Evaluation of the carbapenem-inactivation method for the detection of carbapenemase-producing Enterobacteriaceae

L. Gauthier, R.A. Bonnin, L. Dortet, T. Naas
EA7361, Université Paris-Sud, Université Paris-Saclay, LabEx Lermi, Bacteriology-Hygiene unit, APHP, Hôpital Bicêtre, Associated French NRC, Le Kremlin-Bicêtre, France

INTRODUCTION

The emergence and dissemination of carbapenemase producing enterobacteria (CPE) has become a major challenge for public health. This implies an urgent need for accurate and fast diagnostic tests to identify these multi-drugs resistant bacteria. The CIM (Carbapenem Inactivation Method) test, which is based on in vitro inactivation of the meropenem contained in a 10 µg charged disk by carbapenemase-producing strains, was reported to be 100% sensitive and specific for CPE detection (1). In addition, it does not require trained personnel or specific equipment, and is cost-effective.

OBJECTIVES

We evaluated the CIM test to detect CPEs from cultured colonies. We also tested etapenem and imipenem disks in order to evaluate whether sensitivity and specificity may be obtained changing the carbapenem substrate.

METHODS

A total of 256 enterobacterial isolates were used to evaluate the performance of the CIM test in comparison with a home-made technique of the Carba NP test. Antimicrobial susceptibility was determined by disk diffusion method and minimal inhibitory concentration (MIC) using the E-test technique.

The updated version of the Carba NP test was used and interpreted as previously described (2).

The CIM test was performed using meropenem disk as described (1) with overnight culture (Fig. 1). Absence / presence of an inhibition zone was used as judgment criterion (Fig. 2).

Identification of the carbapenemase genes was made using an in-house PCR sequencing.

RESULTS

Evaluation of the best substrate for the CIM test Based on the results of the retrospective study, sensitivity and specificity were 92.1% and 100% using meropenem disk, 81.1% and 100% using etapenem disk, and 100% and 65.6% using imipenem disk. Using imipenem disk, false-positive results and microcolonies in the inhibitory diameter were especially observed with Amp-C over-producers. At the opposite, false-negative results were observed using etapenem disks, however with a decreased inhibitory diameter compared to non carbapenemase producers.

Evaluation of the CIM test using meropenem disks Among the 256 isolates tested prospectively and retrospectively, sensitivity was 96.3% for the CIM test versus 96.9% for the Carba NP test, and the specificity was 98.9% for the CIM test versus 100% for the Carba NP test. False-negative results were obtained with the CIM on NDM-1 producers (2 K. pneumoniae, 1 P. stuartii and 1 P. rettgeri), which were positive with the Carba NP test, and on 2 VIM producers (1 P. mirabilis VIM-1, Carba NP test positive, 1 E. cloacae VIM-2, Carba NP test negative).

However, the CIM was positive for 48-1 like producing isolates which gave uncertain or false-negative results with the Carba NP test (1 K. pneumoniae OXA-181, 1 E. coli OXA-181 and 4 E. coli OXA-244). All non-carbapenemase producers were negative using the Carba NP test, only one strain gave a false-positive result with the CIM (a C. freundii overexpressing its cephalosporinase).

CONCLUSIONS

We have confirmed that the CIM test might be a cheap and useful tool for the reliable confirmation of carbapenemase-producing Enterobacteriaceae, especially in clinical microbiological laboratories with limited resources, no trained personnel, and no specialized equipment to detect carbapenemase activity. However, if it detects some OXA-48 like producing isolates which gave false-negative results with the Carba NP test, false-negative results were obtained with NDM-1 producing strains.

The main disadvantage of the CIM test resides in the need for two hours incubation and a subsequent overnight culture. Finally, molecular confirmatory tests are necessary to identify the carbapenemase-producing genes, and to validate the CIM-positive results.

REFERENCES