX Check-Points CPO assay and a faint New Delhi metallo-β-lactamase band with the GeneFields CPE assay. The **dagger** indicates that confirmatory testing might include PCR for differentiation of Imipenemase-type metallo-β-lactamase and Verona integron-encoded metallo-β-lactamase with the BD MAX Check-Points CPO assay, PCR for identification of Verona integron-encoded metallo-β-lactamase with the GeneFields CPE assay, multiplex PCR for identifying rare carbapenemases, and whole-genome sequencing for identification of newly emerging carbapenemases. CPB, carbapenemase-producing bacteria; mCIM, modified carbapenem inactivation method.

Detection of the Ambler Classes A, B, and D Carbapenemases

Interestingly, when considering the five major carbapenemases (KPC, NDM, VIM, IMP, and OXA-48 like), all the evaluated assays showed 100% sensitivity, with the exception that the GeneFields CPE test was not developed to detect VIM and the BD MAX Check-Points CPO assay did not differentiate between VIM and IMP producers (Table 3 and Supplemental Table S1).

When considering the specificity of target carbapenemases, mCIM, CARBA-5, and GeneXpert Carba-R assays showed high specificity (99.3% to 100%) for all the target genes. The specificity of the BD MAX Check-Points CPO assay was 100% for IMP and/or VIM (95% CI, 97%–100%) and OXA-48 like (95% CI, 97.5%–100%), 97.8% (95% CI, 93.8%–99.5%) for KPC, and 97.1% (95% CI, 92.5%–99.2%) for NDM (Table 3). The specificity of GeneFields CPE tests was 100% (95% CI, 97.4%–100%) for KPC and IMP, 99.3% (95% CI, 96%–100%) for OXA-48 like, and 96.4% (95% CI, 91.8%–98.8%) for NDM.

The Ambler class A GES was only correctly assigned by mCIM assay, with 100% accuracy (95% CI, 97.7%–100%), sensitivity (95% CI, 83.2%–100%), and specificity (95% CI, 97.4%–100%). A comparative analysis elucidating the main features of each assay is described in Table 3.

Development of a Scheme for the Precise Application of the Assays for Detection of Carbapenemases

On the basis of the findings and because of limitations of the tested assays for detection of carbapenemases (Tables 3 and 4), a scheme was developed combining two methods to improve their performance. The overall 100% sensitivity can be achieved with the combination mCIM with either CARBA-5, GeneXpert Carba-R, BD MAX Check-Points CPO, or GeneFields CPE assay (Supplemental Table S1). The five or four major carbapenemases are detected with 100% sensitivity if CARBA-5, GeneXpert Carba-R, BD MAX Check-Points CPO, or GeneFields CPE assay is used as a first assay within 20 to 120 minutes (Figure 1). In case of a positive result, no further testing is required. mCIM is performed as a second phenotypic test, in case of negative or suspicious results. If the mCIM result is also negative, carbapenemase production is highly excluded. Confirmatory testing is required with a positive mCIM result for identification of VIM with the GeneFields CPE assay, rare carbapenemases, or even newly emerging carbapenemases (Figure 1).

Discussion

The worldwide emergence and spread of β-lactam resistance, with a particular focus on CPB, is a troubling nightmare for all health care workers.[2,20,21] Therefore, there is a growing demand for the rapid and accurate detection of carbapenemase producers to perform the proper diagnosis and determination of precise antibiotic therapy. This study evaluated the performance of five important assays for the detection of CPB, including the mCIM, CARBA-5, GeneXpert Carba-R, GeneFields CPE, and BD MAX Check-Points CPO tests. This is the first report to evaluate GeneFields CPE and BD MAX Check-Points CPO assays for the detection of cultured CPB, comparing both assays, and the first to compare both assays with the mCIM, CARBA-5, or GeneXpert Carba-R assay.

