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Blue-Carba, an Easy Biochemical Test for Detection of Diverse Carbapenemase Producers Directly from Bacterial Cultures

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Quick, simple, and reliable methods are needed for laboratory detection of carbapenemases that are widely disseminated among Gram-negative bacteria, in order to improve the detection and surveillance of these clinically relevant bacteria in an epidemiological context (1, 2). Recently, a highly sensitive and specific rapid biochemical test (Carba NP) was described to detect carbapenemase production on *Enterobacteriaceae* and *Pseudomonas* species extracts prepared with a commercial buffer (B-PER II) (3). Here, we propose a modified test (Blue-Carba) that was validated for the detection of carbapenemase-producing strains directly from bacterial cultures.

One hundred one previously characterized *Enterobacteriaceae* ($n = 44$), *Acinetobacter* ($n = 43$), and *Pseudomonas* ($n = 14$) species strains producing Ambler class A, B, and D carbapenemases (KPC, IMP, NDM, VIM, SPM, and OXA) and 49 noncarbapenemase producers (susceptible or nonsusceptible to carbapenems) were tested (Table 1). Carbapenemase production was assessed by standard phenotypic tests, PCR and sequencing, and/or spectrophotometric assays (2, 4). The MICs for carbapenems were determined using Etest (4). The Carba NP method relies on the detection in a bacterial extract of hydrolysis of the carbapenem β-lactam ring through the acidification of a phenol red solution used as color indicator. In the Blue-Carba test variant, bromothymol blue was selected as the indicator, since it includes the optimal pH range (6.0 to 7.6) for most β-lactamases (pH = 6.8), which was a key factor for a direct colony approach. A commercially and widely available imipenem (Tienam 500; Merck Sharp & Dohme, France) was used as the substrate for carbapenemases. The test solution consisted of an aqueous solution of bromothymol blue at 0.04% (Merck Millipore, Germany) adjusted to pH 6.0, 0.1 mmol/liter $ZnSO_4$, and 3 mg/ml of imipenem, with a final pH of 7.0. A negative-control solution (0.04% bromothymol blue solution, pH 7.0) was prepared to control the influence of bacterial components or products in the pH of the solution. A loop (approximately 5 μl) of a pure bacterial culture recovered from Mueller-Hinton agar (bioMérieux, France) was directly suspended in 100 μl of both test and negative-control solutions in a 96-well microtiter plate and incubated at 37°C with agitation (150 rpm) for 2 h. Carbapenemase activity was revealed when the test and negative-control solutions, respectively, were (i) yellow versus blue, (ii) yellow versus green, or (iii) green versus blue. Noncarbapenemase producers remained blue or green on both solutions (Fig. 1). The test was performed in triplicate for all isolates, yielding reproducible results.

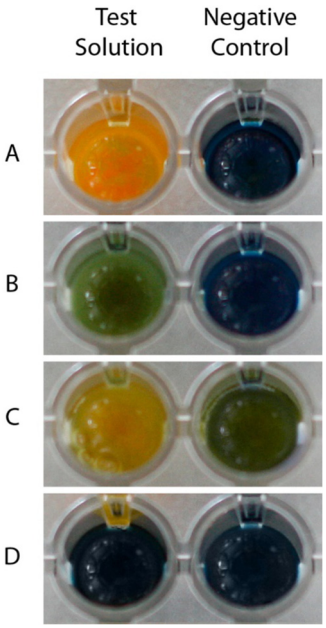

FIG 1 Representative results of the Blue-Carba test obtained from carbapenemase producers (A, B, and C) and non-carbapenemase producers (D) with test solution (left) and negative control solutions (right). (A) NDM-1-producing *E. coli*. (B) OXA-23-producing *A. baumannii*. (C) OXA-48-producing *K. pneumoniae*. (D) *E. coli* ATCC 25922. The images were taken after 2 hours of incubation.

The Blue-Carba test detected all carbapenemase producers (Table 1) with 100% sensitivity and 100% specificity. All noncarbapenemase producers (including extended-spectrum β-lactamase- and/or AmpC-producing isolates), with or without alterations in outer membrane permeability, gave negative results (Table 1). Different times were required to observe a positive result for different carbapenemases types (e.g., KPC or MBL at the first 30 min versus most OXA-type enzymes at 1 h 30 min to 2 h 00 min). Furthermore, a higher inoculum resulted in clearer color changes for OXA types from *Acinetobacter* spp.

Blue-Carba was demonstrated to have specificity and sensitivity (100%) similar to those of Carba NP test, and it presents additional advantages, as follows: (i) increased protocol simplicity due to the direct use of colonies (instead of bacterial extracts); (ii) significantly reduced cost per reaction (over 200×), taking into account the use of Tienam (ca. 10× cheaper than an imipenem monohydrate formula) and the dispensability of the extraction buffer (B-PER II), which is used to obtain bacterial extracts; and (iii) the validation of the test for the detection of OXA-type carbapenemases commonly identified in *Acinetobacter* spp.

