Performance of four different agar plate methods for rectal swabs, synergy disk tests and metallo-β-lactamase Etest for clinical isolates in detecting carbapenemase-producing *Klebsiella pneumoniae*

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The aims of the study were to compare four different agar plate methods in the identification of carbapenemase-producing *Klebsiella pneumoniae* (CP-Kp) from rectal samples and to assess the role of phenotypic methodologies in the identification of carbapenemase type from clinical *K. pneumoniae* isolates. Two chromogenic agars (Brilliance CRE and CHROMagar KPC) were compared to MacConkey agar plates with ertapenem (ERT) or imipenem (IMP) disks for the identification of CP-Kp from 912 rectal swabs. CP-Kp was detected in 329 samples by either agar methodology (299 *K. pneumoniae* carbapenemase positive, 27 Verona integron-encoded metallo-β-lactamase positive and 3 *K. pneumoniae* carbapenemase and Verona integron-encoded metallo-β-lactamase positive). Sensitivity of Brilliance CRE, CHROMagar KPC and MacConkey agar plus IMP or ERT disk (inhibition zone <25 mm) was 96.8, 99.2, 67.2 and 81.8 %, while specificity was 90.9, 78.2, 98.1 and 97.9 %, respectively. Synergy meropenem-disk tests with EDTA or phenylboronic acid were used in order to detect the carbapenemase type as compared to PCR results (*bla* VIM, *bla*KPC and *bla*NDM) from 2515 isolates with reduced susceptibility to any of the Etest-examined carbapenems (ERT, IMP or meropenem). Metallo-β-lactamase MP/MPI Etest was applied in 616 isolates. Sensitivity was 98.4, 90.9 and 82.2 % for phenylboronic acid synergy test, EDTA synergy test and metallo-β-lactamase Etest, respectively, while their specificity was high (>97.5 %). Phenotypic methodologies can provide reliable results for the identification of carbapenemase production among *K. pneumoniae* isolates. Chromogenic agars can be applied in high-risk patients as part of surveillance and infection control programs.

INTRODUCTION

Carbapenemase-producing *Enterobacteriaceae* have rapidly disseminated during the last two decades and have nowadays evolved as a global epidemic (ECDC, 2013; Tzouvelekis et al., 2012). Although different types of carbapenemases have been found in variable species among *Enterobacteriaceae*, carbapenemase-producing *Klebsiella pneumoniae* (CP-Kp) have successfully disseminated since 2000 in Greece (Tzouvelekis et al., 2012). This expansion has serious consequences, since infections by CP-Kp are associated with increased morbidity and mortality owing to limited antibiotic options, mainly consisting of colistin, tigecycline and aminoglycosides (Daikos et al., 2009; Papadimitriou-Olivgeris et al., 2014).

Carbapenemases confer the ability to hydrolyse the carbapenems, which were one of the most commonly used antibiotics to their activity against pathogens producing extended-spectrum β-lactamase (Queenan & Bush, 2007).
The most frequent carbapenemases with worldwide expansion were the K. pneumoniae carbapenemase (KPC; type A), Verona integron-encoded metallo-β-lactamase (VIM; type B), the New Delhi metallo-β-lactamase (NDM; type B) and the oxacillinase-48 (OXA; type D carbapenemase) (Petropoulou et al., 2006). In Europe, CP-Kp are endemic in 3 countries (Greece, Italy and Malta), whereas another 10 countries had inter-regional and regional spread (ECDC, 2013). VIM-producing isolates were mainly disseminated in Greece during the first decade of the twenty-first century, followed by the introduction of KPC-producing isolates from Israel to Greece in 2007 in the course of the aforementioned European dissemination (Tzouvelekis et al., 2012). Other carbapenemases, such as OXA-48 or NDM, have provoked occasional hospital outbreaks, especially in countries of Western Europe and have not reached wide dissemination (ECDC, 2013).

