Verification of the performance of the BD MAX Check-Points CPO Assay on clinical isolates

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Abstract

Objectives: The BD MAX Check-Points CPO Assay (BD MAX CPO Assay; BD, The Netherlands) is a diagnostic test designed for the rapid detection and differentiation of the
blaKPC, blaNDM, blaVIM/blaIMP-1, and blaOXA-48 genes. We veri- fied the performance of the BD MAX CPO Assay to imple- ment it in the clinical laboratory.
Methods: A total of 113 bacterial isolates collected from various clinical specimens harboring carbapenemase genes as confirmed by polymerase chain reaction (PCR) and sequencing were evaluated. The results of the BD MAX CPO Assay were compared to previously confirmed results. Results: All results of the BD MAX CPO Assay were con- cordant with previous results; 61 Klebsiella pneumoniae carbapenemase (KPC), 40 New Delhi metallo-□-lactamase (NDM), three oxacillinase-48 (OXA-48)-like, two imipe- nem (IMP), and four multiple carbapenemase genes (one KPC/NDM, three NDM/OXA) were detected by the BD MAX CPO Assay. Three non-carbapenemase-producing carbap- enem-resistant Enterobacteriaceae isolates were negative. Conclusions: The BD MAX CPO Assay can be used to iden- tify carbapenemase gene in bacterial isolates cultured from clinical specimens in the clinical laboratory.

Keywords: BD MAX Check-Points CPO Assay; carbapen- emase; Enterobacteriaceae; real-time PCR.

Rapid detection of carbapenemase-producing *Enterobacte- riaceae* is important for directing the appropriate treatmentand preventing nosocomial spread of these bacteria [1].

The BD MAX Check-Points CPO Assay (BD MAX CPO Assay;BD, Wageningen, The Netherlands) is a diagnostic test designed for the rapid detection and differentiation of the
bla_{KPC}, bla_{NDM}, bla_{VIM}/bla_{IMP-1}, and bla_{OXA-48} genes. The fol- lowing carbapenemase variants are detected by the assay: *Klebsiella pneumoniae* carbapenemase (KPC)-2–24, New
Delhi metallo-β-lactamase (NDM)-1–16, Verona integron- encoded metallo-β-lactamase (VIM)-1–6, 8–47, imipenem (IMP)-1–4, 6–8, 10, 19, 20, 24–26, 30, 34, 38, 40, 42, 43, 52,
55, and oxacillinase (OXA)-48, 162, 163, 181, 204, 232, 244, 245, 370. It is a qualitative, automated in vitro diagnostic real-time polymerase chain reaction (PCR) test. We veri- fied the performance of the BD MAX CPO Assay with bac-terial isolates from clinical specimens to implement it in the clinical microbiology laboratory.

A total of 113 bacterial isolates were evaluated to assess the reproducibility of the assay (Table 1). Bacterial isolates harboring carbapenemase genes as confirmed by PCR and sequencing were collected from various clinical specimens (45 rectal swabs, 26 urine specimens, 11 res- piratory specimens, six blood cultures, six wounds, and 19 other sites) from April 2013 to November 2018. The antimicrobial susceptibility test was performed using the VITEK2 system (bioMérieux, Marcy l'Etoile, France). To prepare bacterial suspensions, we applied a slightly modified method of preparation of bacterial control sus- pensions for the BD MAX CPO Assay. The isolate was inoc- ulated on MacConkey agar and incubated at 37 °C in 5% CO_2 for 18–24 h. Pure colony was suspended in saline and the concentration was adjusted to a 0.5 McFarland tur- bidity standard (approximately 1.5×10^8 CFU/mL). Serial dilutions of each isolate suspension were performed with saline to obtain the final dilution of 1/5000 from 0.5 McFarland and 50 µL of the specimen was inoculated to the sample buffer tube. The BD MAX System, the equip- ment on which the BD MAX CPO Assay is performed, wasoperated as per the manufacturer's instructions. The results were

compared to previously confirmed results.

