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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-Sensitabs, 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/MPI, 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/MPI 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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Table 1
Comparison of MHT, zinc-supplemented MHT (Zn-MHT), Etests MBL IP/IPI, MP/MPI, Etests MP/MPB, RDS kit, and the CarbaNP test for screening of carbapenemase producing Enterobacteriaceae.

	MHT positive	Zn-MHT positive	Etest KPC MP/MPB number of isolates			Etest MBL MP/MPI number of isolates			Etest MBL IP/IPI number of isolates			RDS	Carba NP test
			Positive	Negative	ND	Positive	Negative	ND	Positive	Negative	ND	Positive	Positive
MBL producers													
VIM (n = 19)	16	16	NA	NA	NA	19	0	0	15	1	4	19	19
NDM (n = 18)	13	17	NA	NA	NA	18	0	0	17	0	1	18	18
IMP (n = 17)	17	17	NA	NA	NA	14	1	2	12	0	5	13	17
KPC producers													
(n = 48)	48	48	44	2	2	NA	NA	NA	NA	NA	NA	46	48
Non-carbapenemase producers with reduced susceptibility to carbapenems													
(n = 19)	2	4	0	18	1	1	14	4	0	6	13	1	0
Ertapenem-susceptible isolates													
(n = 12)	1	1	0	12	0	0	5	7	0	1	11	0	0
SN (%)	91.8	95.1	91.7			94.4			81.5			94.1	100
SP (%)	90.3	83.9	100			97			100			96.7	100

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 were 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 sensitivity of 91.7% and a specificity 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, a great disparity in MIC values of imipenem was observed between single IP strips and double IP/IPI strips (data not shown). As recommended by the manufacturer, the MBL IP/IPI Etest should not be used for determining MIC values, but only for comparison of MIC values with and without EDTA (on each part of the strip). Indeed, among MBL producers, ten strains gave non-determinable results with MIC values of IP <4 μg/mL and IPI <1 μg/mL, and 44 strains gave a positive result, mostly because of the modification of the shape of inhibition ellipse in the presence of EDTA. The three formulations of Etest, showed a high specificity ranging from 97–100% (Table 1).

The Modified Hodge Test (MHT) was performed on MHA as recommended (Clinical & Laboratory Standards Institute, CLSI, 2012), and zinc-modified-MHT (MHT-Zn) with a supplementation with zinc sulfate (100 μg/mL) (Girlich et al., 2012). Sensitivity of the MHT was 91.8% for detection of carbapenemase producers of both Ambler classes, including a sensitivity of 100 % for detection of KPC producers, and 85% for detection of MBL producers. Addition of zinc sulfate increased the sensitivity from 91.8–95.1%, improving detection of MBL producers in particularly of NDM-1 producers from 85–92.6% (Table 1). A Concomitant decreased specificity was observed, from 90.3–83.9% (Table 1), accordingly to results of a previous study (Girlich et al., 2012).

The RDS KPC and MBL confirm kit consisting in four tablets containing meropenem, meropenem + boronic acid, meropenem + dipicolinic acid, and meropenem + cloxacillin was used following the instructions of the manufacturer and results were interpreted as recommended (Rosco Diagnostica). Sensitivity of the RDS was 94.1%, comprising a sensitivity of 96% for detection of class A carbapenemase producers and 92% for detection of class B carbapenemase producers (Table 1). Noticeably, among MBL producers, all NDM- and VIM-producers were efficiently detected with the RDS. Specificity of RDS was 96.7%, with only one isolate with reduced susceptibility to carbapenems 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 other Ambler class A carbapenemases such as GES-types, NmcA, and Sme-1.

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