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Evaluation of the RAPIDEC® CARBA NP and β-CARBA® tests for rapid detection of Carbapenemase-producing *Enterobacteriaceae*

Stefano Mancini [a,b,c,*], Nicolas Kieffer [a,b,c], Laurent Poirel [a,b,c], Patrice Nordmann [a,b,c,d]

[a] Emerging Antibiotic Resistance Unit, Medical and Molecular Microbiology, Department of Medicine, Faculty of Science, University of Fribourg, Switzerland
[b] INSERM European Unit (LEA/IAME Paris, France), University of Fribourg, Switzerland
[c] National Reference Center for Emerging Antibiotic Resistance, Switzerland
[d] Institute of Microbiology, University of Lausanne and University Hospital Center, Lausanne, Switzerland

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ABSTRACT

The analytical performances of the RAPIDEC CARBA NP® (bioMérieux) and the β-CARBA® (Bio-Rad) tests were evaluated for the rapid detection of any known type of carbapenemases in *Enterobacteriaceae*. An international collection of 149 enterobacterial isolates comprising 111 Carbapenemase-Producing *Enterobacteriaceae* (CPE) and 38 non-carbapenemase producers was used. CPE included 32 carbapenemase producers of class A (18 KPC-2, 1 FRI-1, 5 SME and 8 IMI), 33 of class B (13 NDM, 11 VIM and 9 IMP) and 46 of class D (15 OXA-48, 14 OXA-181, 10 OXA-204, 3 OXA-232 and 4 OXA-162). The RAPIDEC CARBA NP® and the β-CARBA® tests were performed in strict accordance to the manufacturer's instructions and results were read within 2 h and 30 min, respectively. RAPIDEC CARBA NP® detected 104/111 CPE isolates compared to 72/111 for the β-CARBA® test. The overall sensitivity and specificity were 93.7% and 100%, respectively, for the RAPIDEC CARBA NP® test, and 64.9% and 90%, respectively, for the β-CARBA® test. The β-CARBA® test failed to detect all the non-KPC class A carbapenemases (14/14) and most (24/31) of the OXA-48-like producers (OXA-162, OXA-181, OXA-204 and OXA-232), and detected 1/1 OXA-163 and 1/1 OXA-405 as carbapenemase producers whereas these enzymes are rather defined as non carbapenemases. RAPIDEC CARBA NP® test exhibited better performances than those of the β-CARBA® test and confirmed to be a reliable tool for the detection of CPE, especially for OXA-48-like producers.

1. Introduction

The emergence and rapid dissemination of Carbapenemase-Producing *Enterobacteriaceae* (CPE) during the last decade represents the most worrisome event in the escalating antibiotic resistance crisis (Nordmann et al., 2011) as CPE have been reported in numerous nosocomial outbreaks worldwide. Most importantly, related infections can be treated only by a few remaining therapeutic options (Canton et al., 2012). Hence, rapid detection of CPE is of paramount importance for the timely choice of the appropriate antibiotic therapy and for the implementation of measures to prevent outbreaks.

Carbapenem resistance may arise from either (i) a concomitant reduced permeability of the outer membrane and an overexpression of enzymes with very low carbapenemase activity (*e.g.* extended-spectrum beta-lactamases and cephalosporinases) or (ii) the expression of enzymes with significant carbapenemase activity, defined as carbapenemases (Nordmann et al., 2012a). A large variety of acquired carbapenemases has been reported worldwide in CPE. The most prevalent carbapenemases belong to the Ambler class A (KPC), class B (VIM, IMP, and NDM) and class D (OXA-48 and OXA-48-like) (Albiger et al., 2015).