This worldwide spread of different carbapenemase genes warrants the application of simple and reliable tests for routine detection, which is the first step in the implementation of infection control policies. PCR for carbapenemase gene detection has been developed and remains the gold standard for the identification of carbapenemase-producing isolates (Nordmann et al., 2011; Queenan & Bush, 2007). Because of the cost, complexity and need for experienced personnel for the PCR method, easy and accurate phenotypic methods are required (Singh et al., 2012). Some of the most commonly applied phenotypic methods with good results are the synergy disk test with EDTA or phenylboronic acid (PBA) and Etest designed to detect the presence of metallo-β-lactamases (MBLs) and/or KPC (Girlich et al., 2013; Giske et al., 2011; Tsakris et al., 2010). As OXA-48 carbapenemase is concerned, its low carbapenemase activity makes it difficult to detect by phenotypic methods, such as chromogenic agars (Viau et al., 2015). Carba NP test is another biochemical assay, which is accurate and rapid in the identification of carbapenemase directly to bacterial colonies (Nordmann & Poirel, 2013). In relation to phenotypic techniques, molecular methods remain the reference standard for the precise identification of carbapenemase genes owing to their higher performance (Viau et al., 2015).

The goals of this study were to compare four different agar plate methods in the identification of CP-Kp from rectal samples and to assess the role of phenotypic methodologies in the identification of carbapenemase type from clinical K. pneumoniae isolates.

**METHODS**

This is a study conducted in the University General Hospital of Patras (UGHP) during a 7 year period (January 2008–December 2014), which comprised two parts. The first part was to assess the performance of four different agar plate methods in identifying CP-Kp isolates from rectal samples obtained from intensive care unit (ICU) patients. In part II, K. pneumoniae isolates from the aforementioned rectal samples and from clinical specimens were further investigated by phenotypic and genotypic methods in order to detect the type of carbapenemases and the presence of bla genes. The study was carried out under the Hospital Surveillance Programme for multidrug-resistant bacterial colonization or infection of hospitalized patients and was approved by the University Hospital Ethics Committee (no. 571).

**Part I.** Nine hundred and twelve double rectal swabs (Stuart agar gel double swabs; Copan) were obtained upon admission and weekly afterwards until discharge from patients admitted to two adult ICUs (ICU A 13 beds and ICU B 6 beds) during a 24 month period (November 2009–October 2011). One swab was inoculated on either selective chromogenic agar. Brilliance CRE (Oxoid) was used for all rectal swabs (n=513) obtained upon admission to both ICUs and those obtained during hospitalization in ICU B, while CHROMagar KPC (CHROMagar) was applied in swabs obtained during hospitalization in ICU A (399 swabs). The second swab was inoculated on two MacConkey agar plates (bioMérieux). Two ertapenem (ERT; 10 µg) disks were placed on the first plate [MacConkey agar plates with ERT disk (MC-Er)], whereas two imipenem (IMP; 10 µg) disks were placed on the second one [MacConkey agar plates with IMP disk (MC-Im)], as previously described (Fig. 1) (Vasoo et al., 2014). All plates were incubated at 37°C for 20 to 24 h. Presumptive carbapenem-resistant K. pneumoniae colonies grown on chromogenic agar plates appear to be in blue or metallic blue colour, while those grown on MC-Er and MC-Im were considered as lactose-fermenting colonies within an inhibition zone of <25 mm. All blue or metallic blue colonies from either chromogenic agar and lactose-fermenting colonies within an inhibition zone of <30 mm by ERT or IMP disk were inoculated in MacConkey agar plates (bioMérieux) and were further incubated at 37°C for 20 to 24 h. These colonies were identified as K. pneumoniae by the VITEK® 2 system (bioMérieux).

