Published in "Antimicrobial Agents and Chemotherapy
doi: 10.1128/AAC.01825-13, 2014"
which should be cited to refer to this work.

Further Proofs of Concept for the Carba NP Test

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Recently, Tijet et al. evaluated the Carba NP test (1), which we developed in order to identify carbapenemase producers. Our first study included 316 carbapenemase- and non-carbapenemase-producing *Enterobacteriaceae* and *Pseudomonas aeruginosa* isolates (2, 3). Tijet et al. confirmed that this biochemical test has an excellent specificity of 100%, together with an excellent positive predictive value (PPV). However, they reported a lower sensitivity than we did (72% versus 100%), mainly due to false-negative results obtained with OXA-48 producers, GES-5-producing *P. aeruginosa* isolates, and a few NDM-1-producing *Providencia* sp. isolates. We believe that the lack of detection of the NDM-producing isolates reported in the Tijet et al. study may be related to the growth conditions of the strains. We have observed that the origin (manufacturer) of the Mueller-Hinton (MH) plates is crucial to obtain a medium that permits a high level of expression of metallo-β-lactamases (MBL). In our experience, the best results have been obtained with the Becton, Dickinson MH plates, which likely contain the optimal zinc concentration for MBL expression (4). Unfortunately, the manufacturer of the MH plates of the Tijet et al. study was not indicated. In addition, we do not understand the high rate of false-negative results obtained with OXA-48-producing isolates. Although the class D β-lactamase OXA-48 is a less potent carbapenemase than are class A carbapenemases, in our experience, a positive result of the Carba NP test has always been obtained after 30 min to 1 h for any OXA-48 producer. Similar positive results have been obtained worldwide where the Carba NP test is now used (personal data). The lack of detection of GES-5-producing *P. aeruginosa* is in accordance with our published results (2).

We have now further improved the Carba NP test by using 1.5-ml Eppendorf tubes instead of 96-well microplates and by using a smaller amount of bacteria (1/4 to 1/3 of a 10 μl calibrated inoculation loop resuspended in 100 μl of Tris-HCl buffer) (4). Those modifications simplify the lysis step of the bacterial extract, eliminating the need of a centrifugation step. This updated protocol is interesting, in particular, when hyper-mucoid strains (mostly *Klebsiella pneumoniae*) are studied. In 2012, the Carba NP test was used on 1,485 non-carbapenem-susceptible enterobacterial isolates that had been sent to the Associated National Reference Center for Antibiotic Resistance (Kremlin-Bicêtre, France) for screening for potential carbapenemase activity. Among those 1,485 isolates, 341 were found to produce a carbapenemase that corresponded to OXA-48-like ($n = 257$), *Klebsiella pneumoniae* carbapenemase (KPC) ($n = 39$), NDM ($n = 27$), VIM ($n = 16$), or IMI ($n = 2$) enzymes. The Carba NP test detected all carbapenemase producers regardless of the type of the carbapenemase and of their mucoid phenotype (5) (overall, there was 100% sensitivity and 100% specificity for carbapenemase producers, including OXA-48 producers, in a comparison with PCR results). Finally, extensive usage of the Carba NP test either by ourselves or by other teams worldwide is validating its usefulness for detecting on a daily basis any type of carbapenemase produced by *Enterobacteriaceae* (6–8).

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For the author reply, see doi:10.1128/AAC.02285-13.