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The Bio-Rad βCARBA Assay Detects a Diverse Range of Carbapenemase-Producing Organisms (CPO)

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BM Willey, X Trimi, R Iaboni, S Rajadurai, DN Grohn, DA Boyd, L. Mataseje, G Ricci, A Mazzulli, D Terenzi, P Lo, M. Mulvey, T Mazzulli, SM Poutanen

Mount Sinai Hospital/UHN, University of Toronto, Toronto, Ontario; William Osler Health Sciences Centre, Brampton, Ontario; and the National Microbiology Laboratory, Winnipeg, Manitoba; CANADA

ABSTRACT

Background: Although PCR-based confirmatory tests are now available, they typically do not detect all possible carbapenemases and are generally too costly for use on every suspected CPO. Phenotypic tests that rely on visualization of pH changes resulting from imipenem hydrolysis have been suggested in standards but have proven unreliable for detecting class D CPO in many clinical microbiology laboratories. This study determined the performance of Bio-Rad's βCARBA assay, which detects CPO via a pH independent colour-change, as it is the chromogenic substrate itself (a proprietary carbapenem) that changes colour.

Methods: 259 species-diverse isolates, highly-characterized by PCR/sequencing, were blinded to prevent bias. They included 221 CPO (108 class A: 99 KPC, 4 SME, 2 IMI-1, 2 GES, 1 NMC-A; 80 class B: 73 NDM, 6 VIM, 1 IMP7; 26 class D; OXA48; OXA181, OXA232, OXA244; 7 class B+D: NDM+OXA181, NDM+OXA232) and 38 non-CPO (derepressed/plasmid-mediated *ampC*, ESBL, *ompC*/*ompF*-mutants, *ompK35*/*ompK36*-mutants, *cphA*, OXA252). On/after recovery from -80°C, ertapenem discs were added to all plates for selective pressure. For βCARBA testing, 259/259 isolates were plated to Oxoid Columbia Sheep Blood agar (CSBA) while 76/259 were plated to Mueller-Hinton agar (MHA), these comprised 58 CPO (11 KPC, 3 SME, 2 GES, 1 IMI1, 1 NMC-A; 12 NDM, 6 VIM, 1 IMP7; 17 OXA48-like, 4 NDM+OXA48-like) and 18 non-CPO. All 335 βCARBA tests were inoculated from growth surrounding ertapenem discs. Incubation, as per Bio-Rad, was at 37°C. Each tube was read independently at 30min by 5 readers for colour-changes from yellow (negative) to orange, red or purple (positives). Consensus reads were analyzed for sensitivity/specificity, and 95% confidence intervals were calculated in www.graphpad.com.

Results: Overall, βCARBA was 98.2% sensitive (95%CI: 95.8-99.3) and 100% specific (95%CI: 92.2-100) detecting 274/279 CPO in 335 tests from both agars combined. βCARBA performed equally well from CSBA and MHA detecting 218/221 (98.6%) and 56/58 (96.6%) of the CPO, respectively. Detection notably included 100% of the troublesome OXA48-like CPO (CSBA: 33/33; MHA: 21/21). False-negatives from both agars included the unique *nmcA*-positive *Enterobacter cloacae* and 1/2 GES *Klebsiella oxytoca* – βCARBA tests remained yellow >45min and again on repeat. The only βCARBA false-positive result was from an *Aeromonas hydrophila* (intrinsic carbapenem-resistance due to *cphA*). This species would be excluded from testing based on its identity, and thus was not included in specificity calculations.

Conclusions: βCARBA is low-complexity assay that is quick and easy to set-up and simple to interpret. When faced with a possible CPO, βCARBA provides a highly sensitive/specific results in 30min from 1uL-loop of organism, unlike similar tests that require larger inoculum and lengthier incubations.

