Brief report

A comparative study between Real-time PCR and LAMP to detect carbapenemase and/or extended-spectrum-β-lactamase genes *in Enterobacterales* directly from bronchoalveolar lavage fluid samples.

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Objectives: To evaluate and compare the efficacy of Real-time PCR (Xpert Carba-R) and LAMP (Eazyplex® SuperBug CRE) for detecting carbapenemase carriage in *Enterobacterales* directly from broncoalveolar lavage (BAL).

Methods: Negative BAL samples were spiked with 21 well-characterized carbapenemase-producing *Enterobacterales* strains to a final concentration of 10^2 to 10^4 CFU/mL. Xpert Carba-R, which detects five targets (*bla*$_{KPC}$, *bla*$_{NDM}$, bla$_{VIM}$, bla$_{OXA-48}$, and *bla*$_{IMP-1}$), and Eazyplex® SuperBug CRE system (Amplex-Diagnostics GmbH, Germany) that detects seven genes (*bla*$_{KPC}$, *bla*$_{NDM}$, bla$_{VIM}$, bla$_{OXA-48}$, *bla*$_{OXA-181}$, *bla*$_{CTXM-1}$, and *bla*$_{CTXM-9}$), were evaluated to detect these genes directly from BAL samples.

Results: Xpert Carba-R showed 100% agreement with carbapenemase characterization by PCR and sequencing for all final bacteria concentration. Eazyplex® SuperBug CRE showed 100%, 80% and 27% agreement with PCR and sequencing when testing 10^4, 10^3 and 10^2 CFU/mL, respectively. False negative results for Eazyplex® SuperBug CRE matched with highest Ct values for Xpert Carba-R. Hands-on time for both assays was about 15 minutes, but Eazyplex® SuperBug CRE results were available within 30 minutes, whereas Xpert Carba-R took around 50 minutes.

Conclusions: We here describe the successful use of two commercial diagnostic tests, Xpert Carba-R and Eazyplex® SuperBug CRE, to detect bacterial carbapenem resistance genes directly in lower respiratory tract samples. Our results could be used as proof-of-concept data for validation of these tests for this indication.

Introduction

Ventilator-associated pneumonia (VAP), the hospital-acquired pneumonia that develops in patients with tracheal intubation or on mechanical ventilation for at least 48 hours, is one of the leading causes of infection and death in the healthcare setting. Patients with VAP present longer periods with mechanical ventilation, as well as longer stay in the ICU and in the hospital.[1]

Unfortunately, the diagnosis of VAP is complicated and there is no a reliable reference test.[2] Microbiological diagnosis is necessary to confirm the clinical suspicion of VAP, but it is not exempt from limitations (negative culture due to empiric treatment, expensive molecular tests, difficulty to differentiate colonization from infection). Early and adequate treatment decreases the mortality,[3] but empirical treatment is usually initiated in patients with suspected VAP before having the definitive diagnosis,[4] which could cause unnecessary health care costs and risk of antibiotic resistance appearance.

Enterobacterales, such as *Escherichia coli* and *Klebsiella pneumoniae*, are among the most frequent pathogens causing VAP.[5] These microorganisms are also in the WHO priority pathogens list due to the carbapenem and 3rd generation cephalosporin resistance.[6]

The application of rapid diagnostic techniques to identify microbial pathogens seems to have a huge impact in the management of patients with VAP, reducing inappropriate or unnecessary antimicrobial treatments and mortality in these patients.[7,8,9]

Xpert Carba-R (Cepheid, Sunnyvale, USA) is an integrated commercial technique (sample extraction, amplification by PCR and detection carried out within a self-contained cartridge) that detects five targets for carbapenemase-producing organisms (bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{OXA-48}, and bla_{IMP-1}). On the other hand, the Eazyplex® SuperBug CRE system (Amplex-Diagnostics GmbH, Germany) combines loop-mediated isothermal amplification (LAMP) of the target and real-time photometric detection of amplified material for rapid and simple detection of extended-spectrum

beta-lactamase (ESBL) and carbapenemase-encoding genes (*bla*$_{CTXM-1}$, *bla*$_{CTXM-9}$, *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, and *bla*$_{OXA-181}$).

The aim of this study was to evaluate and compare the efficacy of Xpert Carba-R and Eazyplex® SuperBug CRE for detecting ESBL and carbapenemase carriage directly from broncoalveolar lavage (BAL) samples inoculated with a well-defined collection of *Enterobacterales* clinical isolates.

