Evaluation of a novel procedure for rapid detection of carbapenemase-producing Enterobacteriaceae (CPE) using the LightMix® modular carbapenemase kits

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Objectives: Evaluation of the LightMix® modular carbapenemase kits for the rapid detection of carbapenemase-producing Enterobacteriaceae (CPE) and the application of these kits to the direct detection of colonized patients and bacteraemias.

Methods: The modular multiplex PCR kits targeting blaKPC, blaNDM, blaVIM, blaIMP and blaOXA-48-like carbapenem resistance genes were evaluated in terms of sensitivity and specificity for carbapenemase resistance in a set of 118 labelled clinical isolates. Among these, 96 were CPE genotypically characterized by PCR and sequencing. The limits of detection were calculated for the different carbapenem resistance genes in terms of cfu/mL. In addition, the kits were used to evaluate colonization of patients by CPE by comparing this assay with the Xpert® Carbo-R Kit on 127 rectal, perirectal and pharyngeal samples. Blood cultures from bacteraemias (4) and spiked blood cultures (23) with genotypically characterized isolates were also evaluated.

Results: The overall sensitivity and specificity of the multiplex PCR assay was 99% and 100%, respectively. The limit of detection for blaKPC, blaVIM, blaIMP and blaOXA-48-like is 60 cfu/mL and for blaNDM, 500 cfu/mL. The colonization and bacteraemia studies revealed a 100% agreement between the results obtained by this assay and the ones obtained by GeneXpert®.

Conclusions: The LightMix® modular carbapenemase kits are highly reliable and utilizable assays for both colonized and septic patients, and can help in the improvement of infection control. Their modular design facilitates cost-effective detection of CPE in hospital settings.

Introduction

Carbapenemase-producing Enterobacteriaceae (CPE) are emerging worldwide.1,2 Accurate detection of carriers is required, allowing rapid implementation of infectious control measures. Detection of CPE in clinical samples is also important, which requires highly reliable methods compatible with use in a clinical setting, which must be easy to perform, fast and cost effective.3 Classical methods for antimicrobial resistance detection are based on measuring the phenotype and expression levels.4–6 Other tests are based on enzyme hydrolysis by the antibiotics (e.g. proteomic methods), or the Carba-NP test,7–9 and others use immunological capture assays (e.g. the K-SetT assay).10,11 However, molecular methods are able to detect specific carbapenemase genes directly from rectal swabs, stools or other colonization sources and have proven excellent for the surveillance of carriers.12–15

The aim of this study was to evaluate whether the LightMix® modular carbapenemase kits (TIB Molbiol, Berlin, Germany) can be used to identify CPE by using a modular multiplex PCR targeting blaKPC, blaNDM, blaVIM, blaIMP and blaOXA-48-like carbapenem resistance genes, and to evaluate the possible application of these kits as a novel diagnostic method for CPE detection.

Materials and methods

Analytical evaluation

For initial studies of the evaluation of the kits, a total of 118 non-repeat Enterobacteriaceae (Tables 1 and 2), characterized by PCR and DNA sequencing,16–18 were streaked on a Blood Agar Plate (Becton Dickinson, NY, USA). After an overnight incubation at 37°C, DNA was extracted, by boiling for 30 min at 96°C. The LightMix® modular assays were run on a LightCycler® 480 II instrument (Roche Diagnostics).

According to the manufacturer, the KPC assay will detect KPC-1 to KPC-11, the NDM assay detects NDM-1 to NDM-13, NDM-16 and NDM-17, the VIM assay detects variants 1–36, the IMP assay detects IMP-17, -22 and -25-type, and the OXA-48 assay detects OXA-48.
and the OXA-48 assay detects major OXA-48-type members, in particular OXA-162, -163, -244, -245, -247 and most likely OXA-181, -204 and -232.

The experimental procedure was always performed with positive and negative controls contained in the kit. Reactions were performed in a LightCycler® Multiwell Plate 96 (Roche Diagnostics). For each reaction, 15 µL reaction mixture consisted of 1 µL of PCR-grade water (Roche Probes Master Kit), 1 µL of Uracil-DNA Glycosylase (Roche Diagnostics), 0.5 µL of each primer containing primers and probes (VIM, NDM, OXA-48, KPC and IMP), 0.5 µL of control reactionconst of each resistance gene were: 488 for VIM, 510 for NDM, 580 for OXA-48, 610 for KPC and 640 for IMP. For interpretation of results, a run was considered valid when the internal control was detected at a quantification cycle (Cp) < 37 cycles. All samples were considered as negative when Cp > 37. The negative control must show no signal. For high positive samples (Cp < 25) the internal control can be expected not to be visible. The isolates were subjected to analysis to evaluate the sensitivity and specificity of the multiplex PCR assay. One isolate of each carbapenemase gene group (Klebsiella pneumoniae blaOXA-24, K. pneumoniae blaNDM-1, Escherichia coli blaKPC-2 and K. pneumoniae blaIMP-2) was randomly selected to determine the limit of detection (LOD) in terms of cfu/mL. Linear regression analysis was performed in serial 10-fold dilutions in water, from 10^7 to 10 cfu/mL, analysing each one five times. These dilutions were tested in the same way as the samples. LOD values were determined at a threshold of 40 Cp.

