JCM Journals.ASM.org

Evaluation of the Recently Launched Rapid Carb Blue Kit for Detection of Carbapenemase-Producing Gram-Negative Bacteria

Ângela Novais,[a] Michael Brilhante,[a] João Pires,[a,b,c] Luísa Peixe[a]

UCIBIO/REQUIMTE, Faculdade de Farmácia da Universidade do Porto, Porto, Portugal[a]; Institute for Infectious Diseases, University of Bern, Bern, Switzerland[b]; Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland[c]

The detection of carbapenemase-producing bacteria is still a challenge for many laboratories worldwide, especially in low-income countries, though critical from a clinical or epidemiological point of view (1). The Blue-Carba test is an inexpensive biochemical method for the detection of carbapenemase producers, based on the color change of a pH indicator (bromothymol blue) when a carbapenemase hydrolyzes imipenem (2). Based on this principle, the Rapid Carb Blue kit was recently launched by Rosco Diagnostica using tablets containing imipenem or not (Rosco Diagnostica A/S, Taastrup, Denmark). We aimed to evaluate the performance of the Rapid Carb Blue kit in a representative collection of carbapenemase and noncarbapenemase producers.

Seventy-five clinical isolates from different hospitals and countries and diverse *Enterobacteriaceae*, *Pseudomonas* spp., and *Acinetobacter* spp. producing a wide diversity of class A ($n = 13$; KPC and GES), class B ($n = 28$; VIM, IMP, NDM, and SMP), class D ($n = 32$; OXA), or class B and D ($n = 2$; NDM and OXA) carbapenemases were tested (2) (Table 1). They displayed variable MIC values to imipenem (0.5 to >32 μg/ml), ertapenem (0.25 to >32 μg/ml), and meropenem (0.063 to >32 μg/ml) as determined by Etest (2, 3). In addition, 30 noncarbapenemase producers susceptible or nonsusceptible to carbapenems (including extended-spectrum β-lactamase [ESBL]- or AmpC-producing isolates with or without alterations in outer membrane permeability or efflux) were tested (Table 1). Carbapenemase production was confirmed by standard phenotypic methods (disk diffusion using different inhibitors), PCR, and sequencing of diverse carbapenemase genes (bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, bla_{SPM}, bla_{GES}, and bla_{OXA}) and/or spectrophotometric assays using imipenem as the substrate (1).

An in-house Blue-Carba test was performed as previously described (2). Briefly, a 5-μl loop of the bacterial culture isolated in Mueller-Hinton agar was suspended in two different 0.04% bromothymol blue solutions containing (test) or not (control) 3 mg/ml imipenem (Tienam 500; Merck Sharp & Dohme, France) plus 0.1 mmol/liter $ZnSO_4$, and color changes were registered after an incubation period at 37°C for 2 h (2). The Rapid Carb Blue kit test was performed according to the manufacturer's recommendations (reference 98023, issued 30 September 2014). Bacterial suspensions (a >4 McFarland standard) were prepared from isolates grown on Mueller-Hinton agar in 0.9% KCl solution (pH 8.5). In two separate tubes, 200 μl of the suspension was vortexed briefly with an imipenem (2×)-bromothymol blue tablet and a Carb Negative Control Blue Diatab, respectively, and further centrifuged for supernatant clearance. Results were interpreted after 15 min, 30 min, and 1 h of incubation at 37°C, and a change in color from blue to yellow, from blue to light green, or from green to yellow in the supernatant or at the interface of the precipitate was considered a positive result. All tests were read by two different readers, blinded regarding the β-lactamase content of the strains tested. The sensitivity, specificity, and positive and negative predictive values of the tests were calculated, considering PCR and spectrophotometric assays (when PCR was negative and isolates were resistant to carbapenems) as the reference methods.