**Part II.** Part II comprised K. pneumoniae collected from part I and also K. pneumoniae isolates from clinical specimens (blood, urine, tip of catheters, sputum, bronchial secretions, pus, pleural and peritoneal fluid; one per patient) from patients hospitalized in the UGHP during a 7 year period (January 2008–December 2014). The susceptibility to IMP, meropenem (MER) and ERT was determined by Etest (bioMérieux) and was interpreted according to the EUCAST (2016).

All isolates collected during part I and those from clinical specimens displaying MIC	extsubscript{IMP} ≥1 mg l\textsuperscript{-1}, MIC	extsubscript{MER} ≥0.5 mg l\textsuperscript{-1} or MIC	extsubscript{ERT} ≥0.25 mg l\textsuperscript{-1} were further tested in order to verify the production of carbapenemase. Phenotypic identification of carbapenemases was performed by the EDTA synergy test (MER/MER-EDTA) and the PBA synergy test (MER/MER-PBA), which distinguished the production of MBL and serine carbapenemase, as previously described by Petropoulou et al. (2006) and Tsakris et al. (2011). Strains with reduced susceptibility to carbapenems and negative synergy tests with both EDTA and PBA were further screened with the combined disk test (MER/MER-EDTA-PBA) in order to screen for the co-production of MBL and KPC carbapenemases (Miragou et al., 2013). Production of MBL was assessed in a proportion of isolates using the MBL MP/MP Etest (bioMérieux). Since this Etest cannot be used in isolates exhibiting MIC to MBL <4 mg l\textsuperscript{-1}, only isolates with MIC ≥4 mg l\textsuperscript{-1} were included (Galani et al., 2008). All tested isolates were kept frozen in Trypsitase soy broth for molecular analysis.

**PCR for carbapenemase gene detection.** Detection of bla genes, encoding important carbapenemase types circulating in Greece, was performed by PCR, using specific primers for blax	extsubscript{NDM} and blax	extsubscript{KPC} for all isolates during the study period (2008–2014). Isolates recovered from 2013 and afterwards were additionally tested for the presence of blax	extsubscript{KPC} according to published protocols (Nordmann et al., 2011; Queenan & Bush, 2007).

**Statistical analysis.** SPSS version 19.0 (SPSS) software was used for data analysis. Specificity, sensitivity and positive and negative predictive values (PPV and NPV) were calculated in order to assess the performance of the four agar plate methods in the identification of the presence of CP-Kp from rectal swabs, carbapenem MICS in the detection of...
carbapenemase production among *K. pneumoniae* isolates and phenotypic methods (PBA or EDTA synergy disk test and MBL MP/MPI Etest) in the identification of KPC or MBL carbapenemases. A rectal sample was considered positive if any of the agar plate yielded a CP-Kp as established by PCR. The gold standard comparator for PBA synergy test was positivity of *bla*KPC PCR, whereas for EDTA synergy test and MBL MP/MPI Etest, it was positivity of *bla*VIM or *bla*NDM PCR. The accuracy of the aforementioned methods was investigated using receiver operating characteristic analysis. *P* value of <0.05 was considered significant.

**RESULTS**

**Part I**

Among 912 rectal swabs obtained from 370 patients hospitalized in two adult ICUs of UGHP, 329 swabs were positive for CP-Kp: 299 *bla*KPC positive, 27 *bla*VIM positive and 3 *bla*KPC and *bla*VIM positive. Brilliance CRE was applied in 513 specimens and correctly detected 232 CP-Kp (219 KPC, 10 VIM and 3 KPC and VIM positive), whereas it could not identify two KPC-positive *K. pneumoniae*. False-positive results were obtained from 36 specimens (7 were due to carbapenemase-negative *K. pneumoniae* and 29 to lactose-non-fermenting bacteria).

CHROMagar KPC was applied in the remaining 399 rectal swabs and correctly identified 232 CP-Kp (219 KPC, 10 VIM and 3 KPC and VIM positive), whereas it could not identify two KPC-positive *K. pneumoniae*. False-positive results were obtained from 36 specimens (7 were due to carbapenemase-negative *K. pneumoniae* and 29 to lactose-non-fermenting bacteria).