INTRODUCTION

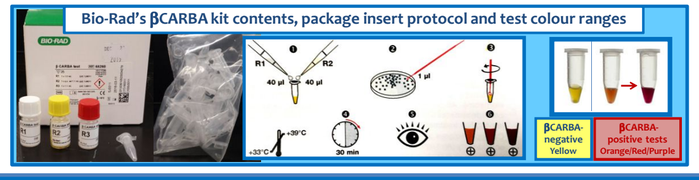
Rapid and accurate detection of CPO is critical to patient care and to limit dissemination. CPO are multidrug resistant Gram-negative bacilli (MDR-GNB) that present an urgent public health threat as they are typically resistant to all available antimicrobials including carbapenems which are generally considered among the last resort agents for treating serious infections.

Molecular methods are considered "gold standard" for confirming carbapenemase genes, yet no currently available PCR assay detects all CPO. This is because novel genotypes frequently arise without apparent warning, and known genotypes have been noted to diversify via genetic drift at rates which have reduced molecular test sensitivities. Availability of a routine PCR test for the limited number of common genotypes is important in certain situations to enable rapid detection (<4h) of CPO directly from specimens. But for day to day use, availability of a reliable rapid test capable of detecting phenotypic evidence of any carbapenemase in a suspicious surveillance or clinical isolate will reduce the overall risk of missing new or unusual genotypes, and enable improved patient management by allowing important infection control decisions to be made without delay. This was the idea behind the CARBA-NP assay, but in most labs, this pH indicator-based phenotypic test proved unreliable for detecting Class D type CPO.

Generally, specimens received for CPO surveillance are first screened using selective culture while CPO in clinical specimens are first detected via routine susceptibility testing. Suspected CPO are then ruled out depending on the laboratory by any of a number of methods including inhibitor testing, limited range PCR, or referral to reference laboratories for more extensive PCR. This may result in long reporting delays during which time dissemination of an unrecognized CPO may take place.

This study determined the utility of a low-complexity phenotypic CPO detection test, Bio-Rad's βCARBA assay. In contrast to the CARBA-NP, the βCARBA assay detects hydrolysis of a proprietary colorimetric carbapenem which undergoes a colour-change within 30min exposure to a CPO. This study was designed to assess the practicality, sensitivity, specificity, and cost-effectiveness of implementing the βCARBA into routine workflow of a large clinical microbiology laboratory to detect CPO from isolates grown on a variety of agars.

Bio-Rad's βCARBA kit contents, package insert protocol and test colour ranges



METHODS

Table 1 describes the isolate characteristics and distribution by agar type used in the study. On and after recovery from -80°C, ertapenem discs were added to all subculture plates to maintain selective pressure. In preparation for blinded testing, all 259 isolates were plated to Oxoid's Columbia-based 5% Sheep Blood agar (Blood agar), of which 76 were also plated to Oxoid's Mueller-Hinton Plus agar (MHA). plates as before had an ertapenem disc placed on the inoculated area to maintain antibiotic pressure. Using fresh cultures of the total 335 isolates, Bio-Rad βCARBA tests were performed as per package inserts. Briefly, all liquid from Reagent 1 was transferred to dissolve the dehydrated contents of Reagent 2. Then 40µL ea. Regents 2 and 3 were pipetted into a micro-tube (provided in kit). A heavy 1 mL loop of colonies derived from growth closest to ertapenem discs on each plate was inoculated into each tube and mixed thoroughly. At 30 min incubation at 37°C (water-bath), tubes were read independently by 5 readers for colour-changes: Yellow = CPO-negative; Orange, Red or Purple = CPO-positive. Consensus reads were analyzed for sensitivity and specificity, 95% confidence intervals were calculated in www.graphpad.com.

