

ICAAC/ICC 2015

September 17–21, 2015 | San Diego, CA
San Diego Convention Center

Comparison of the In-house Carba NP (CNP) and the Blue-Carba Test (BCT) for the Detection of Carbapenemase-producing Gram Negative Bacilli (GNB)

Keywords: Carbapenemase; CARBA NP; BLUE CARBA

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Financial Disclosures: F. Pasteran, None..

O. Veliz, None..

P. Ceriana, None..

E. Albornoz, None..

M. Rapoport, None..

A. Corso, None.

Biography: No Abstract Present.

Abstract: Background : the CNP and the BCT are biochemical tests for rapid detection (<2 h) of carbapenemase production on GNB based on in vitro hydrolysis of imipenem by bacterial colonies, which is detected by changes in pH values using the indicator phenol red or bromothymol blue, respectively. The CNP, but not the BCT, requires a prior step of protein extraction with a lysis buffer. Up to date, there is no study comparing the performance of both in-house tests. Aim: to compare the performance of the CNP and the BCT in detecting the presence of carbapenemase-producing GNB. Methods : A panel of 140 clinical strains from diverse locations in Latin America (21 countries) was included: 90 Enterobacteriaceae (16 species), 44 Pseudomonas spp. (9 species) and 6 Acinetobacter spp. PCR/DNA sequencing were considered the gold standard for β -lactamases characterization. The BCT was performed according to a modified protocol by Pasteran F. et al. Two protocols of the CNP were tested in parallel: the latest version proposed by Poirel L. et al (CNP-Original) and the CLSI version (CNP-CLSI), recently issued in M100-S25. Tests were performed in duplicates. Results : Table. False negative results (n): a) CNP-Original: KPC-2 (1), GES-5 (1), NDM-1 (3), VIM-2 (1), OXA-48 (2), OXA-247 (1); b) CNP-CLSI: KPC-2 (3), GES-5 (1), NDM-1 (3), VIM-2 (2), VIM-11 (1), OXA-48 (2), OXA-247 (1), OXA-438 (1); c) BCT: OXA-247 (1), OXA-438 (1). Conclusions : The BCT resulted in a superior performance to detect those carbapenemases with low hydrolytic activity (GES) or those strains with extremely low imipenem MICs (carbapenem-susceptible VIM- or KPC-producers). Both CNP versions, but not the BCT, miss detected several Proteae isolates harboring NDM-1, in spite of the presence of 0.1 mmol/liter ZnSO₄ in the working mix. All tests missed some Class D carbapenemase. All carbapenemase nonproducers were negative by all methods, which confirmed the high specificity and positive predictive values (100%) of these tests for their use in the routine labs. Tests performance: No. of isolates with a positive test/No. of isolates tested (%). Group (No. of isolates)

Beta-lactamases (No. of isolates)	CNP-Original	CNP-CLSI	BCT
Class A carbapenemases (n=30)	KPC-2 (22), Sme (3), NMC-A (2), KPC-3 (1), GES-3 (1), GES-5 (1)	28/30 (93%)	26/30 (87%)
		30/30 (100%)	

Class B

carbapenesases (n=30) NDM-1 (11), VIM-2 (6), IMP-1 (3), IMP-8 (3), IMP-13 (1), IMP-16 (2) IMP-18 (1), SPM (1), VIM-1 (1), VIM-11 (1) 26/30 (87%) 24/30 (80%) 30/30 (100%)

Class D

carbapenesases (n=10) OXA-48 (5), OXA-247 (2), OXA-438 (2), OXA-181 (1) 7/10 (70%)
6/10 (60%) 8/10 (80%)

All carbapenemase producers (n=70) not applicable 61/70 (87%) 56/70 (80%) 68/70 (97%)

Carbapenemase

nonproducers (n=70) ESBLs (20), AmpC (15), efflux + porins loss (30), wild-type (5) 0/70 (0%)
0/70 (0%) 0/70 (0%)