All results of the BD MAX CPO Assay were concord- ant with previous results; 61 KPC, 40 NDM, three OXA-48 like, two IMP, and four multiple carbapenemase genes (one KPC/NDM, three NDM/OXA) were detected by the

Table 1: Bacterial isolates used for the evaluation of the BD MAX Check-Points CPO Assay.

Carbapenemase type species identification	No. of isolates	MIC, µg/mL[a]		Results of the BD MAX CPO Assay	No. of isolates
		Imipenem (no. of isolates)	Ertapenem (no. of isolates)		
KPC-2	61			KPC POS	61
Citrobacter spp.	2	8 (1), ≥16 (1)	4 (1), ≥8 (1)		
Enterobacter spp.	1	≥16 (1)	4 (1)		
Escherichia coli	13	8 (2), ≥16 (11)	4 (8), ≥8 (5)		
Klebsiella pneumoniae	45	8 (2), ≥16 (43)	4 (6), ≥8 (39)		
NDM	40			NDM POS	40
NDM-1	36				
Citrobacter spp.	4	≥16 (4)	4 (1), ≥8 (3)		
Enterobacter spp.	8	8 (1), ≥16 (7)	4 (2), ≥8 (6)		
Escherichia coli	7	8 (1), ≥16 (6)	4 (4), ≥8 (3)		
Klebsiella oxytoca	4	8 (1), ≥16 (3)	4 (1), ≥8 (3)		
Klebsiella pneumoniae	11	8 (1), ≥16 (10)	≥8 (11)		
Serratia marcescens[b]	2	≥16 (2)	≥8 (2)		
NDM-3	1				
Escherichia coli	1	Not tested	Not tested		
NDM-4	2				
Escherichia coli	1	≥16 (1)	≥8 (1)		
Klebsiella pneumoniae	1	≥16 (1)	≥8 (1)		
NDM-5	1				
Escherichia coli	1	≥16 (1)	≥8 (1)		
OXA-48 like	3			OXA POS	
OXA-181	2				
Escherichia coli	1	1 (1)	≥8 (1)		
Klebsiella pneumoniae	1	2 (1)	4 (1)		
OXA-232	1				
Klebsiella pneumoniae	1	≥16 (1)	≥8 (1)		
IMP	2			VIM and/or IMP POS	2
IMP-1	1				
Citrobacter spp.	1	0.5 (1)	4 (1)		
IMP-4	1				
Enterobacter spp.	1	≥16 (1)	≥8 (1)		
Multiple carbapenemase genes	4				
KPC-2/NDM-1	1			KPC POS/NDM POS	1
Klebsiella oxytoca	1	≥16 (1)	≥8 (1)		
NDM-1/OXA-232	1			NDM POS/OXA POS	1
Klebsiella pneumoniae	1	≥16 (1)	≥8 (1)		
NDM-5/OXA-48	1			NDM POS/OXA POS	1
Escherichia coli	1	8 (1)	≥8 (1)		
NDM-5/OXA-181	1			NDM POS/OXA POS	1
Klebsiella pneumoniae	1	Not tested	Not tested		
Negative	3			NEG	3
Escherichia coli	2	≥16 (2)	4 (1), ≥8 (1)		
Klebsiella pneumoniae	1	1 (1)	≥8 (1)		
Sum	113				113

[a]Imipenem and ertapenem minimum inhibitory concentrations (MIC) were tested by the VITEK2 system (bioMérieux). [b]*Serratia* were recently classified under the new family of the order *Enterobacterales*: *Yersiniaceae*. POS, positive; NEG, negative.

BD MAX CPO Assay. Three non-carbapenemase-produc- ing carbapenem-resistant *Enterobacteriaceae* isolates were all negative (Table 1). Sensitivity and specificity were 100%.