MC-Er and MC-Im were applied in all samples and detected 277 CP-Kp (252 KPC, 22 VIM and 3 KPC and VIM positive) and 228 CP-Kp (204 KPC, 21 VIM and 3 KPC and VIM positive), respectively, within a zone of inhibition <30 mm.

Performance of agar plate methods for the identification of CP-Kp from rectal swabs is depicted in Table 1. For the MC-Er and MC-Im, a zone of inhibition of <25 mm for both agar plate methodologies showed the best performance for the detection of CP-Kp. Sensitivity was 96.8, 99.2, 81.8 and 67.2% for Brilliance CRE, CHROMagar KPC, MC-Er and MC-Im, while specificity was 90.9%, 78.2, 97.9 and 98.1%, respectively. MC-Er and MC-Im showed higher PPV and lower NPV as compared to chromogenic agars.

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**Fig. 1.** Specimens’ flowchart. MER, meropenem.
**Table 1. Performance of agar plate methods for the identification of CP-Kp from rectal swabs**

<table>
<thead>
<tr>
<th>Agar plate methods</th>
<th>Number</th>
<th>TP</th>
<th>FP</th>
<th>TN</th>
<th>FN</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brilliance CRE</td>
<td>513</td>
<td>92</td>
<td>38</td>
<td>380</td>
<td>3</td>
<td>96.8%</td>
<td>90.9%</td>
<td>70.8%</td>
<td>99.2%</td>
<td>92.0%</td>
</tr>
<tr>
<td>CHROMagar KPC</td>
<td>399</td>
<td>232</td>
<td>36</td>
<td>129</td>
<td>2</td>
<td>99.2%</td>
<td>78.2%</td>
<td>86.6%</td>
<td>98.5%</td>
<td>90.5%</td>
</tr>
<tr>
<td>MacConkey agar plus IMP disk (&lt;25 mm)</td>
<td>912</td>
<td>221</td>
<td>11</td>
<td>572</td>
<td>108</td>
<td>67.2%</td>
<td>98.1%</td>
<td>95.3%</td>
<td>84.1%</td>
<td>87.0%</td>
</tr>
<tr>
<td>MacConkey agar plus ERT disk (&lt;25 mm)</td>
<td>912</td>
<td>269</td>
<td>12</td>
<td>571</td>
<td>60</td>
<td>81.8%</td>
<td>97.9%</td>
<td>95.7%</td>
<td>90.5%</td>
<td>92.1%</td>
</tr>
</tbody>
</table>

TP, true positive; FP, false positive; TN, true negative; FN, false negative.

**Part II**

From the aforementioned four agar plate methods, a total of 870 *K. pneumoniae* were collected. Moreover, 2953 *K. pneumoniae* strains (one per patient) were isolated from clinical specimens during 2008 to 2014. In total, 2480 (835 isolates from rectal swabs from part I and 1645 from clinical specimens, respectively) showed MIC<sub>ERT</sub> ≥0.25 mg l<sup>-1</sup>, MIC<sub>IMP</sub> ≥1 mg l<sup>-1</sup> or MIC<sub>MER</sub> ≥0.5 mg l<sup>-1</sup>. The MIC distribution to each tested carbapenem of *K. pneumoniae* isolates according to presence of carbapenemases is shown in Fig. 2, whereas the performance of different cut-off values is shown in Table 2. EUCAST MIC breakpoints for non-susceptibility among *Enterobacteriaceae* to IMP, MER and ERT are >2, >2 and >0.5 mg l<sup>-1</sup> and would have failed to detect 289 (12.2%), 315 (13.3%) and 13 (0.5%) CP-Kp isolates, respectively.

