Comparison of two chromogenic media and enrichment broth for the detection of carbapenemase-producing Enterobacteriaceae on screening rectal swabs from hospitalized patients

The rapid dissemination of carbapenemase-producing Enterobacteriaceae (CPE) constitutes a major threat to patient care and public health (Nordmann et al., 2011; Livermore, 2012). Direct screening by rectal swabbing of high-risk patients is essential to detect asymptomatic carriers, who constitute the main reservoir of CPE (Gagliotti et al., 2013). The rapid detection of CPE allows faster implementation of infection control strategies in order to prevent their dissemination inside the hospital. The Belgian epidemiology of CPE is characterized by a predominance of OXA-48-like carbapenemases (>80%) that weakly hydrolyse carbapenems and may therefore be difficult to detect in screening cultures (Huang et al., 2011, 2013; Glupczynski et al., 2012; Poiré et al., 2012). The aim of this study was to compare the performance of two chromogenic media and an enrichment broth for CPE detection on rectal swab samples from hospitalized patients.

Rectal swab (Copan Italia) samples (n=730) from patients (n=480) hospitalized at a tertiary-care academic hospital (Hôpital Erasme, Brussels, Belgium) were collected prospectively between December 2012 and March 2013. The active surveillance policy of our hospital is to screen for multidrug-resistant Gram-negative bacilli (MDR-GNB) at the intensive care station (all admitted patients twice a week) and at the haematological department (once a week for all admitted patients). All patients admitted to our hospital at risk of carrying an MDR-GNB are also screened (e.g. those travelling abroad, known MDR-GNB carriers or transferred from another hospital). The rectal swabs were homogenized by vortexing for 15 s in 1 ml sterile 0.85% saline and 100 μl of this suspension was inoculated into MacConkey broth (Recton Dickinson), without additional antibiotics and plated on primary chromID CARBA (bioMérieux) and Brilliance CRE (Oxoid, Thermo Fisher Scientific). The media were incubated at 35 °C and examined after 24 and 48 h of incubation. The MacConkey broths were subcultured after overnight incubation onto secondary chromogenic plates and examined after 24 h. All growing colonies were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Daltonics).

Susceptibility testing to meropenem and ertapenem was performed by a disk diffusion method according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) on all growing Enterobacteriaceae isolates (EUCAST, 2012). All isolates were stored at −80 °C until further characterization. Enterobacteriaceae isolates showing a positive carbapenemase screening cut-off according to EUCAST were characterized further by determination of their MICs to carbapenems using a microdilution method and by the detection of carbapenemase production using a RAPIDEC Carba NP test (bioMérieux) (EUCAST, 2013; Poiré & Nordmann, 2015). Confirmation of a carbapenemase was assessed by an in-house multiplex PCR targeting the genes encoding blakPC, blakOXA-48, blakNDM, blakTIM and blakIMP on all growing Enterobacteriaceae including isolates showing negative EUCAST screening cut-off (Bogaerts et al., 2013).

For each medium, true positive results were defined as typical colonies confirmed as CPE by PCR and the RAPIDEC Carba NP test; false-positive results were defined as typical colonies not confirmed as CPE. The statistical significance (P<0.05) of inter-medium disagreement was estimated by the McNemar test or the exact McNemar test (Stata 12 software; StataCorp).

During this clinical evaluation, 480 hospitalized patients were screened for CPE carriage by sampling rectal swabs (n=730) (one to eight samples per patient with a median of 1.00 per patient) (Fig. 1). Among 86 Enterobacteriaceae that grew from 72 samples (50 patients) on chromogenic agars or after enrichment, 37 isolates showed a positive carbapenemase screening cut-off in 29 rectal swabs (20 patients). RAPIDEC Carba NP tests performed on these isolates were positive for 19 isolates (Citrobacter freundii, n=7; Escherichia coli, n=6; Klebsiella pneumoniae, n=5; Enterobacter cloacae, n=1) from 14 samples (nine patients). Carbapenemase phenotypic tests were in complete agreement with the multiplex PCR results detecting 17 OXA-48-like and two VIM CPE. The MICs for ertapenem ranged from 0.25 to 32 mg l⁻¹ (median=8 mg l⁻¹) and for meropenem from 0.25 to 8 mg l⁻¹ (median=1 mg l⁻¹) for the OXA-48-like isolates. The two VIM-positive C. freundii showed MICs of 4 and 8 mg l⁻¹ for ertapenem and 4 mg l⁻¹ for meropenem.

CPE isolates (n=19) were significantly better detected on primary Brilliance CRE (n=15) than on chromID CARBA (n=5) (P=0.004) (Table 1). The two VIM-positive isolates were recorded on both primary plates. Out of the 17 OXA-48-like carbapenemase isolates, 13 were detected on the Brilliance CRE primary plate, while only three were isolated on the chromID CARBA primary plate. Prolonged incubation at 48 h did not allow detection of any further CPE isolates but lowered the specificities of both media. Three additional OXA-48-like CPE isolates of three patients were detected only after enrichment (P>0.05): two isolates only on Brilliance CRE and one isolate on both media.