Nowadays several methods are available for the rapid detection of carbapenemase producers including: (I) molecular techniques to identify the most common carbapenemase genes (Findlay et al., 2015), (II) the MALDI-TOF technology that allows the detection of carbapenem degradation products (Hrabak et al., 2013), (III) a recently developed electrochemical assay that allows the detection of carbapenemase-producers (Bogaerts et al., 2016), (IV) immunochromatographic assays detecting KPC, IMP-like, OXA-48 and OXA-48-like β-lactamases (Glupczynski et al., 2016; Kitao et al., 2011) and (V) colorimetric assays relying on the color change of a pH indicator to detect carbapenemase activity (CARBA NP test (Nordmann et al., 2012b) and copies or derivatives (Compain et al., 2016; Noel et al., 2016; Pires et al., 2013)).

Abbreviations: CPE, Carbapenemase-producing *Enterobacteriaceae*; MALDI-TOF, Matrix-assisted laser desorption/ionization-time-of-flight mass spectrometer; ESBL, Extended-spectrum β-lactamases.

* Corresponding author. Tel.: +41-26-300-9583.
E-mail address: stefano.mancini@unifr.ch (S. Mancini).

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Here, we compared the performances of two commercially-available colorimetric assays, the RAPIDEC® CARBA NP and the recently developed β-CARBA® test, by using a common panel of CPE.

2. Materials and methods

2.1. Strain collection

A collection of 149 clinical enterobacterial isolates was used to assess the performances of the RAPIDEC® CARBA NP (bioMérieux, La Balmes-grottes, France) and the β-CARBA® (Bio-Rad, Marnes-la-Coquette, France) tests. All the strains were characterized for their β-lactamase content by PCR and sequencing of the corresponding genes. The clinical isolates of worldwide origin were recovered from various clinical samples (sputum, blood cultures, urines, gut flora, *etc.*) and consisted in 38 carbapenemase-negative isolates and 111 CPE. The carbapenemase-negative isolates included 18 extended-spectrum β-lactamases (ESBL) producers (CTX-M, OXA-163, OXA-405 and GES-1), 13 isolates possessing plasmid-encoded or chromosomally-encoded overproducing AmpC, and 7 wild-type carbapenem-susceptible isolates. The CPE comprised 18 KPC producers, 1 FRI-1 producer, 5 SME producers, 8 IMI producers, 13 NDM-1 producers, 11 VIM producers, 9 IMP producers, 14 OXA-48 producers and 31 OXA-48 like producers (Table 1).

2.2. Antibiotic susceptibility testing

Etest strips (bioMérieux) were used to determine the minimum inhibitory concentrations (MICs) of meropenem and ertapenem. Results were interpreted according to the EUCAST guidelines (http://www.eucast.org/ast_of_bacteria/). The breakpoints for susceptibility/resistance of meropenem and ertapenem were ≤2/>8 μg/ml and ≤0.5/>1 μg/ml, respectively.

2.3. RAPIDEC® CARBA NP test

The RAPIDEC® CARBA NP was performed with colonies collected from URI*Select*™ 4 plates (Bio-Rad) after 16 hours of incubation at 37 °C. The tested bacteria were taken with the extremity of a stick provided in the kit and resuspended in well C of the RAPIDEC® CARBA NP kit to obtain a turbidity corresponding to that of the control. Results were interpreted by multiple operators in a blinded manner after 2 h of incubation in accordance with the interpretation's guidelines. Results were considered positive in case of color shifts from red to yellow, to orange or to red-orange.

2.4. β-CARBA® test

The β-CARBA® test was performed using a 1 μl loop full with colonies grown for 16 hours at 37 °C on URI*Select*™ 4 plates, as indicated in the kit's manual. As recommended in the interpretation's guidelines, results were read blindly by multiple interpreters within 30 min of incubation. Results were considered positive in case of color changes from yellow to clear orange, to red or to purple.

3. Results

3.1. Performances of the RAPIDEC® CARBA NP and β-CARBA® tests

Of the 149 tested isolates, 111 were CPE. RAPIDEC® CARBA NP yielded positive results for 104 out of the 111 CPE isolates, while the β-CARBA® test yielded positive results for only 72/111 isolates. Sensitivity and specificity of the RAPIDEC® CARBA NP test were 93.7% and 100%, respectively, values that are in good agreement with those indicated in the manufacturer's specifications (specificity 97.8% and sensitivity 97.8%). By contrast, the sensitivity and specificity of the β-CARBA® test were 64.9% and 90%, respectively, which are significantly inferior to those reported in the kit's specifications (specificity 89.4% and sensitivity 97.8%).

