Reply to "Further Proofs of Concept for the Carba NP Test"

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We acknowledge the letter by Dortet et al. (1) in response to our evaluation of the Carba NP test (2), performed in two independent Canadian laboratories (the National Microbiology Laboratory, the national reference lab, and Public Health Ontario, a provincial reference lab).

In their letter (1), the authors state that the manufacturer of the Mueller-Hinton agar (MHA) medium plays a crucial role in the detection of metallo-β-lactamase (MBL) activity. In our study, we used two different sources of MHA: Acumedia (Neogen Corp., Lansing, MI, USA) and Oxoid (Thermo Scientific, Nepean, ON, Canada). MHA was used not only for growing the isolates included in our study but also for susceptibility profile determinations by the agar dilution method and Etest. Most of the MBL producers studied (49 out of 52, including VIM, IMP, and NDM producers) were detected in both laboratories. We strongly believe that two of the false-negative results obtained were a consequence of incomplete lysis: one NDM-1-producing *Providencia rettgeri* isolate displaying mucoid colonies and one IMP-27-producing *Proteus mirabilis* isolate with swarming motility. We transferred the NDM-1 plasmid from *P. rettgeri* to *Escherichia coli* J53 by conjugation and cloned and expressed IMP-27 in *E. coli* TOP10, and in both cases, we obtained quick positive results using the same MHA that was used for the clinical isolates. The MHA source might explain the false-negative result for one NDM-1-producing *Providencia stuartii* isolate, which expressed high-level carbapenem resistance. However, the previous results suggest that the MHA does not play a major role in MBL activity detection.

In our evaluation, we obtained lower sensitivity (72.5%, using the published protocols, which increased to 80% with a more concentrated bacterial extract) (2) than the 100% (94% in the case of *Pseudomonas aeruginosa*) previously reported (3, 4). Our results were due mainly to the inability of the Carba NP test to detect OXA-48-like producers. In our experience (2), the sensitivity of the test for OXA-48-like producers ($n = 39$) was 55.7% (31 false negatives) using the original protocol and 70.9% (16 false negatives) using our modifications. We confirmed these results by exchanging blind samples between our laboratories, including 24 OXA-48-like-producing strains with false-negative results (2). Furthermore, we tested six additional OXA-163- and OXA-247-producing enterobacteria (β-lactamases with weak carbapenemase activity detected by the modified Hodge test) using our modified protocol, and all of them were also falsely negative by the Carba NP test (personal data). The last results expand the OXA-48-like alleles not detected by the test. Finally, the studies referenced by Dortet et al. (5–7) focus mainly on KPC and NDM producers, isolates that we were easily able to detect in our study (only in the study reported in reference 7 was one OXA-48-like producer included).

We agree with Dortet et al. that the Carba NP test is rapid, inexpensive, and easy to perform, but we want to highlight the limitations related to its sensitivity, particularly against β-lactamases with weak carbapenemase activity (e.g., OXA-48-like, SME-1, or GES-5) or expressed in mucoid cells. We encourage other investigators to conduct further studies using the Carba NP test with carbapenemases displaying weak activity against the carbapenems or displaying a mucoid phenotype. In addition, we hope that the modifications that the authors will soon publish can solve the detection problems that we found in our evaluation.

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