### Evaluation for CPE bacteraemia confirmation

Twenty-three blood cultures spiked with genotypically characterized isolates were tested, plus four blood cultures from bacteremic ill patients. Spiked blood cultures were generated by inoculating blood culture flasks (Bactec TM, Aerobic/F Culture Vials, Becton Dickinson, Germany) containing 10 mL of human blood and 200 µL of a suspension of the respective isolate derived from a fresh overnight culture (with a turbidity equivalent to that of a 0.5 McFarland standard). The spiked isolates were four blaKPC, three blaOXA-24, four blaNDM, four blaIMP, five blaOXA-48 and three negative controls: one blaOXA-24, one blaKPC and one blaOXA-48. Incubation was performed in an automated system (Bactec FX, Becton Dickinson, Germany) until the flask was flagged positive. In blood cultures obtained directly from the patients, the procedure was applied after the flask flagged was positive and once the GeneXpert procedure had been positive. To 200 µL of sample (PBS from swab or blood culture) were added 180 µL of MagNA Pure Bacteria Lysis Buffer (Roche Diagnostics) and 20 µL of Proteinase K (20 µg/µL, Sigma–Aldrich, Germany). After incubation at 65°C for 10 min the solution was submitted to automatic DNA extraction, using the MagNA Pure Compact Nucleic Acid Isolation Kit (Roche Diagnostics). After 25 min, the extraction was complete and the multiplex PCR was performed as explained above.

### Clinical evaluation for CPE patient colonization screening

In total, 127 rectal, perirectal and pharyngeal samples were analysed during a period of 2 months from February to March 2016 in the Complejo Hospitalario Universitario A Coruña using the LightMix® modular carbapenemase kits. The results were compared with those obtained using the Xpert® Carba-R Kit (Cepheid, Sunnyvale, USA).13 When two swabs were collected from the same location and patient, one was used for testing by GeneXpert® following the manufacturer’s instructions and the second was used for testing by the LightMix® modular carbapenemase kits. The second swab was vortexed in 600 µL of PBS, from which 200 µL was used for extraction. If only one swab was available, 200 µL of sample was collected from the Xpert Carba-R Sample Reagent (5 mL, Cepheid).

### Results and discussion

The analytical evaluation revealed complete agreement between the results obtained for isolates tested by PCR and sequencing and the LightMix® modular kits, with an overall sensitivity of 99% and
Table 2. CPE (n=96) used for evaluating the LightMix® modular carbapenemase kits

| Species (n) | bla\textsubscript{KPC} | bla\textsubscript{NDM} | bla\textsubscript{VIM} | bla\textsubscript{IMP} | bla\textsubscript{OXA-48} | bla\textsubscript{OXA-181} | bla\textsubscript{OXA-247} | bla\textsubscript{OXA-248} | bla\textsubscript{OXA-249} | bla\textsubscript{OXA-250} | bla\textsubscript{OXA-251} | Total |
|-------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-------|
| Klebsiella pneumoniae | 12 | 1 | 2 | 2 | 9 | 2 | 25 | 1 | 1 | 1 | 1 | 1 | 16 |
| Enterobacter cloaceae | 2 | 1 | 3 | 1 | 3 | 2 | 1 | 1 |
| Enterobacter aerogenes | 1 | 2 | 1 | 1 |
| Serratia marcescens | 1 | 2 | 1 | 1 |
| Citrobacter freundii | 1 | 1 | 1 | 1 |
| Escherichia coli | 6 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Klebsiella oxytoca | 1 | 1 | 1 | 1 | 1 |
| Total | 16 | 5 | 23 | 11 | 41 |

\textsuperscript{a}Resistance genes submitted for evaluation of the LightMix® modular carbapenemase kits, previously characterized by PCR and sequencing.

\textsuperscript{b}Positive controls used for the evaluation of the LightMix® modular carbapenemase kits.

\textsuperscript{c}Number of isolates (n) of each species corresponding to the resistance genes characterized.

\textsuperscript{d}Enterobacteriaceae expressing non-carbapenemase resistance mechanisms, used as negative controls, remained negative. The LightMix® modular assay and the PCR sequencing.

\textsuperscript{e}The four blood cultures from bacteraemic patients were considered five targets for the full panel of carbapenem gene detection. The Carba-R Kit was positive for the full panel of carbapenem genes.

\textsuperscript{f}The Carba-R Kit was positive for the full panel of carbapenem genes.

\textsuperscript{g}According to the manufacturer, all swab and blood culture samples carrying 500 cfu/mL. Although the sensitivity is lower than those provided by the manufacturer, all swab and blood culture samples carrying the resistance mechanisms, used as negative controls, remained negative. The Carba-R Kit was in 100% agreement. Positive and negative predictive values of the multiplex PCR assay were 100%.

\textsuperscript{h}The four blood cultures from bacteraemic patients were considered five targets for the full panel of carbapenem gene detection. The Carba-R Kit was positive for the full panel of carbapenem genes.