Non-carbapenemase-producing Enterobacteriaceae (NCPE) grew more frequently on Brilliance CRE (60/78) than on chromID CARBA (11/19) (Table 1). NCPE were either resistant (Brilliance CRE
51/78; chromID CARBA 9/19) or susceptible (Brilliance CRE 9/78; chromID CARBA 2/19) to third-generation cephalosporins (data not shown). The sensitivities and specificities of the two chromogenic media at 24 and 48 h of incubation with and without MacConkey broth are shown in Table 1.

In an epidemiological setting of low prevalence (<2%) of CPE with a predominance of OXA-48-like producers, Brilliance CRE displayed a better sensitivity but a lower specificity than chromID CARBA for CPE screening. This confirmed the lack of specificity of Brilliance CRE described by other authors (Cohen Stuart et al., 2013). The presence of a carbapenemase should be assessed phenotypically (e.g. RAPIDEC Carba NP) and genotypically. Extending the duration of culture to 48 h is not recommended due to the growth of extended-spectrum \(\beta\)-lactamase- and/or AmpC-producing isolates, particularly for Brilliance CRE agar. In this study, Brilliance CRE presented better performance than chromID CARBA in recovering OXA-48-like CPE. A recently developed commercial medium, chromID OXA-48 (bioMérieux) showed excellent performance in detecting CPE harbouring OXA-48-like carbapenemase (Girlich et al., 2013; Zarakolu et al., 2015). Therefore, a combination of two media such as chromID CARBA and chromID OXA-48

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**Fig. 1.** Detection of carbapenemase-producing *Enterobacteriaceae* on rectal swab samples from patients admitted to a teaching hospital. *The presence of a carbapenemase was assessed by an in-house multiplex PCR targeting the genes encoding blaKPC, blaOXA-48, blaNDM, blaVIM and blalMP.*
should be used for an accurate detection of the different types of CPE (Girlich et al., 2013; Zarakoli et al., 2015).

The aim of using MacConkey enrichment broth was to boost the growth of Gram-negative rods prior to subculturing on selective chromogenic medium. Previous evaluations of non-selective broth supplemented with a carbapenem as recommended by the Centers for Disease Control and Prevention did not improve the performance of direct inoculation on screening agars (CDC, 2009; Lolans et al., 2010; Girlich et al., 2014; Papadimitriou-Olivgeris et al., 2014). In our study, the addition of an enrichment step without antimicrobials increased systematically, but not statistically significantly, the sensitivity of both chromogenic screening media. However, the use of an enrichment step extends the time to final reporting by an additional 24 h.

In conclusion, in a setting with a predominance of OXA-48like-producing Enterobacteriaceae among CPE, chromID CARBA and Brilliance CRE do not show enough sensitivity to accurately detect OXA-48-like CPE in rectal swabs. Further clinical studies are needed to assess the additional value of enrichment broths and selective media for the detection of CPE, particularly OXA-48-like-producing Enterobacteriaceae.

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Abbreviations: CPE, carbapenemase-producing Enterobacteriaceae; NCPE, non-carbapenemase-producing Enterobacteriaceae; MDR-GNB, multidrug-resistant Gram-negative bacilli.

Table 1. Analytical performance of two chromogenic CPE screening media with or without enrichment culture (MacConkey broth)

<table>
<thead>
<tr>
<th>Sample</th>
<th>CPE</th>
<th>NCPE</th>
<th>Total</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>CPE</th>
<th>NCPE</th>
<th>Total</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
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<tbody>
<tr>
<td>Time</td>
<td></td>
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<tr>
<td>After 24 h</td>
<td>5 (71.4%)</td>
<td>2 (28.6%)</td>
<td>7</td>
<td>26.3</td>
<td>NA</td>
<td>15 (34.1%)</td>
<td>29 (65.9%)</td>
<td>44</td>
<td>78.9</td>
<td>NA</td>
</tr>
<tr>
<td>After 48 h</td>
<td>5 (73.0%)</td>
<td>4 (0.6%)</td>
<td>1 (0.1%)</td>
<td>5 (0.7%)</td>
<td>28.6</td>
<td>99.9</td>
<td>11 (1.5%)</td>
<td>26 (3.6%)</td>
<td>37 (5.1%)</td>
<td>78.6</td>
</tr>
<tr>
<td>Total after enrichment</td>
<td>5 (480)</td>
<td>3 (0.6%)</td>
<td>5 (1.0%)</td>
<td>8 (1.7%)</td>
<td>33.3</td>
<td>98.9</td>
<td>7 (1.5%)</td>
<td>26 (5.4%)</td>
<td>30 (6.3%)</td>
<td>66.7</td>
</tr>
<tr>
<td>Patients (n=5480)</td>
<td>3 (0.6%)</td>
<td>5 (1.0%)</td>
<td>8 (1.7%)</td>
<td>33.3</td>
<td>98.9</td>
<td>7 (1.5%)</td>
<td>26 (5.4%)</td>
<td>30 (6.3%)</td>
<td>66.7</td>
<td>94.7</td>
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References


