Evaluation of Etest® strips for detection of KPC and metallo-carbapenemases in Enterobacteriaceae

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The performance of Etest KPC and MBL strips (bioMérieux) was evaluated as compared to other phenotypic tests for detecting carbapenemases of the KPC-type and metallo-β-lactamases, respectively, on 133 well-characterized enterobacterial isolates. KPC and meropenem-containing MP/MPI Etest had high sensitivity (≥92%) and specificity (≥97%).

The rapid spread of carbapenemase-producing Enterobacteriaceae (CPE) is a worldwide major challenge for the treatment and control of many nosocomial and now community-acquired infections (Nordmann et al., 2011). These isolates produce different types of carbapenemases, the most frequent being Klebsiella pneumoniae carbapenemases (KPC; Ambler class A), metallo-β-lactamases (MBL; Ambler class B, metallo-enzymes), and oxacillinases (OXA-48-type; Ambler class D) (Ambler et al., 1991). KPC-type enzymes hydrolyze all β-lactams including carbapenems, and are inhibited by aminophenyl boronic acid (APBA) (Poirel et al., 2007). MBLs hydrolyze all β-lactams, except aztreonam and are inhibited by divalent cation chelators such as EDTA or dipicolinic acid (DPA).

MBLs and KPC detection by simple and reliable phenotypic tests is needed for infection control and prevention (Giske et al., 2011; Landman et al., 2005; Liao et al., 2011). Nevertheless, isolates expressing these enzymes may be reported as susceptible to carbapenems due to heterogeneous and variable levels of expression of β-lactam resistance. The modified Hodge test (MHT) is usually considered as the phenotypic reference method for confirmation of carbapenemase production despite the lack of discrimination between the three different classes of carbapenemases (KPC, MBL, and OXA) and difficulties in interpretation of the results. Commercial diagnostic tablets from Rosco (RDS) (Rosco Diagnostica Neo-Sensi tabs, Eurobio, Courtaboeuf, France) consist in meropenem disks supplemented with class A (APBA) or class B (DPA) β-lactamase inhibitors. In this study, a prototype of Etest® KPC (bioMérieux, La Balme-les-Grottes, France), containing meropenem and boronic acid, and two commercially available Etest® MBL strips (bioMérieux, La Balme-les-Grottes, France), IP/IPI, containing imipenem and EDTA and MP/MI, containing meropenem and EDTA, were compared to the MHT, zinc-supplemented-MHT (Girlich et al., 2012), the RDS, and the Carba NP test (rapid detection of any carbapenemase activity) (Nordmann et al., 2012) for detecting KPC or MBL-producing Enterobacteriaceae.

One-hundred thirty-three Enterobacteriaceae isolates, characterized at the molecular level, were used in this study. Forty-eight Enterobacteriaceae isolates produced KPC-type β-lactamases, and 54 produced MBLs of the VIM- (n = 19), IMP- (n = 18), and NDM-type (n = 17). Thirty-one non-carbapenemase producers (non CPE) were used as controls, including nineteen isolates with decreased susceptibility to ertapenem (Jacoby et al., 2004) and 12 carbapenem-susceptible isolates. Strains were as follows: K. pneumoniae (n = 75), Escherichia coli (n = 23), Enterobacter cloacae (n = 21), E. aerogenes (n = 1), Serratia marcescens (n = 6), Citrobacter freundii (n = 4), Proteus spp. (n = 2), and Salmonella typhimurium (n = 1).

Etest® MBL strips IP/IPI containing imipenem (4–256 μg/mL)/imipenem (1–64 μg/mL) + EDTA (constant level), and MP/MI containing meropenem (0.125–8 μg/mL)/meropenem (0.032–2 μg/mL) + EDTA (constant level), were used for detection of MBL-producer, whereas Etest® KPC strips MP/MPB containing meropenem (0.25–16 μg/mL)/meropenem (0.064–4 μg/mL) + a boronic acid derivative (constant level) were used for detection of KPC-producers.

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Etest assays were performed as recommended by the manufacturer (bioMérieux). MIC was read on each side of the strip. A >3-fold decrease of the carbapenem MIC in the presence of the inhibitor or the presence of a deformed ellipse on the IPI, MPI or MPB side of the strip was interpreted as a positive test for MBL and KPC, respectively. All other cases were considered as negative or non-determinable (ND), i.e., with MICs over or below the limit detection values of the strips (≥16/≤4 or ≤4/≤1). KPC Etest strips showed a high specificity of 91.7% and a sensitivity of 90.3% (Table 1). For detection of MBL producers, meropenem containing strips were more efficient than those containing imipenem, with a respective sensitivity of 94.4% and 81.5% (Table 1). Among the MBL producers, all NDM- and VIM-producers were detected with meropenem-containing Etest MBL, whereas IMP-producers were detected at 82% (Table 1). Noticeably, among MBL producers, all NDM- and VIM-producers were detected as MBL-producer, probably due to a non specific effect of DPA. The recently developed Carba NP test, based on the rapid detection of the hydrolysis of imipenem by a change in the pH value of the indicator (red to yellow/orange) was used as previously described (Nordmann et al., 2012). Sensitivity and specificity of the Carba NP test was 100% on the tested strains (Table 1).

The main advantage of the MBL and KPC Etests (and the RDS) over the MHT and the Carba NP test is the rapid discrimination between class A and class B carbapenemase producers. However, the Carba NP test II, which includes inhibition by tazobactam and EDTA, may be also used for detection of carbapenemase types (Dortet et al., 2012). One limitation of this study is that class A carbapenemase producers from other types than KPC have not been studied. Indeed, boronic acid contained in KPC Etest would be likely also a good inhibitor of both Ambler class A carbapenemases such as GES-types, NmcA, and Sme-1.

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**References**


