We compared the diagnostic accuracy of the Carba NP test with that of a straightforward matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) method for detecting carbapenemase-producing Enterobacteriaceae (CPE). Using PCR as the reference method, both tests demonstrated a sensitivity of 87% and a specificity of 100%. MALDI-TOF MS offers a potential alternative for the rapid detection of CPE in the clinical laboratory setting.

The global spread of carbapenemase-producing Enterobacteriaceae (CPE) is a major threat to public health (1). The efficient laboratory detection of these agents requires a rapid phenotypic test that provides accurate results while awaiting confirmation by PCR. In this study, a method was developed for detecting CPE using an imipenem hydrolysis assay and matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS), using the Vitek MS research use only (RUO) system (bioMérieux, Marcy l’Etoile, France). Using PCR as a reference method, the diagnostic accuracy of MALDI-TOF MS was compared with that of the Carba NP test (CNP).

The investigation was conducted at Melbourne Pathology and Swinburne University of Technology, Melbourne, Australia, between October 2013 and April 2014. A panel of 105 genetically characterized Enterobacteriaceae was assembled from nine Australian laboratories. All strains were identified by MALDI-TOF MS (Vitek MS [bioMérieux]) using the Vitek MS Database version 2.0 (bioMérieux) and had MICs for meropenem determined by Etest (bioMérieux). Molecular characterization had been performed by various methods (2–11); at a minimum, each strain had been subjected to PCR, including targets for \( \text{bla}_{KPC} \), \( \text{bla}_{NDM} \), \( \text{bla}_{IMP} \), \( \text{bla}_{VIM} \), and \( \text{bla}_{OXA-48} \) (2–11). The entire collection was assembled prior to the study, and all of the strains obtained were included.

The CNP was performed according to a previously published method (12) on strains cultured on Columbia blood agar (CBA) for 18 to 24 h. The results were read by a microbiologist who was blinded to all other results in the study.

The MALDI-TOF MS method was adapted from a previous method (12) and included modifications for the detection of CPE. The spectra generated by MALDI-TOF MS were analyzed using the Vitek MS software (bioMérieux) and compared with the Carba NP test results.

FIG 1 Spectra in MALDI-TOF MS method for CPE detection. Top, imipenem; middle, imipenem with \( K. \) pneumoniae harboring \( \text{bla}_{IMP} \); bottom, imipenem with PCR-negative \( K. \) pneumoniae. a, area under the curve.
The results for all the strains are presented in Table 1. Overall, the gene targets tested. The collection included 11 different species. bapenemase-producing strains (20 KPC, 9 NDM, 9 IMP, 2 VIM,ing results to the value of 5% that was chosen prospectively. curve analysis revealed no alternative cutoff value with superior comparisons; data not shown). No peaks were seen in the range of much simpler single-peak method obtained with Launchpad for ing SARAMIS Premium yielded identical results to those of the confidence interval [CI], 74.3 to 93.9%) and 100% (95% CI, 93.9 to 100%), respectively. After resolution of the 12 discrepant strains, two strains that were received as PCR positive were found to be negative by the reference PCR method (11). These were analyzed as non-CPE, in accordance with the testing protocol. In total, there were 46 carbapenemase-producing strains (20 KPC, 9 NDM, 9 IMP, 2 VIM, and 6 OXA producers) and 59 strains that were negative for the gene targets tested. The collection included 11 different species. The results for all the strains are presented in Table 1. Overall, the sensitivity and specificity of both tests were found to be 87% (95% confidence interval [CI], 74.3 to 93.9%) and 100% (95% CI, 93.9 to 100%), respectively.

For MALDI-TOF MS, more complex spectral comparisons us- ing SARAMIS Premium yielded identical results to those of the much simpler single-peak method obtained with Launchpad for all strains tested and by all methods used (Cohen’s kappa, 1 for all comparisons; data not shown). No peaks were seen in the range of 200 Da to 700 Da that were consistently present in any group of CPE types and consistently absent among the non-CPE. The ROC curve analysis revealed no alternative cutoff value with superior results to the value of 5% that was chosen prospectively.

The CNP is a rapid easy-to-use phenotypic test of carbapenemase production. It has consistently been found to be 100% specific for detecting carbapenemases in Enterobacteriaceae (12, 14); sensitivity estimates, however, range from 72.5 to 100% (12, 14). Previously, the main limitation found has been a reduced ability to detect OXA-48-producing strains (14, 15). Unfortunately, the present investigation provides further evidence of this shortcoming.

MALDI-TOF MS performed well overall and detected 97% of the meropenem-resistant strains by CLSI criteria (16). Additionally, it correctly identified all four OXA-48 producers. Given the previously published success of MALDI-TOF MS for detecting OXA production (13), this aspect of its performance warrants further investigation. While the reason MALDI-TOF MS failed to
identify carbapenemase production by the three strains of *Proteus* spp. is not entirely clear, it may be related to improved enzyme detection after cell lysis, which forms a part of the CNP but not the MALDI-TOF MS method.

The main limitation of this study was the relatively small sample size. In particular, it was possible to incorporate only two VIM-producing strains. Additionally, care must be taken when generalizing these results to settings with differing prevalences of the various carbapenemase types described.

For clinical laboratories using Vitek MS that have access to the appropriate software, the method described can be relatively easily implemented and offers an alternative to the CNP. The main advantages of MALDI-TOF MS are the objective endpoint and the possibility of better detection of OXA-48 producers. The main disadvantage of MALDI-TOF MS is the extra time required for incubation (4 h versus 2 h), processing of target plates, and interpretation of the results (total hands-on time of several minutes). This could be minimized with efficient incorporation into the laboratory workflow and greater automation of the testing procedure by the manufacturer.

In conclusion, both the CNP and MALDI-TOF MS performed well. MALDI-TOF MS offers a potential alternative for the rapid detection of CPE in the clinical laboratory setting.

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