In order to detect the presence of carbapenemases, all 870 isolates from part I and the 1645 isolates from clinical specimens were further evaluated by phenotypic and molecular methodologies (Fig. 1). Among the 2515 isolates, 2366 were CP-Kp (2076 bla<sub>KPC</sub>, 232 bla<sub>VIM</sub>, 54 bla<sub>ERC</sub> and bla<sub>NDM</sub>). PBA and EDTA synergy tests were applied in all samples. PBA synergy test correctly detected 2096 KPC-producing *K. pneumoniae* isolates (2076 bla<sub>KPC</sub>-positive and 20 bla<sub>KPC</sub> and bla<sub>VIM</sub>-positive), while it failed to detect 34 bla<sub>KPC</sub>- and bla<sub>VIM</sub>-positive isolates. EDTA synergy test correctly detected 260 MBL-producing *K. pneumoniae* isolates (232 bla<sub>VIM</sub>, 24 bla<sub>KPC</sub> and bla<sub>VIM</sub> and 4 bla<sub>NDM</sub>), while it failed to detect 30 bla<sub>KPC</sub>- and bla<sub>VIM</sub>-positive isolates. In total, 26 bla<sub>KPC</sub>- and bla<sub>VIM</sub>-positive isolates were negative by both PBA and EDTA synergy tests. When further screened with the combined disk test (MER/MER-EDTA-PBA), they were all found positive, indicating a co-production of VIM and KPC carbapenemases.

MBL MP/MPI Etest was applied in 616 isolates, of which 213 were MBL producing (155 bla<sub>VIM</sub>, 54 bla<sub>KPC</sub> and bla<sub>VIM</sub> and 4 bla<sub>NDM</sub>). It correctly identified 175 isolates (153 bla<sub>VIM</sub>, 18 bla<sub>KPC</sub> and bla<sub>VIM</sub> and 4 bla<sub>NDM</sub>), while it was false positive for four bla<sub>KPC</sub>-positive isolates.

The performance of phenotypic methods for the identification of CP-Kp is shown in Table 3. Sensitivity was 98.4%, 90.9 and 82.2% for PBA synergy test, EDTA synergy test and MBL MP/MPI Etest, respectively, while specificity of the aforementioned methodologies was 100, 100 and 99.0%, respectively, PPV of all methodologies was excellent (>97.5%), and NPV was good (>90.0%).

**DISCUSSION**

In the present study, different phenotypic methodologies were evaluated accurately in detecting the presence of CP-Kp from surveillance rectal cultures or clinical samples from patients hospitalized in a university hospital with high rates of CP-Kp isolation (Spyropoulou et al., 2015).

Both chromogenic agars tested (Brilliance CRE and CHROMagar KPC) showed a very good performance for detecting CP-Kp isolates from rectal swabs of ICU patients. Their sensitivity was excellent (>95%) and comparable to that from previous studies (Adler et al., 2011; Cohen Stuart et al., 2013; Moran Gilad et al., 2011). The specificity of Brilliance CRE was very good (90%) and higher than that of CHROMagar KPC (78%). There is a great disparity in the literature concerning the performance of the aforementioned chromogenic agars, which depends on the comparing methodology. Thus, performance is lower when compared to real-time PCR (Singh et al., 2012; Vasoo et al., 2014) and equal when compared to other agar plate methods (Nordmann et al., 2012) and the epidemiology of carbapenemase genes (high performance of chromogenic agars for bla<sub>KPC</sub>, good for bla<sub>VIM</sub> and bla<sub>NDM</sub> and low for bla<sub>OXA-48</sub>) (Day et al., 2013; Nordmann et al., 2012; Singh et al., 2012).

