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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 bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP} and bla_{OXA-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® Carba-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 bla_{KPC}, bla_{VIM}, bla_{IMP} and bla_{OXA-48}-like is 60 cfu/mL and for bla_{NDM} 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-SeT 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 bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP} and bla_{OXA-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 most described variants, but will miss IMP-9, -16, -18, -22 and -25-type,

Table 1. Non-CPE ($n=22$) used for evaluating the LightMix® modular carbapenemase kits

	Resistance genes[a]						
	Negative controls[b]						
	bla_{EBC}	bla_{CMY}	bla_{DHA}		bla_{K-1}	bla_{CTX-M}	
Species (n)[c]	bla_{EBC}	bla_{CMY-2}	bla_{DHA-1}	bla_{DHA-6}	bla_{OXY-1}	$bla_{CTX-M-14}$	$bla_{CTX-M-15}$
Klebsiella pneumoniae			1	1		2	7
Enterobacter cloacae	3					1	
Enterobacter aerogenes							
Serratia marcescens							
Citrobacter freundii							
Escherichia coli		2					
Klebsiella oxytoca					5		
Total	3	2	2		5	10	

[a]Resistance genes submitted for evaluation of the LightMix® modular carbapenemase kits, previously characterized by PCR and sequencing.
[b]Negative controls used for the evaluation of the LightMix® modular carbapenemase kits. Among them, all *E. cloacae* bla_{EBC} ($n=3$) and two *K. pneumoniae* $bla_{CTX-M-15}$ displayed reduced susceptibility to carbapenems.
[c]Number of isolates (n) of each species corresponding to the resistance genes characterized.

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 reagent containing primers and probes (VIM, NDM, OXA-48, KPC and IMP), 0.5 μL of control reaction and 10 μL of Master (LC480 Probes Master, Roche Diagnostics). To 15 μL of reaction mixture, pipetted into each well, 5 μL of control or sample was added, to give a final volume of 20 μL. The plate was sealed, centrifuged and the run started. The protocol consists of three steps: denaturation at 95°C for 5 min, then cycling for 45 cycles of 95°C for 5 s, 60°C for 15 s and 72°C for 15 s, and finally cooling at 40°C for 30 s. The multiplex PCR was completed in <90 min.

For reading results, the analysis was performed using the second derivative maximum method with colour compensation. The channels used for 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* bla_{VIM-1}, *K. pneumoniae* bla_{NDM-1}, *Escherichia coli* bla_{OXA-48}, *Enterobacter cloacae* bla_{KPC-2} and *K. pneumoniae* bla_{IMP-22}) 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.

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).[13,19] 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).

Evaluation for CPE bacteraemia confirmation

Twenty-three blood cultures spiked with genotypically characterized isolates were tested, plus four blood cultures from bacteraemic 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 bla_{KPC}, three bla_{VIM}, four bla_{NDM}, four bla_{IMP}, five bla_{OXA-48} and three negative controls: one bla_{SHV-12}, one bla_{CMY-2} and one $bla_{CTX-M-15}$. 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.

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

	Resistance genes[a]																								
	Positive controls[b]																								
	bla_{KPC}		bla_{NDM}			bla_{VIM}			bla_{IMP}				bla_{OXA-48}-like												
Species (*n*)[c]	bla_{KPC-2}	bla_{KPC-3}	bla_{NDM-1}	bla_{NDM-7}	bla_{VIM-1}	bla_{VIM-2}	bla_{IMP-1}	bla_{IMP-13}	bla_{IMP-22}	bla_{IMP-28}	bla_{OXA-48}	$bla_{OXA-181}$	$bla_{OXA-232}$	$bla_{OXA-204}$	$bla_{OXA-162}$	$bla_{OXA-163}$	$bla_{OXA-405}$	$bla_{OXA-244}$	$bla_{OXA-245}$	bla_{OXA-48} + bla_{VIM-1}
Klebsiella pneumoniae	12	1	2	2	9				2		25			1	1	1			2	1
Enterobacter cloacae	2		1		3			1	3		2									
Enterobacter aerogenes											1									
Serratia marcescens	1				2												1			
Citrobacter freundii					1	1														
Escherichia coli					6		1		1	1	3	1	1					1		
Klebsiella oxytoca					1		1			1										
Total	16		5		23			11							41					

[a]Resistance genes submitted for evaluation of the LightMix® modular carbapenemase kits, previously characterized by PCR and sequencing.
[b]Positive controls used for the evaluation of the LightMix® modular carbapenemase kits.
[c]Number of isolates (*n*) of each species corresponding to the resistance genes characterized.

specificity of 100%. The assay was able to detect even bla_{NDM-7}, bla_{IMP-22}, $bla_{OXA-181}$ and $bla_{OXA-232}$, which the manufacturer did not claim the kit could detect, but was unable to detect $bla_{OXA-204}$. Enterobacteriaceae expressing non-carbapenemase resistance mechanisms, used as negative controls, remained negative with the LightMix® modular assay.

According to the manufacturer, using a plasmid DNA dilution, the LightMix® modular assay detects 5 genome equivalent copies per reaction or less, and about 10 copies for NDM genes. We were able to detect the resistance genes in all serial suspensions down to a dilution of 60 cfu/mL, except for bla_{NDM-1} with a LOD of 500 cfu/mL. Although the sensitivity is lower than those provided by the manufacturer, all swab and blood culture samples carrying a carbapenemase enzyme, even an NDM-type, tested positive.

Of the 127 rectal, perirectal and pharyngeal samples analysed, 1 was positive for bla_{VIM}, 3 were positive for bla_{NDM} and 28 were positive for bla_{OXA-48}-like, and all the remaining samples were negative. The LightMix® modular carbapenemase kits and the GeneXpert® Carba-R Kit were in 100% agreement. Positive and negative predictive values of the multiplex PCR assay were 100%.

Spiked blood cultures were analysed with a 100% agreement between the LightMix® modular assay and the PCR sequencing. The four blood cultures from bacteraemic patients were considered as bla_{OXA-48}-like by both the LightMix® Modular assay and the Xpert® Carba-R assay.

A limitation of the LightMix® modular carbapenemase kits (as well for the Xpert® Carba-R Kit) is that they give false positive results for $bla_{OXA-163}$, $bla_{OXA-405}$ and will certainly give these for $bla_{OXA-247}$ enzymes, which are OXA-48-like enzymes devoid of any carbapenemase activity.[20] Another limitation is the need for DNA extraction before running the PCR, extending the hands-on time of the procedure and the time taken for detection, and therefore increasing the secondary costs. However, the main advantage of the LightMix® modular carbapenemase kits is the direct cost of the technique, which is 75% lower than for the Xpert® Carba-R Kit (14€/determination compared with 55€ for the Xpert® Carba-R Kit). In addition, their modular design allows fewer targets to be run, according to the user's choice, lowering the price per test. If only one target is detected, the price is 5€/determination, increasing by 2€/determination up to the five targets for the full panel of carbapenem gene detection.

In conclusion, the LightMix® modular carbapenemase kits offer a reliable and novel pathway to detect CPE directly from rectal, perirectal and pharyngeal swab samples and from blood cultures, and should prove a valuable diagnostic tool in healthcare settings.

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Transparency declarations

None to declare.

References

1 Nordmann P, Poirel L. The difficult-to-control spread of carbapenemase producers among Enterobacteriaceae worldwide. *Clin Microbiol Infect* 2014; **20**: 821–30.

2 Canton R, Akóva 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 Pasteran 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 Oviaño 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.S.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 ceftazidime 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.