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LETTER TO THE EDITOR

Evaluation of the Blue-Carba Test for Rapid Detection of Carbapenemases in Gram-Negative Bacilli

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The Blue-Carba test (BCT) is a biochemical test for rapid (<2 h) detection of carbapenemase production in Gram-negative bacilli directly from bacterial culture (1). It is based on the *in vitro* hydrolysis of imipenem by bacterial colonies (direct inoculation without prior lysis), which is detected by changes in pH values revealed by the indicator bromothymol blue (blue to green/yellow or green to yellow). It was reported to be 100% sensitive and specific for *Enterobacteriaceae*, *Pseudomonas* spp., and *Acinetobacter* spp. harboring carbapenemases (1). We evaluated a simplified protocol of the BCT against various Gram-negative species, including combinations of bacterial species/resistance mechanisms that were not previously evaluated.

A total of 300 clinical isolates were included (188 carrying known carbapenemase-encoding genes and 112 without carbapenemase production) (Table 1). The isolates, belonging to the collection of the National and Regional Reference Lab, correspond to submissions from very diverse locations in Latin America (21 countries, 1,226 labs surveyed from 2010 to 2014) (2) and thus should represent minimal clonal and enzyme bias. PCR analysis was considered the gold standard for β-lactamases characterization (for the primer pairs used, see Table S1 in the supplemental material) (3). Outer membrane protein analysis was performed by SDS-PAGE (4, 5). Efflux pump inhibitors were used for phenotypic screening of efflux pump activity (6). Chromosomally mediated cephalosporinase hyperproduction was evaluated by spectrophotometric analyses as described previously (3). Identification to the species level was confirmed by matrix-assisted laser desorption ionization–time of flight mass spectrometry (Bruker, Germany). Imipenem MICs (agar dilution) and the modified Hodge test (MHT) were performed and interpreted by using the Clinical and Laboratory Standards Institute guidelines (6).

The BCT was performed by using a modified protocol as follows. Strains were grown on Mueller-Hinton agar, Trypticase soy agar, and Columbia agar with 5% sheep blood plates. Subsequently, a 5-μl loop of a pure bacterial culture from each type of agar was resuspended in the test mixture, which consisted of an aqueous solution of bromothymol blue at 0.04% (Sigma) and 0.1 mmol/liter $ZnSO_4$ (Sigma) with (reaction tube) or without (control tube) supplementation with 6 mg of Tienam (MSD) (equivalent to 3 mg of imipenem) and adjusted to a final pH of 7.0. Unsupplemented mixture solution was stored at 4°C (pH adjustments were needed every 4 to 6 weeks). The test solution was supplemented with Tienam immediately before being used. Strains were tested in duplicate. Two independent readers interpreted the results (interpretation discrepancies were recorded as equivocal results).

The results of BCT according to our modified protocol were 100% coincident on all three agar types used for bacterial subcultures. The BCT detected all class A carbapenemases (Table 1), including those of two *Klebsiella pneumoniae* KPC-2 producers with imipenem MICs of 0.12 μg/ml (M15222) and 0.03 μg/ml (M13403) (7) and enzymes with slow imipenem hydrolysis, such as the GES-type ($n = 3$) or Sme-type ($n = 3$) enzymes. All metallo-beta-lactamases (MBLs) tested positive (Table 1), including those from three strains with an imipenem MIC of 0.25 μg/ml. The BCT detected all *Acinetobacter* species isolates with acquired OXA carbapenemases but only 9 of 14 OXA-48-like enzyme-producing *Enterobacteriaceae* isolates (Table 1). Misdetections occurred among all of those OXA-48-like enzyme-producing *Enterobacteriaceae* isolates with MICs of <1.0 μg/ml; one OXA-48 producer and one OXA-247 producer (MIC, 0.5 μg/ml), both with equivocal results, and three OXA-163 producers (MICs, 0.12 to 0.5 μg/ml) were negative. Therefore, the overall sensitivity and negative predictive value of the BCT were 97 and 96%, respectively. All carbapenemase nonproducers were negative by the BCT (Table 1), which confirmed the high specificity and positive predictive value (100%) of the method described previously (1).