\textsuperscript{i}According to the manufacturer, all swab and blood culture samples carrying 500 cfu/mL. Although the sensitivity is lower than those provided by the manufacturer, all swab and blood culture samples carrying the resistance mechanisms, used as negative controls, remained negative. The Carba-R Kit was in 100% agreement. Positive and negative predictive values of the multiplex PCR assay were 100%.

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Detection of CPE using the LightMix® modular carbapenemase kits

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Transparency declarations
None to declare.

References

1. Nordmann P, Poirot L. The difficult-to-control spread of carbapenemase producers among Enterobacteriaceae worldwide. Clin Microbiol Infect 2014; 20: 821–30.
2. Canton R, Akova M, Carmeli Y et al. European Network on Carbapenemases. Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. Clin Microbiol Infect 2012; 18: 413–31.
3. Bou G, Vila J, Seral C et al. Detection of carbapenemase-producing Enterobacteriaceae in various scenarios and health settings. Enferm Infecc Microbiol Clin 2014; 32: 24–32.
4. Pastoran F, Gonzalez LJ, Albornoz E et al. Triton Hodge test: improved protocol for modified Hodge test for enhanced detection of NDM and other carbapenemase producers. J Clin Microbiol 2016; 54: 640–9.
5. Heinrichs A, Nonhoff C, Roisin S et al. Comparison of two chromogenic media and enrichment broth for the detection of carbapenemase-producing Enterobacteriaceae on screening rectal swabs from hospitalized patients. J Med Microbiol 2016; 65: 438–41.
6. Simner PJ, Gilmour MW, DeGagne P et al. Evaluation of five chromogenic agar media and the Rosco Rapid Carb screen kit for detection and confirmation of carbapenemase production in Gram-negative bacilli. J Clin Microbiol 2015; 53: 105–12.
7. Sparbier K, Schubert S, Weller U et al. Matrix-assisted laser desorption ionization-time of flight mass spectrometry-based functional assay for rapid detection of resistance against β-lactam antibiotics. J Clin Microbiol 2012; 50: 927–37.
8. Oviño M, Barba MJ, Fernández B et al. Rapid detection of OXA-48-producing Enterobacteriaceae by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF). J Clin Microbiol 2016; 54: 754–9.
9. Dortet L, Agathine A, Naas T et al. Evaluation of the RAPIDEC® CARBA NP, the Rapid CARB Screen® and the Carba NP test for biochemical detection of carbapenemase-producing Enterobacteriaceae. J Antimicrob Chemother 2015; 70: 3014–22.
10. Meunier D, Vickers A, Pike R et al. Evaluation of the K-SeT R.E.S.I.T. immunochromatographic assay for the rapid detection of KPC and OXA-48-like carbapenemases. J Antimicrob Chemother 2016; 71: 2357–9.
11. Dortet L, Jousset A, Sainte-Rose V et al. Prospective evaluation of the OXA-48 K-SeT assay, an immunochromatographic test for the rapid detection of OXA-48-type carbapenemases. J Antimicrob Chemother 2016; 71: 1834–40.
12. Tenover FC, Canton R, Kop J et al. Detection of colonization by carbapenemase-producing Gram-negative bacilli in patients by use of the Xpert MDRO assay. J Clin Microbiol 2013; 51: 3780–7.
13. Tato M, Ruiz-Garbajosa P, Traczewski M et al. Multisite evaluation of Cepheid Xpert Carba-R assay for the detection of carbapenemase-producing organisms in rectal swabs. J Clin Microbiol 2016; 54: 1814–9.
14. Ellington MJ, Findlay J, Hopkins KL et al. Multicentre evaluation of a real-time PCR assay to detect genes encoding clinically relevant carbapenemases in cultured bacteria. Int J Antimicrob Agents 2016; 47: 151–4.
15. Findlay J, Hopkins KL, Meunier D et al. Evaluation of three commercial assays for rapid detection of genes encoding clinically relevant carbapenemases in cultured bacteria. J Antimicrob Chemother 2015; 70: 1338–42.
16. Oteo J, Saez D, Bautista V et al. Carbapenemase-producing Enterobacteriaceae in Spain in 2012. Antimicrob Agents Chemother 2013; 57: 6344–7.
17. Oteo J, Navarro C, Cercenado E et al. High-level of cefotaxime and cefazidime resistance in Escherichia coli: spread of clonal and unrelated isolates between the community, long-term care facilities and hospital institutions. J Clin Microbiol 2006; 44: 2359–66.
18. Pérez-Pérez FJ, Hanson ND. Detection of plasmid-mediated AmpC β-lactamase genes in clinical isolates by using multiplex PCR. J Clin Microbiol 2002; 40: 2153–62.
19. Dortet L, Fusaro M, Naas T. Improvement of the Xpert Carba-R kit for the detection of carbapenemase-producing Enterobacteriaceae. Antimicrob Agents Chemother 2016; 60: 3832–7.
20. Dortet L, Oueslati S, Jeannot K et al. Genetic and biochemical characterization of OXA-405, an OXA-48-type extended-spectrum β-lactamase without significant carbapenemase activity. Antimicrob Agents Chemother 2015; 59: 3823–8.