Of interest, the mCIM and CARBA-5 assay was the most accurate test for the detection of CPB and the five major carbapenemases (KPC, NDM, IMP, VIM, and OXA-48

Table 4 Comparison of the Features of the Five Evaluated Assays, Including the Strength and the Limitations of Every Assay

Feature	mCIM	CARBA-5	GeneXpert Carba-R	BD MAX Check-Points CPO	GeneFields CPE
Detection principles	Carbapenem inactivation assay (phenotypic)	Immunochromatographic assay	Automated qPCR assay	Real-time PCR assay	Nucleic acid chromatography assay
Target gene	All carbapenemases (positive/negative)	OXA-48, KPC, NDM, VIM, IMP	OXA-48, KPC, NDM, VIM, IMP	OXA-48, KPC, NDM, VIM, and/or IMP	OXA-48, KPC, NDM, IMP
Sample (in this study)	0.5 McFarland bacterial solution	Bacterial colony	0.5 McFarland bacterial solution	1:400 of 0.5 McFarland bacterial solution	DNA
Time required for	5	5	5	10	30
Preparation, minutes	4 hours and overnight	15 minutes	50 minutes	120 minutes	90 minutes
		1	1	1	1
Incubation/reaction	5	21 minutes	56 minutes	131 minutes	121 minutes
Reading the result, minutes	22 hours 10 minutes				
Total time					
Interpretation	Visual by measuring the inhibition zone	Visual by detection of the specific band	Automatic interpretation	Automatic interpretation	Visual by detection of the specific band
Advantages	Simple, inexpensive, accurate, differentiates CPB/non-CPB	Simple, fast, high accuracy, identifies five major carbapenemases with high sensitivity and specificity	Simple, relatively fast, high accuracy, identifies five major carbapenemases with high sensitivity and specificity	Relatively simple, relatively fast, identifies five major carbapenemases with high sensitivity	Relatively simple, relatively fast, identifies four major carbapenemases with high sensitivity
Limitations	Could not identify the type of carbapenemases and relatively long time	Identifies only five carbapenemases with the possibility of missing other carbapenemases	Identifies only five carbapenemases with the possibility of missing other carbapenemases, requires additional equipment	Identifies only five carbapenemases with the possibility of missing other carbapenemases, requires additional equipment, could not differentiate VIM and IMP, low specificity with double-carbapenemase producers	Identifies only four carbapenemases with the possibility of missing other carbapenemases, requires additional equipment, low specificity for NDM

CPB, carbapenemase-producing bacteria; IMP, Imipenemase-type metallo-β-lactamase; KPC, *K. pneumoniae* carbapenemases; mCIM, modified carbapenem inactivation method; NDM, New Delhi metallo-β-lactamase; OXA, oxacillinase group of β-lactamase; qPCR, real-time quantitative PCR; VIM, Verona integron-encoded metallo-β-lactamase.

like). These results are in agreement with previous reports elucidating the high sensitivity (97% to 99%) and specificity (99% to 100%) of mCIM for the phenotypic detection of carbapenemase-producing Enterobacteriaceae[18] and 98% sensitivity and 95% specificity for the identification of carbapenemase-producing *Pseudomonas aeruginosa*.[22] Moreover, these results confirmed the previous finding that the overall sensitivity and specificity of the CARBA-5 test are 97.3% and 99.7%, respectively.[23] Recently, Boutal et al[24] (2018) reported that the sensitivity of this assay reached 100% and that the specificity ranged from 95.3% to 100%. Similar to the findings of the current study, Lucena Baeza et al[6] (2019) reported that, with the exception of one *Klebsiella pneumoniae* isolate coproducing OXA-232 and NDM-1, the CARBA-5 test could detect all KPC, OXA-48−like, NDM, and VIM producers. Although the current study showed that the CARBA-5 assay could detect all IMP-1 and IMP-6 producers, other studies reported the limitations of this test for the detection of IMP-13/IMP-14[6,23] and IMP-28/IMP-50.[6] Another limitation of this test

is the detection of low-activity carbapenemases, such as OXA-163 and OXA-405.[24]

The GeneXpert Carba-R assay proved to be the third most accurate test for the detection of CPB. These results are in agreement with the manufacturer's specification as well as with previous studies reporting the high sensitivity and specificity of this assay, which ranged from 100% sensitivity and specificity for the Cepheid GeneXpert Carba-R assay[25] to 97.8% sensitivity and 95.3% specificity for the GeneXpert Carba-R kit version 2.[26] In a multicenter study performed in two health care facilities in the United States, the overall sensitivity and specificity of this assay were found to be 100% and 97.1% to 98.1%, respectively.[27] Recently, Lucena Baeza et al[6] (2019) reported high sensitivity (88.2%) and specificity (100%) for this assay for the detection of carbapenemases, and it was the only assay among those evaluated that detected carbapenemases in bacteria coproducing different carbapenemases. Of interest, in this experiment, all the evaluated assays could detect all carbapenemases in coproducer isolates.