All carbapenemase producers and noncarbapenemase producers from the collection tested in this study were detected by the in-house Blue-Carba test (100% sensitivity and 100% specificity) (Table 1), confirming its good performance as reported by us and other authors (2, 4). Also, high specificity and sensitivity (though variable with the collection analyzed) were reported for the Carba NP test (a similar test variant using a different pH indicator and a previous β-lactamase extraction step) (1, 5, 6), revealing the usefulness of these acidimetric tests, which can also be applied directly from clinical samples and in a wide range of culture media (1, 7). The Rapid Carb Blue kit detected 93.3% of carbapenemase producers, false-negative results being obtained for 2 *Pseudomonas aeruginosa* isolates producing VIM-2 or GES-6 and 3 *Acinetobacter baumannii* isolates producing OXA-23, OXA-40, or GES-14 (Table 1). None of the noncarbapenemase producers yielded a false-positive result (100% specificity). We hypothesize that the lower sensitivity of the kit variant is due to the lower inocula used (compared with the in-house test protocol) and/or the higher instability of imipenem in tablets. Positive and negative predictive values were 100% and 85.3%, respectively. High specificity and a lower sensitivity were also reported for the Rapid Carb Screen kit (Rosco Diagnostica A/S, Taastrup, Denmark) compared with the in-house Carba NP test (6).

In conclusion, we report high sensitivity (93.3%) and specificity (100%) for the detection of carbapenemase producers by the Rapid Carb Blue kit, a performance slightly inferior to that of the in-house protocol. Both test variants are reliable and cost-effective and can be easily adapted to routine microbiology laboratories for quick detection of carbapenemase-producing Gram-negative bacteria.

Accepted manuscript posted online 15 July 2015

Citation Novais Â, Brilhante M, Pires J, Peixe L. 2015. Evaluation of the recently launched Rapid Carb Blue kit for detection of carbapenemase-producing Gram-negative bacteria. J Clin Microbiol 53:3105–3107. doi:10.1128/JCM.01170-15.

Editor: P. Bourbeau

Address correspondence to Luisa Peixe, lpeixe@ff.up.pt.

doi:10.1128/JCM.01170-15

TABLE 1 Carbapenemase and noncarbapenemase producers tested in both Blue-Carba test variants

Producer type and acquired β-lactamase (*n*)	Species (*n*)	Test result[a]	
		In-house test	Rapid Carb Blue kit
Carbapenemase producers			
Class A			
KPC-2, KPC-3 (11)	*Klebsiella pneumoniae* (8)	+	+
	Enterobacter cloacae (2)	+	+
	Escherichia coli (1)	+	+
GES-6, GES-14 (2)	*P. aeruginosa* (1)	+	−
	A. baumannii (1)	+	−
Class B			
NDM-1 (9)	*Escherichia coli* (4)	+	+
	Klebsiella pneumoniae (3)	+	+
	Enterobacter cloacae (2)	+	+
VIM-1 (4)	*Klebsiella pneumoniae* (2)	+	+
	Enterobacter cloacae (2)	+	+
VIM-2 (10)	*P. aeruginosa* (7)	+	+ (6/7)
	Pseudomonas pseudoalcaligenes (1)	+	+
	Klebsiella pneumoniae (1)	+	+
	Enterobacter cloacae (1)	+	+
VIM-34 (1)	*Klebsiella pneumoniae* (1)	+	+
IMP-5 (3)	*A. baumannii* (1)	+	+
	Acinetobacter bereziniae (2)	+	+
SPM-1 (1)	*P. aeruginosa* (1)	+	+
Class D			
OXA-23 (8)	*A. baumannii* (8)	+	+ (7/8)
OXA-40 (8)	*A. baumannii* (7)	+	+ (6/7)
	Acinetobacter haemolyticus (1)	+	+
OXA-58 (7)	*A. baumannii* (6)	+	+
	Acinetobacter pittii (1)	+	+
OXA-72 (1)	*A. baumannii* (1)	+	+
OXA-48 (8)	*Klebsiella pneumoniae* (8)	+	+
OXA-48 + NDM-1 (2)	*Klebsiella pneumoniae* (2)	+	+
Noncarbapenemase producers			
Class A			
CTX-M-15, CTX-M-1, CTX-M-9 (5)[b]	*Escherichia coli* (3)	−	−
	Klebsiella pneumoniae (1)	−	−
	Klebsiella oxytoca (1)	−	−
SHV-5, SHV-12 (2)	*Escherichia coli* (2)	−	−
TEM-24, TEM-199 (2)	*Klebsiella pneumoniae* (1)	−	−
	Proteus mirabilis (1)	−	−
GES-1 (1)	*Escherichia coli* (1)	−	−
Class C			
CMY-2 (1)	*Escherichia coli* (1)	−	−
DHA-1 (1)[b]	*Klebsiella pneumoniae* (1)	−	−
ACT-4 (1)[b]	*Enterobacter asburiae* (1)	−	−
Class A + class C			
SHV-12 + DHA-1 (1)	*Klebsiella pneumoniae* (1)	−	−
Class D			
OXA-30 (1)	*Salmonella enterica* (1)	−	−
OXA-51 (3)	*A. baumannii* (3)	−	−
None (5)[b]	*P. aeruginosa* (3)	−	−
	P. aeruginosa (1)	−	UN
	Escherichia coli (1)	−	−
None (7)[b]	*Enterobacter aerogenes* (2)	−	−
	Enterobacter cloacae (2)	−	−
	Klebsiella pneumoniae (2)	−	−
	Escherichia coli (1)	−	−