Table 1. Characteristics of 259 bacteria and agars used to evaluate Bio-Rad's βCARBA assay

Ambler (No.)	CPO Genotypes (No.)	Species identification	Blood agar (No.)	MHA subset (No.)
Class A CPO (108)	*blaKPC (99)*	*Citrobacter freundii*	2	0
		Enterobacter aerogenes	3	1
		Enterobacter cloacae	35	2
		Escherichia coli	12	3
		Klebsiella oxytoca	1	1
		Klebsiella pneumonia	46	4
	blaGES5 (2)	*Klebsiella oxytoca*	2	2
	blaIMI1 (1)	*Enterobacter cloacae*	1	1
	blaNMCA (2)	*Enterobacter cloacae*	2	1
	blaSME (4)	*Serratia marcescens*	4	3
Class B CPO (80)	*blaIMP7 (1)*	*Pseudomonas aeruginosa*	1	1
	blaNDM (73)	*Acinetobacter baumannii*	1	1
		Citrobacter freundii	1	1
		Enterobacter cloacae	3	1
		Escherichia coli	30	2
		Klebsiella pneumoniae	33	3
		Morganella morganii	4	3
		Proteus mirabilis	1	1
	blaVIM (6)	*Citrobacter freundii*	1	1
		Enterobacter cloacae	4	4
		Pseudomonas putida	1	1
Classes B + D CPO (7)	*blaNDM+blaOXA181 (1)*	*Escherichia coli*	1	1
	blaNDM+blaOXA181 (3)	*Klebsiella pneumoniae*	3	2
	blaNDM+blaOXA232 (1)	*Escherichia coli*	1	0
	blaNDM+blaOXA232 (2)	*Klebsiella pneumoniae*	2	1
Class D CPO (26)	*blaOXA48 (15)*	*Escherichia coli*	8	6
		Klebsiella pneumoniae	7	4
	blaOXA181 (6)	*Escherichia coli*	2	0
		Klebsiella pneumoniae	4	3
	blaOXA232 (4)	*Escherichia coli*	1	0
		Klebsiella pneumoniae	3	2
	blaOXA244 (1)	*Escherichia coli*	1	1
Non-CPO (38)	*ompC-ompF (3)*	*Enterobacter cloacae*	1	1
		Escherichia coli	3	3
	ompK35-ompK36 (6)	*Klebsiella pneumoniae*	6	6
	Weak OXY promoter (1)	*Klebsiella oxytoca*	1	1
	Other mechanisms ()	*Enterobacteriaceae*	26	7
	blaOXA252 (1)	*Shewanella putrifaciens*	1	0

RESULTS

Table 2: Summary of CPO detection results obtained using Bio-Rad's βCARBA assay when challenged by 259 distinct isolates cultivated on two common laboratory agars

CPO Genotypes Tested	No. (%) Blood agar/ No. (%) βCARBA-Pos	No. (%) Mueller-Hinton agar/ No. (%) βCARBA-Pos	Tn. (%) Tested/ Tn. (%) βCARBA-Pos
blaKPC	99 (38.2)/99 (100)	11 (14.5)/11 (100)	110 (32.8)/110 (100)
blaNDM	73 (28.2/73 (100)	12 (15.8)/12 (100)	85 (25.4)/85 (100)
blaOXA48-like	26 (10)/26 (100)	17 (22.4)/17 (100)	43 (12.8)/43 (100)
blaNDM+OXA48-like	7 (2.7)/7 (100)	4 (5.3)/4 (100)	11 (3.3)/11 (100)
blaVIM	6 (2.3)/6 (100)	6 (7.9)/6 (100)	12 (3.6)/12 (100)
blaSME	4 (1.5)/4 (100; 1 weak)	3 (4)/3 (100)	7 (2.1)/7 (100)
blaGES5	2 (0.8)/0 (0)	2 (2.6)/1 (50)	4 (1.2)/1 (25)
blaNMC$_A$	2 (0.8)/1 (50)	1 (1.3)/ 0 (0)	3 (1.5)/1 (33)
blaIMI1	1 (0.4)/1 (100)	1 (1.3)/1 (100)	2 (0.6)/2 (100)
blaIMP7	1 (0.4)/1 (100)	1 (1.3)/1 (100)	2 (0.6)/2 (100)
Non-CPO	37 (14.3)/0 (0)	18 (23.7)/0 (0)	55 (16.4)/0 (0)
Non-CPO (intrinsic *cphA*)	1 (0.4)/1 (100)	ND	1 (0.3)/1 (100)
Total CPO tested	221 (85.3)/218 (98.6)	58 (76.3)/56 (96.6)	279 (83.3)/274 (98.2)
Total non-CPO tested	38 (14.7)/1 (2.6)	18 (23.7)/0 (0)	56 (16.7)/1 (1.8)
Total isolates tested	259	76	335

RESULTS

Table 2 (bottom left) summarizes βCARBA results obtained from blood and Mueller-Hinton agars, while Table 3 (below) displays overall βCARBA sensitivity and specificity with (95%CI) for detecting 274/279 98.2% CPO in 335 tests from both agars combined.