The BCT is an accurate and inexpensive way to unequivocally identify class A and B carbapenemases as described previously (1). Unlike other similar tests, such as the Carba NP (6, 8), the BCT detected KPC/MBL production in isolates with extremely low imipenem MICs. However, in our study, the BCT gave suboptimal results for the identification of OXA-48-like enzyme producers, with the largest amount of misdetections among the OXA-163 producers. The Carba NP has also shown limitations in the detection of OXA-48-like enzyme-producing *Enterobacteriaceae*, with reported sensitivities of 11 to 100% (6, 8–10). The results observed in this work could be linked to the low hydrolytic profile of OXA-163 producers, although there is still an open debate on its role as a carbapenemase. Whereas *in vitro* kinetic assays showed weak carbapenemase activity (11), *in vivo* data have suggested that OXA-163 *per se* could cause carbapenem treatment failure or favor the intrapatient selection of new OXA variants (12, 13).

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TABLE 1 Isolates tested,[i] imipenem susceptibility, and test results

Group and β-lactamase (no. of isolates)	Bacterial species included (no. of isolates)	% of isolates		No. of isolates BCT positive/no. (%) of isolates tested[a]
		Imipenem nonsusceptible	MHT positive	
Class A carbapenemase producers				
KPC-2 (51)	*K. pneumoniae* (34), *E. coli* (5), *E. cloacae* (4), *P. aeruginosa* (3), *C. freundii* (2), *C. braakii* (1), *K. oxytoca* (1), *L. adecarboxylata* (1)	88	100	51/51 (100)
KPC-3 (1)	*K. pneumoniae* (1)	100	100	1/1 (100)
Sme-1b (3)	*S. marcescens* (3)	100	0	3/3 (100)
GES-3 (2)	*E. cloacae* (1), *K. pneumoniae* (1)	100	100	2/2 (100)
GES-5 (1)	*P. aeruginosa* (1)	100	100	1/1 (100)
IMI-1/NMC-A (2)	*E. cloacae* (2)	100	100	2/2 (100)
All (60)		90	95	60/60 (100)
Class B carbapenemase producers				
NDM-1 (14)	*K. pneumoniae* (4), *E. coli* (3), *P. rettgeri* (3), *A. pittii* (2), *A. baumannii* (1), *P. mirabilis* (1)	100	21	14/14 (100)
IMP-1 (9)	*A. ursingii* (4), *A. lwoffii* (2), *A. junii* (2), *A. johnsonii* (1)	89	100	9/9 (100)
IMP-8 (8)	*E. cloacae* (4), *E. coli* (2), *C. freundii* (1), *K. oxytoca* (1)	88	100	8/8 (100)
IMP-13 (5)	*P. aeruginosa* (5)	60	40	5/5 (100)
IMP-16 (4)	*P. aeruginosa* (4)	50	50	4/4 (100)
IMP-18 (1)	*P. aeruginosa* (1)	100	100	1/1 (100)
VIM-2 (26)	*P. aeruginosa* (7), *P. putida* (7), *P. monteilii* (4), *E. cloacae* (1), *K. pneumoniae* (1), *P. chlororaphis* (1), *P. fulva* (1), *P. fluorescens*, (1), *P. oleovorans* (1), *P. rettgeri* (1), *P. stutzeri* (1)	92	92	26/26 (100)
VIM-11 (3)	*P. aeruginosa* (2), *E. cloacae* (1)	67	100	3/3 (100)
VIM-1 (1)	*E. coli* (1)	100	100	1/1 (100)
SPM-1 (5)	*P. aeruginosa* (5)	100	100	5/5 (100)
All (76)		89	89	76/76 (100)
Class D carbapenemase producers				
OXA-48 (5)	*E. coli* (3), *K. oxytoca* (1), *K. pneumoniae* (1)	60	100	4/5 (80)
OXA-163 (5)	*K. pneumoniae* (3), *E. cloacae* (1), *K. georgiana* (1)	20	100	2/5 (40)
OXA-247 (2)	*E. coli* (1), *K. pneumoniae* (1)	50	100	1/2 (50)
OXA-181 (1)	*K. pneumoniae* (1)	100	100	1/1 (100)
OXA-438 (1)[b]	*E. coli* (1)	100	100	1/1 (100)
All *Enterobacteriaceae* OXA producers (14)		46	100	9/14 (64)
OXA-58 (20)	*A. baumannii* (7), *A. lwoffii* (6), *A. junii* (5), *A. pittii* (1), *A. ursingii* (1)	90	90	20/20 (100)
OXA-23 (14)	*A. baumannii* (9), *A. lwoffii* (3), *A. junii* (2)	100	100	14/14 (100)
OXA-23 + OXA-58 (3)	*A. baumannii* (3)	100	100	3/3 (100)
OXA-143 (1)	*A. baumannii* (1)	100	100	1/1 (100)
All *Acinetobacter* OXA producers (38)		94	94	38/38 (100)
Carbapenemase nonproducers				
ESBLs + porin loss (38)[c]	*K. pneumoniae* (30), *E. coli* (4), *E. cloacae* (2), *S. marcescens* (2)	87	45	0/38 (0)
ESBLs (11)[d]	*A. baumannii* (4), *P. aeruginosa* (4), *S. flexneri* (2), *P. putida* (1)	0	0	0/11 (0)
AmpCs + porin loss (16)[e]	*E. cloacae* (10), *E. aerogenes* (3), *C. freundii* (2), *E. coli* (1)	88	81	0/16 (0)
AmpCs (12)[f]	*P. aeruginosa* (9), *E. coli* (1), *K. pneumoniae* (1), *P. mirabilis* (1)	0	0	0/12 (0)
Narrow spectrum (5)[g]	*A. baumannii* (2), *P. aeruginosa* (2), *E. coli* (1)	0	0	0/5 (0)
None (30)[h]	*P. aeruginosa* (29), *E. coli* (1)	77	0	0/30 (0)
All nonproducers (112)		62	27	0/112 (0)