3.2. Detection of the ambler class a, B and D type carbapenemases

Among the Ambler class A type carbapenemase producing isolates, all the 18 KPC-2 producers were correctly assigned by both the RAPIDEC® CARBA NP and β-CARBA® tests. However, while RAPIDEC® CARBA NP test correctly identified the FRI-1 (1/1), SME (4/4) and IMI (8/8) producers as carbapenemase producers, the β-CARBA® test failed to detect all of them. All the Ambler class B producers (13 NDM, 11 VIM and 9 IMP) were correctly assigned by both the RAPIDEC® CARBA NP and β-CARBA® tests, in accordance with the results obtained by others (Compain et al., 2016; Dortet et al., 2015a; Noel et al., 2016). Similar results were obtained with the 15 Ambler class D OXA-48 producers, whose carbapenemase production was correctly assigned by both tests. Contrasting results were instead observed with the remaining Ambler class D carbapenemase producers. While RAPIDEC® CARBA NP detected carbapenemase production of 24/31 OXA-48 like producers (14 OXA-181, 10 OXA-204, 3 OXA-232 and 4 OXA-162), only 6/31 were correctly detected by the β-CARBA® test. Of note, extension of the incubation time to 2 hours significantly increased the number of OXA-48-like carbapenemases correctly detected by the β-CARBA® test (19/31) and therewith the sensitivity of the assay (76.6%), consistent with previous observations (Compain et al., 2016). Also, use of larger bacterial inocula (approximately double the initial amounts) did also positively impact on the detection of OXA-48-like carbapenemases by using the CARBA® test (data not shown). However, whether the use of a higher bacterial inoculum might negatively affect on the overall specificity of the β-CARBA® test was not investigated.

4. Discussion

4.1. Interpretation of the results

Several works have investigated the analytical performances of RAPIDEC® CARBA NP and have reported different sensitivities in detecting CPE, ranging from 90.2% to 99% (Bernabeu et al., 2017; Dortet et al., 2015a; Hombach et al., 2015; Kabir et al., 2016; Noel et al., 2016; Poirel and Nordmann, 2015). On the other hand, with the exception of the work carried out by Noël et al., where they report a value of 84% (Noel et al., 2016), the sensitivity of the RAPIDEC® CARBA NP test is always close to 100%. By contrast, only three works have so far assessed the performances of the β-CARBA® test in detecting CPE, reporting discordant sensitivity values of 85.1% (Bernabeu et al., 2017), 85.4% (Compain et al., 2016) and 97.3% (Noel et al., 2016), while the specificity values appear more consistent, being 92.7%, 96% and 97.7%, respectively. The evaluation of the analytical performances of such tests is notoriously affected by the distribution of the carbapenemase types in the tested collection of CPE, and most importantly by the proportion of OXA-48-like carbapenemase producers, which are known to be more difficult to detect. Here, a large number of OXA-48-like producers (41% of the tested CPE) was included to assess more accurately the sensitivity of the assays, but also to take into account the growing proportion of this group of enzymes in many parts of the world and in particular in Europe, as reported in a recent survey on the epidemiology of CPE conducted in 38 European countries during 2014–2015 (Albiger et al., 2015). Another factor that can influence the evaluation of the performance of such assays is the way how doubtful results are categorized. With regard to the RAPIDEC® CARBA NP test, divergence in the interpretation of the results can occur when dealing with carbapenemase-producing isolates causing a slight color change (red to orange), an observation classified as a weak positive result according to the manufacturer's instructions but that operators may falsely consider as negative results. Concerning the β-CARBA® test, ambiguity can derive from faint color variations and more precisely from yellow to dark