According to the package insert data, the BD MAX CPO Assay showed good performance on the rectal swab, with a sensitivity of 96.6–100% and a specificity of 98.3–100% according to the carbapenemase type [2]. The performance was comparable with that of the Xpert Carba-R Assay (Cepheid, Sunnyvale, CA, USA). However, that performance was evaluated with the rectal swab, not the bacterial isolates. It cannot be a proof of performance with the bacterial isolates. No study for the evaluation of the performance of the BD MAX CPO Assay on the bacterial isolates has been published. Several studies have evaluated the performance of the Xpert Carba-R Assay for the bacterial isolates and/or rectal swabs [3–6]. Similar to the BD MAX CPO Assay, the Xpert Carba-R Assay is a qualitative, on-demand real-time PCR test for the rapid detection and differentiation of the bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{OXA-48}, and bla_{IMP-1} genes. The following carbapenemase variants are detected by the assay: KPC-2–16, NDM-1–9, VIM-1, 2, 4–20, 23–38, IMP-1–4, 6, 8–11, 19–22, 24, 25, 27, 28, 30, 31, 33, 37, 40, 42, and OXA-48, 162, 163, 181, 204, 232, 244, 245, 247. This assay is performed on pure colonies and rectal swabs. Despite the limitations and variations of these studies, overall it showed 96.6–100% of sensitivity and 95.3–99.13% of specificity.

Rapid and accurate detection of carbapenemase genes is crucial for patient care, infection control, and surveillance. The BD MAX CPO Assay can be performed in approximately 2.5 h, and the time is a little shorter when testing small numbers. It is faster compared to growth-based methods for the confirmation of carbapenemase production including the modified Hodge test and modified carbapenem inactivation method which take 18–24 h [7]. There are Carba NP and lateral flow immunoassays for faster methods. Carba NP and variants take 30 min to 2 h. The new immunochromatographic RESIST-4 O.K.N.V. assay (Coris, Gembloux, Belgium) generates results within 15 min.

The BD MAX CPO Assay automates the testing process and minimizes operator intervention from the time the sample is placed onto the BD MAX System until results are available. Accordingly, less trained staff can easily perform the test and the risk of contamination and human error can be minimized. The assay can test 24 samples at a time and one sample can be tested without reagent The BD MAX CPO Assay has several limitations. Most of all, this assay does not identify bacteria. Moreover, detection of these gene sequences does not indicate the presence of viable organisms. Therefore, when the assay is performed on rectal swab specimens from patients, concomitant cultures are necessary to recover organisms for epidemiological typing, antimicrobial susceptibility testing, and further confirmatory bacterial identification. And the VIM and IMP genes are combined in one optical channel of the BD MAX system; therefore, the BD MAX CPO Assay does not distinguish between the bla_{VIM} and bla_{IMP} genes.

Because of the absence or low prevalence of VIM and IMP carbapenemases in isolates found in clinical specimens in our laboratory, only two isolates of IMP-producing *Enterobacteriaceae* were included in this study. Considering the low prevalence of VIM and IMP carbapenemases in our region, it would not be critical. This study also included only a small number of OXA-48-like genes; three alone and two in combination with other carbapenemase genes. Further studies are needed to evaluate the performance for those loss. And assays performed on the BD MAX System other than the BD MAX CPO Assay can be proceeded at the same time, which helps to meet the various needs of the clini-cians in the clinical laboratory. carbapenemases. Especially, studies for the evaluation of carbapenemase detection directly from rectal swabs are needed.

In this study, we verified the performance of the BD MAX CPO Assay to introduce it into routine use in the clinical microbiology laboratory for the identification of carbapenemase genes in the bacterial isolates. In conclusion, the BD MAX CPO Assay can be used to identify carbapenemase gene in bacterial isolates in the clinical laboratory. This can allow for the earlier implementation of appropriate treatment and infection control as it is less labor-intensive than comparable methods and yields reliable results quickly.

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It showed complete concordance with comparable methods for the major carbapenemases. We concluded that it can be used for carbapenemase identification in the bacterial isolates cultured from clinical specimens.

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