The use of MacConkey agar with carbapenem disks should be discouraged for the detection of CP-Kp, since their sensitivity was low. Previous studies showed similar sensitivity and specificity (Lolans et al., 2010; Moran Gilad et al., 2011; Vasoo et al., 2014). One main disadvantage is that they failed to detect 30 CP-Kp from rectal swabs due to the fact that only lactose-negative isolates were present on the MacConkey agar, probably due their higher load as compared to *K. pneumoniae*. This result means that the rectal flora may play an important role in the use of these methods. The use of ERT disk as compared to that of IMP showed better results, which is in accordance to previous findings that suggest ERT as a better screening substrate, compared to
Fig. 2. MIC distribution of *K. pneumoniae* isolates according to carbapenemase presence of (a) IMP, (b) MER and (c) ERT. The flat line depicts the breakpoint chosen by the authors for further epidemiological testing, while the dotted line depicts the non-susceptibility breakpoint according to the EUCAST MBL: VIM- or NDM-positive *K. pneumoniae* isolates.
**Fig. 2.** (cont.)

IMP or MER, in populations with high prevalence of carbapenemases (McGettigan et al., 2009; Vading et al., 2011).

In our study, we used as screening cut-off values for clinical *K. pneumoniae* isolates MIC$_{\text{ERT}} \geq 0.25$ mg l$^{-1}$, MIC$_{\text{IMP}} \geq 1$ mg l$^{-1}$ or MIC$_{\text{MER}} \geq 0.5$ mg l$^{-1}$, which are similar to that proposed by EUCAST in December 2012 (EUCAST, 2012). Clinical breakpoints should be used to guide clinicians in carbapenem treatment of patients with infections by CP-Kp, since the MIC of carbapenems and not the presence of carbapenemase gene was associated with survival (Daikos et al., 2009). It is important in epidemiological detection of carbapenemase-producing isolates to use lower cut-off values from the clinical breakpoints, since EUCAST breakpoints for non-susceptibility for *Enterobacteriaceae* to IMP, MER and ERT failed to detect 289 (12.2 %), 315 (13.3 %) and 13 (0.5 %) CP-Kp isolates, respectively. As previously shown, ERT showed the best accuracy in detecting CP-Kp and is considered as the best indicator in settings with high rates of CP-Kp, such as our institution (McGettigan et al., 2009; Vading et al., 2011).

The use of the combined disk tests (either PBA or EDTA) showed excellent performance in the identification of type of carbapenemase that *K. pneumoniae* isolates carried. The misidentification was mainly with isolates carrying both

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**Table 2.** Performance of carbapenem MIC cut-off values for the detection of carbapenemase-producing isolates among 3823 *K. pneumoniae* isolates

<table>
<thead>
<tr>
<th>Carbapenems</th>
<th>MIC (mg l$^{-1}$)</th>
<th>TP</th>
<th>FP</th>
<th>TN</th>
<th>FN</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMP</td>
<td>≥1</td>
<td>2274</td>
<td>116</td>
<td>1341</td>
<td>92</td>
<td>96.1 %</td>
<td>92.4 %</td>
<td>95.1 %</td>
<td>93.6 %</td>
<td>94.6 %</td>
</tr>
<tr>
<td></td>
<td>&gt;2</td>
<td>2077</td>
<td>4</td>
<td>1453</td>
<td>289</td>
<td>87.8 %</td>
<td>99.8 %</td>
<td>99.8 %</td>
<td>83.4 %</td>
<td>92.3 %</td>
</tr>
<tr>
<td>MER</td>
<td>≥0.5</td>
<td>2362</td>
<td>196</td>
<td>1261</td>
<td>4</td>
<td>99.8 %</td>
<td>86.6 %</td>
<td>92.3 %</td>
<td>99.6 %</td>
<td>94.8 %</td>
</tr>
<tr>
<td></td>
<td>&gt;2</td>
<td>2051</td>
<td>1</td>
<td>1456</td>
<td>315</td>
<td>86.7 %</td>
<td>99.9 %</td>
<td>100 %</td>
<td>82.2 %</td>
<td>91.7 %</td>
</tr>
<tr>
<td>ERT</td>
<td>≥0.25</td>
<td>2364</td>
<td>114</td>
<td>1343</td>
<td>2</td>
<td>99.2 %</td>
<td>92.2 %</td>
<td>95.4 %</td>
<td>99.9 %</td>
<td>97.0 %</td>
</tr>
<tr>
<td></td>
<td>&gt;0.5</td>
<td>2353</td>
<td>7</td>
<td>1450</td>
<td>13</td>
<td>99.5 %</td>
<td>99.5 %</td>
<td>99.7 %</td>
<td>99.1 %</td>
<td>99.5 %</td>
</tr>
</tbody>
</table>