[a] Scores when different from 100% are represented in parentheses. UN, uninterpretable.
[b] Includes isolates nonsusceptible to carbapenems with deficiencies in membrane permeability and/or increased efflux.

ACKNOWLEDGMENTS

This work was supported by the European Union (FEDER funds) through Programa Operacional Factores de Competitividade—COMPETE and from National Funds (FCT, Fundação para a Ciência e a Tecnologia) through grant numbers PEst-C/EQB/LA0006/2013 and UID/Multi/04378/201317 and also by funds from the European Union (FEDER funds) under the framework of QREN through Project NORTE-07-0124-FEDER-000066.

We are also grateful to Rosco Diagnostica for providing us with the kits.

REFERENCES

1. **Hrabák J, Chudáčková E, Papagiannitsis CC.** 2014. Detection of carbapenemases in *Enterobacteriaceae*: a challenge for diagnostic microbiological laboratories. Clin Microbiol Infect **20:**839–853. http://dx.doi.org/10.1111/1469-0691.12678.
2. **Pires J, Novais A, Peixe L.** 2013. Blue-carba, an easy biochemical test for detection of diverse carbapenemase producers directly from bacterial cultures. J Clin Microbiol **51:**4281–4283. http://dx.doi.org/10.1128/JCM.01634-13.
3. **CLSI.** 2011. Performance standards for antimicrobial susceptibility testing; 20th informational supplement. CLSI document M100-S21. Clinical and Laboratory Standards Institute, Wayne, PA.
4. **Pasteran F, Veliz O, Ceriana P, Lucero C, Rapoport M, Albornoz E, Gomez S, Corso A, ReLAVRA Network Group.** 2015. Evaluation of the Blue-Carba test for rapid detection of carbapenemases in Gram-negative bacilli. J Clin Microbiol **53:**1996–1998. http://dx.doi.org/10.1128/JCM.03026-14.
5. **Osterblad M, Hakanen AJ, Jalava J.** 2014. Evaluation of the Carba NP test for carbapenemase detection. Antimicrob Agents Chemother **58:**7553–7556. http://dx.doi.org/10.1128/AAC.02761-13.
6. **Yusuf E, Van Der Meeren S, Schallier A, Piérard D.** 2014. Comparison of the Carba NP test with the Rapid CARB Screen kit for the detection of carbapenemase-producing *Enterobacteriaceae* and *Pseudomonas aeruginosa*. Eur J Clin Microbiol Infect Dis **33:**2237–2240. http://dx.doi.org/10.1007/s10096-014-2199-3.
7. **Dortet L, Bréchard L, Poirel L, Nordmann P.** 2014. Impact of the isolation medium for detection of carbapenemase-producing *Enterobacteriaceae* using an updated version of the Carba NP test. J Med Microbiol **63:**772–776. http://dx.doi.org/10.1099/jmm.0.071340-0.