

Topic

18. Diagnostics, other than Molecular: Diagnostic/laboratory methods (other than molecular)

Abstract title

Rapid Detection of Carbapenemase-Producing Gram Negative Bacilli from Blood Cultures Using the Blue-Carba Test (BCT)

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The BCT is a biochemical test for rapid detection (<2 h) of carbapenemase production on gram-negative bacilli. It is based on *in vitro* hydrolysis of imipenem by bacterial colonies (direct inoculation without prior lyses), which is detected by changes in pH values using the indicator bromothymol blue. The BCT has not been evaluated using directly clinical samples.

Objective: to determine the ability of the BCT to detect carbapenemase producers directly from positive blood cultures.

Methods: A panel of 60 clinical strains (35 *Enterobacteriaceae*, 15 *Pseudomonas*, 10 *Acinetobacter*), previously characterized by PCR/DNA sequencing, was used for spiking blood cultures. Blood cultures were made from 10 mL sterile total human blood inoculated with 100 CFU/mL of each strain. Bactec (BD) blood culture bottles were incubated until a positivity vial was detected by the system. About 8 mL was retrieved from each positive vial and placed into a sterile tube containing a separator gel (Vacutainer). Tubes were centrifuged at 10000 rpm for 15 min. The bacterial pellet was visible at the outer surface of the separator gel. The supernatant was removed and the bacterial pellet was washed with 1 mL of sterile water. This mixture was transferred to an eppendorf and centrifuged at 10000 rpm for 2 min. The pellet was tested for BCT as described by Pires J. *et al.* and monitored throughout 2 h.

Results: Table. Sensitivity and specificity of the BCT from a positive blood culture was 95% and 100%, respectively.

Group	Resistant mechanism (No.)	BCT results from blood cultures*	BCT results from solid media*	Time for BCT carbapenemase detection from the blood culture vial (range, in minutes)#
Class A Carbapenemase	KPC-2 (10) KPC-3 (1) Sme-1b (1) GES-3 (1) GES-5 (1) IMI (1)	14/15	15/15	30-45
Class B Carbapenemase	NDM-1 (4)	15/15	15/15	75-120

	VIM-2 (3) IMP-8 (2) IMP-1 (1) IMP-13 (1) IMP-16 (1) IMP-18 (1) VIM-11 (1) SPM-1 (1)			
Class D Carbapenemase	OXA-48 (5) OXA-181 (1) OXA-247 (1) OXA-23 (1) OXA-58 (1) OXA-143 (1)	9/10	9/10	120-150
Carbapenemase nonproducers	ESBLs (5) AmpCs (5) Efflux/porins (5) Others (5)	0/20	0/20	NA

*No. of isolates with a positive BCT /No. of isolates tested. #Time 0: blood culture vial signaled as positive by the system. NA: not applicable. False negative results: one *P. aeruginosa* GES-5 isolate and one *Escherichia coli* OXA-48 isolate.

Conclusions: for labs concerned with the widely disseminated KPC and NDM producers, the BCT could be an accurate and cost-effective method to rapidly identify these carbapenemases directly from blood cultures. A negative result with the BCT could require additional testing. Using the protocol developed here, the total time for identification of a carbapenemase producer responsible for a bacteraemia can be reduced from 24–48 h to 30-150 min.

Keywords

carbapenemase
KPC
OXA-48
NDM
blue-carba
rapid test