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TABLE 1 Acquired-carbapenemase and noncarbapenemase-producing isolates tested

Group of acquired β-lactamase[a]	Variant	Species (no. of isolates)	MIC (μg/ml)[b]			Blue-Carba result	Reference or source
			IPM	MEM	ERT		
Carbapenemase producers							
Class A							
KPC	KPC-2	*Klebsiella pneumoniae* (2)	8–16	8–16	4–16	+	This study; Rafael Cantón
	KPC-3	*K. pneumoniae* (7)	0.5–>8	0.5–>8	1–>8	+	Rafael Cantón
Class B							
IMP	IMP-5	*Acinetobacter baumannii* (1)	>32	>32	NA	+	5
		Acinetobacter bereziniae (1)	>32	>32	NA	+	This study
NDM	NDM-1	*Escherichia coli* (4)	6–64	16–>32	>16	+	Laurent Poirel; Katie Hopkins and Neil Woodford
		K. pnemuoniae (3)	16–64	32–>32	>16	+	Katie Hopkins and Neil Woodford
VIM	VIM-1	*K. pneumoniae* (13)	0.5–>32	0.063–1	1–32	+	This study; Rafael Cantón
	VIM-2	*K. pneumoniae* (1)	1	0.25	0.25	+	This study
		Pseudomonas aeruginosa (12)	16–>32	1–>32	NA	+	This study; 6, 7
		Pseudomonas pseudoalcaligenes (1)	>32	>32	NA	+	8
	VIM-34	*K. pneumoniae* (2)	1	0.5	0.5	+	This study
SPM	SPM-1	*P. aeruginosa* (1)	>32	>32	NA	+	Laurent Poirel
Class D							
OXA	OXA-23	*A. baumannii* (14)	>32	>32	NA	+	Paolo Visca; 9, 10
	OXA-40	*A. baumannii* (17)	>32	>32	NA	+	9
		Acinetobacter haemolyticus (1)	>32	>32	NA	+	11
		Acinetobacter baylyi (2)[c]	>32	>32	NA	+	11
	OXA-48	*K. pneumoniae* (12)	0.5–>32	0.5–>32	1–>32	+	Rafael Cantón; Laurent Poirel
	OXA-58-like	*A. baumannii* (6)	0.5–>32	1–8	NA	+	Paolo Visca; 9
	OXA-72	*A. baumannii* (1)	>32	>32	NA	+	Ivana Goic-Barisic
Noncarbapenemase producers							
Class A							
CTX-M	CTX-M-1	*E. coli* (1)	≤1	≤1	≤0.25	−	This study
	CTX-M-2	*E. coli* (1)	≤1	≤1	≤0.25	−	This study
	CTX-M-9	*E. coli* (1)	≤1	≤1	≤0.25	−	This study
		Salmonalla enterica (1)	≤1	≤1	≤0.25	−	12
	CTX-M-14	*E. coli* (1)	≤1	≤1	≤0.25	−	This study
	CTX-M-15	*E. coli* (2)	≤1	≤1	≤0.25	−	13
		Klebsiella oxytoca (1)	≤1	≤1	≤0.25	−	This study
		K. pneumoniae (2)	≤1	≤1	≤0.25	−	This study
	CTX-M-15 + SHV-12	*E. coli* (1)	≤1	≤1	≤0.25	−	13
	CTX-M-15	*K. pneumoniae* (4)[d]	0.125–8	0.25–8	0.03–32	−	14
	CTX-M-32	*E. coli* (1)	≤1	≤1	≤0.25	−	This study
GES	GES-1	*K. pneumoniae* (1)	≤1	≤1	≤0.25	−	This study
SHV	SHV-2	*K. pneumoniae* (1)	≤1	≤1	≤0.25	−	This study
	SHV-12	*E. coli* (1)	≤1	≤1	≤0.25	−	This study
		K. pneumoniae (2)	≤1	≤1	≤0.25	−	This study
TEM	TEM-10	*K. pneumoniae* (1)	≤1	≤1	≤0.25	−	This study
		Serratia marcescens (1)	≤1	≤1	≤0.25	−	This study
	TEM-24	*K. pneumoniae* (1)	≤1	≤1	≤0.25	−	This study
	TEM-52	*K. pneumoniae* (1)	≤1	≤1	≤0.25	−	This study
	TEM-199	*Proteus mirabilis* (1)	≤1	≤1	≤0.25	−	This study
Class C							
AmpC	DHA-1	*K. pneumoniae* (1)[d]	0.125	0.016	>32	−	This study
Class A + C	SHV-12 + DHA-1	*K. pneumoniae* (1)	≤1	≤1	≤0.25	−	This study
Class D							
OXA	OXA-1/-30	*S. enterica* (1)	≤1	≤1	≤0.25	−	15
		A. baumannii (4)	0.5–>32	0.5–>32	NA	−	Paolo Visca; Jaroslav Hrabák; Harald Seifert
		E. coli (1)	0.06–0.25	0.008–0.06	0.004–0.015	−	Reference strain ATCC 25922
		E. coli (1)	2	0.5	4	−	This study
		Enterobacter aerogenes (1)	16	2	2	−	This study
		E. aerogenes (1)[d]	4	0.5	2	−	14
		Enterobacter cloacae (3)	0.5–2	0.25	2–32	−	This study
		K. pneumoniae (2)	0.125	0.125–4	0.5–8	−	This study
		K. pneumoniae (2)[d]	1	2	16	−	14
		P. aeruginosa (4)	>32	>32	NA	−	This study
		P. aeruginosa (1)[d]	0.25	0.25	NA	−	José Claudio Pérez-Díaz

[a] β-Lactamases conferring resistance to extended-spectrum β-lactams.
[b] IPM, imipenem; MEM, meropenem; ERT, ertapenem; NA, not applicable.
[c] Transformant strains.
[d] Isolates have deficiency in membrane permeability.

In conclusion, Blue-Carba is an easier and cheaper alternative to the Carba NP test, allowing the detection of carbapenemase activity directly from bacterial cultures of *Enterobacteriaceae*, *Pseudomonas*, and *Acinetobacter* species.

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