βCARBA performed well from Blood/MHA, from which CPO detection was 218/221 (98.6%)/ 56/58 (96.6%) overall, and for OXA48-types, it was 33/33 (100%)/21/21 (100%), respectively.

False-negatives from both agars were rarely encountered in unusual class A genotypes only, namely in *K. oxytoca* with *blaGES5* and *E. cloacae with blaNMC$_A$* which were not or only poorly detected by βCARBA from either agar (Table 2). In these isolates, the βCARBA tests reproducibly were yellow beyond 45 min. Sequencing confirmed their alleles to be *blaGES5* - a genotype with recognized carbapenemase activity. Similarly, sequences were determined for the 3 *E. cloacae* with closely related *blaNMC/IMI* genes. Although only represented by a few isolates in this study, it appeared that *blaIMI1* readily hydrolyzed the proprietary carbapenem utilized in the βCARBA assay while *blaNMC$_A$* encountered difficulties. In all cases, the β–lactamases produced by these isolates failed to hydrolyze the particular carbapenem used in the assay regardless of evidence of growth to ertapenem on both test agars, and phenotypic meropenem resistance as demonstrated by MHA using the screen breakpoint of ≤25mm to indicate a suspect CPO.

The only βCARBA "false-positive" result was from an *Aeromonas hydrophila*. Since this species is intrinsically carbapenem-resistant due to chromosomal *cphA*, it is not considered a true CPO in the epidemiological sense. If encountered, this species should be excluded from further CPO testing based on its identity. For this reason, it was excluded from βCARBA specificity calculations. But as it mounted a reliably strong βCARBA-positive reaction, this species could be retained as a safe quality control isolate for this and equivalent tests, so to be readily discernable from potentially cross-contaminating CPO in clinical laboratories.

Table 3: Overall Bio-Rad βCARBA test CPO detection performance from two common agars

βCARBA Sensitivity for any CPO	98.2% (95% CI: 95.8-99.3)
βCARBA Specificity (excluding intrinsic mechanisms)	100% (95% CI: 92.2-100)

DISCUSSION & CONCLUSION

Bio-Rad's βCARBA phenotypic CPO detection assay

- ✓ Simple to use and very easy to interpret (<1 minute hands-on time)
- ✓ Clear results available within 30 minutes
- ✓ Only 1µl loop of bacterial colonies required to set up test
- ✓ Accurate results from most common agars - Mueller-Hinton agar, Columbia-based sheep blood agar (insert precludes use of βCARBA from colonies on MacConkey-based agars citing unreliable results)
- ✓ Highly specific (100%) - no false-positive at 30min with the exception of genera with intrinsic resistance (exclude these based on identity)
- ✓ Highly sensitive (98.2%) for overall CPO detection, with 100% detection in the most common CPO such as *blaKPC*, *blaNDM*, *blaVIM*, and very notably in the troublesome *blaOXA48*-like genotypes
- ❖ Unreliable for detecting the rare *blaNMC-A* (1/9) and *blaGES5* (0/2) CPO

In conclusion, βCARBA is a cost-effective, low-complexity assay as it is relatively inexpensive, quick and easy to use, extremely simple to interpret, thus improves laboratory workflow. When faced with a possible CPO, βCARBA provides highly sensitive/specific results in 30min from only 1µL loop of organism, unlike similar phenotypic tests that require more colonies and lengthier incubations.

Acknowledgements

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bwilley@mtsinai.on.ca