[a] A color change from blue or green (control tube) to green/yellow or yellow (reaction tube), respectively, was interpreted as a positive result; when both tubes (control and reaction) remained either blue or green, the test was interpreted as negative. No invalid results were obtained (a yellow control tube).
[b] Novel OXA-48-like variant (GenBank accession no. KP410734).
[c] CTX-M-2, $n = 31$; CTX-M-15, $n = 5$; SHV-18, $n = 1$; SHV-5, $n = 1$.
[d] GES-1, $n = 5$; CTX-M-2, $n = 2$; PER-2, $n = 2$; VEB-1, $n = 2$.
[e] Hyperproduction of chromosomally mediated cephalosporinase, $n = 15$; CMY-2, $n = 1$.
[f] Hyperproduction of chromosomally mediated cephalosporinase, $n = 9$; CMY-2, $n = 2$; DHA-1, $n = 1$.
[g] OXA-1, $n = 2$; OXA-51 (without upstream IS*AbaI*), $n = 2$; TEM-1, $n = 1$.
[h] Nonenzymatically carbapenem-resistant isolates and quality control strains, i.e., *Pseudomonas aeruginosa* carbapenem resistant because of efflux overproduction ($n = 5$), porin loss ($n = 3$), or dual mechanisms ($n = 20$) and quality control strains *P. aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922.
[i] Tested were 155 *Enterobacteriaceae*, 89 *Pseudomonas*, and 56 *Acinetobacter* isolates.

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In conclusion, for labs concerned with the widely disseminated class A KPC producers, class D MBL (e.g., NDM) producers, and class D OXA carbapenemase-producing *Acinetobacter*, the BCT could be an accurate and cost-effective way to rapidly identify potential carrier isolates. For some mechanisms, such as OXA-48-like enzyme-producing *Enterobacteriaceae*, the total number of isolates studied was low; however, preliminary results suggest that in scenarios with an increasing prevalence of this mechanism, a negative result with the BCT could require additional tests such as the MHT (which showed 100% sensitivity in this study), preferably in combination with temocillin or piperacillin-tazobactam susceptibility results (14).

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