TP, true positive; FP, false positive; TN, true negative; FN, false negative.
KPC and VIM carbapenemases for which EDTA-MER and PBA-MER were negative in 30 and 34 isolates, respectively. Similar results concerning strains with KPC and VIM co-production have been previously reported (Miriagou et al., 2013; Zioga et al., 2010). Those were accurately detected when the assay included the EDTA-PBA-MER disk. Although co-presence of blaKPC and blaVIM was reported first in Greek hospitals, nowadays, reports from around the world indicate their worldwide dissemination (Giakkoupi et al., 2009; Martinez et al., 2015; Steinmann et al., 2011; Wang et al., 2012). In hospitals where the incidence of co-carriage of KPC and VIM carbapenemases among K. pneumoniae isolates is high or when results of the conventional disk test are ambiguous, it is prudent to add a fourth disk with EDTA-PBA-MER in order not to miss their co-presence. The MBL MP/MPI Etest conferred very good results, as previously shown by Girlich et al. (2013), but its performance was influenced by the high occurrence of co-carriage of KPC and VIM carbapenemases.

The main limitation of the study is that the assays were tested in only one centre with a high occurrence of KPC and VIM carbapenemases among K. pneumoniae isolates and a lower incidence of NDM carbapenemase, while no OXA-48-producing isolate was detected during the study period (Spyropoulou et al., 2015). Moreover, no genotypic method (PCR, real-time PCR or DNA hybridization techniques) was used for the direct rapid detection of carbapenemase genes directly from rectal swabs (Vianu et al., 2016). Although molecular methods have shown excellent performance, their higher price and the incapacity of further epidemiological study render their use problematic (Vianu et al., 2016).

To conclude, phenotypic methodologies can provide reliable results for the identification of carbapenemase production among K. pneumoniae isolates. Chromogenic agars can be applied in high-risk patients, such as ICU patients, as part of surveillance and infection control programs. Clinical laboratories from countries where CP-Kp isolates have been reported should apply the EUCAST screening cut-off values of carbapenems for the identification of isolates suspected of being carbapenemase producing, which should be tested by phenotypic methods (EDTA and PBA synergy tests, Etest) or PCR in order to certify the type of carbapenemase gene.

### Table 3. Performance of phenotypic methods for the identification of CP-Kp

<table>
<thead>
<tr>
<th>Phenotypic methods</th>
<th>n</th>
<th>Carbapenemase sought by test</th>
<th>TP</th>
<th>FP</th>
<th>TN</th>
<th>FN</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylboronic acid</td>
<td></td>
<td>synergy test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2310</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>99.6</td>
</tr>
<tr>
<td>EDTA synergy test</td>
<td></td>
<td>MBL (n=286)</td>
<td>260</td>
<td>0</td>
<td>229</td>
<td>38</td>
<td>82.2</td>
<td>99.0</td>
<td>97.8</td>
<td>91.3</td>
<td>93.2</td>
</tr>
<tr>
<td>MBL MP/MPI Etest</td>
<td></td>
<td>MBL (n=313)</td>
<td>175</td>
<td>4</td>
<td>399</td>
<td>38</td>
<td>91.3</td>
<td>90.9</td>
<td>91.3</td>
<td>91.3</td>
<td>93.2</td>
</tr>
</tbody>
</table>

TP, true positive; FP, false positive; TN, true negative; FN, false negative.

### ACKNOWLEDGEMENTS

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