For the first time, in this study, the performance of the GeneFields CPE and the BD MAX Check-Points CPO assays for the detection of CPB was compared. Both assays were previously evaluated for detection of CPB from stool specimens or rectal swabs,[7,10,28] but their performance for detection of cultured CPB was still untested. Although the accuracy of both tests (94.3% for BD MAX Check-Points CPO and 86.2% for GeneFields CPE) is lower than that of mCIM, CARBA-5, and GeneXpert Carba-R assays, it is still high in comparison to that of other phenotypic or molecular tests for the detection of CPB.[6,9] The findings of the BD MAX Check-Points CPO assay are comparable to the manufacturer's specification and with the findings of the BD MAX assay for the detection of CPB from rectal swabs.[7] Furthermore, a multiplex SYBR Green real-time PCR for the BD MAX system was previously developed, and the assay correctly identified all carbapenemase-positive and carbapenemase-negative isolates.[28] On the other hand, the GeneFields CPE assay showed lower overall sensitivity (77.4%) and similar specificity (98.5%) for the detection of cultured CPB in comparison to the detection of CPB directly from stool specimens, with 93.3% and 99.1% sensitivity and specificity, respectively.[10] These differences could be attributed not only to the culture method but also to the type of carbapenemase, the incubation temperature, and time having a considerable effect on the performance of the assay.[6,8]

The current study showed high accuracy (86.2% to 100%), sensitivity (77.4% to 100%), and specificity (98.5% to 100%) for all the evaluated assays (Table 3). In addition, all assays successfully detected carbapenemases in double carbapenemase-producing isolates. However, the evaluated assays had variable limitations in terms of the efficient diagnosis of CPB with accurate identification of the specific carbapenemases (Tables 3 and 4). For instance, all the assays were designed for the identification of the four or five major carbapenemases, with the possibility of missing the rare or newly emerged carbapenemases. The GeneFields CPE assay was not designed for VIM identification, which is one of the major carbapenemases, particularly in Europe and Africa.[2] Moreover, this assay showed low specificity for NDM, as a false-positive faint or intermediate-strength blue line was identified, especially for isolates harboring other carbapenemases. The BD MAX Check-Points CPO assay was not designed for VIM and IMP differentiation, and the results are expressed as VIM and/or IMP. Furthermore, the false identification of bacteria harboring double carbapenemases is problematic with this assay, as seven isolates were identified to harbor two carbapenemases by BD MAX Check-Points CPO assay, whereas they only harbor a single carbapenemase according to PCR.

On the basis of these findings, a scheme was proposed for competent carbapenemase detection with combining mCIM with either test of the other evaluated assays (Figure 1). With this scheme, the five or four major carbapenemases can be identified within 20 to 130 minutes with 100% sensitivity. Furthermore, this scheme overcomes the limitation of every assay and improves the overall sensitivity for detection of CPE to 100% compared with a single assay (77.4% to 100%) within a day. This scheme has the merit of being reliable, rapid, and accurate for detection of CPE, especially for epidemiologic studies, where precise identification of carbapenemase type is crucial.

Conclusion

Overall, this study confirmed the high accuracy, sensitivity, and specificity of all the evaluated assays for the detection of CPB. These assays could be reliable tools for the rapid and accurate diagnosis of carbapenemases. Therefore, they could be suitable for routine use in microbiology laboratories to overcome the obstacles of other molecular methods, such as the need for sophisticated techniques and long detection times. Furthermore, the rapid and optimized detection of CPB has considerable value for the application of prompt infection control measures, providing a guide for precise antibiotic therapy, and represents a frontier line to prevent the dissemination of resistance determinants. However, the limitations of the evaluated assays and most commercially available tests, especially for the detection of rare and newly emerging carbapenemases, are an intricate problem. Therefore, this study proposes a simple and rapid scheme for precise use of the evaluated assays for the accurate detection of carbapenemases and to obtain the maximum benefits.

Supplemental Data

Supplemental material for this article can be found at *http://doi.org/10.1016/j.jmoldx.2020.05.012*.

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