Material and Methods

Bacterial isolates and samples

Twenty-one *Enterobacterales strains* producing the following carbapenemases were included: four strains producing NDM, five strains producing OXA-48, one strain producing both NDM and OXA-48, one strain producing OXA-181, five strains producing KPC and five strains producing VIM (Table 1). Some of the strains were also carrying ESBLs (CTXM-1 or CTXM-9). These organisms were identified using MALDI-TOF (Bruker Daltonics, Bremen, Germany). The presence of genes encoding carbapenemases and ESBL (*bla*$_{\text{CTXM-1}}$, *bla*$_{\text{CTXM-9,}}$ *bla*$_{\text{KPC}}$, *bla*$_{\text{NDM}}$, *bla*$_{\text{VIM}}$, *bla*$_{\text{OXA-48}}$, *bla*$_{\text{IMP-1}}$ and *bla*$_{\text{OXA-181}}$) was confirmed by PCR amplification with specific primers and Sanger DNA sequencing.[10] Negative BAL samples by bacterial culture were collected from the Clinical Microbiology Laboratory at the Hospital Clinic of Barcelona (Spain) after being processed for routine techniques. They were mixed to obtain a homogeneous matrix and stored at -80ºC until use. Negative samples were homogenized by vortexing and spiked with a dilution of each of the selected strains to a final concentration of 10^2 to 10^4 CFU/mL. Two protocols depending on the method used are described in Figure 1.

Sample preparation for Xpert Carba-R

Spiked samples were mixed with sample reagent of the Xpert Carba-R assay (vol:vol), vortexed and incubated at room temperature for ten minutes, vortexing each five minutes. Then, 1.7 mL of the sample reagent was transferred into the cartridge and the assay was run on the GeneXpert platform (Cepheid, Sunnyvale, USA) according to the manufacturer's instructions.

Sample preparation for Eazyplex® SuperBug CRE

Same volume of spiked sample (850 µL) was centrifuged at 14,000 x g for 5 min., the pellet was resuspended in 500 µL of resuspension and lysis fluid (RALF), and incubated in a thermal block (99°C) for 2 min. After centrifugation, 25 µL of the supernatant were added to each tube of the strip containing the ready-to-use mastermix. The strip was immediately placed into the Genie II instrument (OptiGene, Horsham, United Kingdom). The reaction mixtures were incubated at 66°C for 30 min with fluorescence monitoring. The Eazyplex® SuperBug CRE test strip contains eight wells with six oligonucleotide primers in each well, allowing the simultaneous specific amplification of seven different resistance genes, plus an internal control.

Analysis

We evaluated the agreement between the results obtained by conventional methods and rapid test applied directly to the clinical sample. Time to obtain the results and agreement were compared between Xpert Carba-R and Eazyplex® SuperBug CRE. Statistical analysis was performed with the software R (version 3.4.4).

Results and discussion

Considering the Xpert Carba-R, 100% agreement with PCR and sequencing was observed with all concentrations (Table 1). Regarding Eazyplex® SuperBug CRE, the concordance was 100%, 80% and 27% when testing 10^4, 10^3 and 10^2 CFU/mL, respectively. False negative results for Eazyplex® SuperBug CRE matched with highest Ct values for Xpert Carba-R (median Ct of 33.2 and 28.3 for negative and positive Eazyplex SuperBugCRE resultls, respectively; $p<0.001$). At 10^2 CFU/mL, most of the genes were not detected in contrast to Xpert Carba-R. This is an integrated assay that includes a DNA extraction step, which increases the amount of DNA for the amplification step compared to LAMP assay.

Hands-on time for both assays was about 15 minutes, but with an easier-to-use sample preparation for Xpert Carba-R. Regarding time-to-results, Eazyplex SuperBugCRE results were available within 30 minutes, whereas Xpert Carba-R took around 50 minutes.

In order to distinguish between infection and colonization, it is established that a concentration of microorganisms in BAL $\geq 10^4$ CFU/mL is indicative of infection,[12] although several factors can modify this interpretation. In a previous study evaluating Xpert Carba-R on BAL samples, a cutoff value to distinguish between colonization (Ct ≤24.7 corresponded to a count of $\geq 10^5$ CFU/mL) and infection (Ct >26.9 corresponded to a count of $<10^4$ CFU/mL) was proposed.[11] Although we observed a good correlation between the Ct value for Xpert Carba-R and the bacteria concentration ($r=-0.81$, $p<0.001$), due to the limited number of samples we could not establish such a cutoff. This was not observed for Eazyplex® SuperBug CRE results ($r=0.45$, $p<0.001$). Although both assays were able to detect the target genes at bacterial concentrations below the diagnostic limit for infection in BAL, the Xpert Carba-R showed a limit of detection 10-fold lower than that of the Eazyplex® SuperBug CRE.

We here describe the successful use of two commercial diagnostic tests to detect bacterial carbapenem resistance genes directly in lower respiratory tract samples: Xpert Carba-R designed to

identify patients showing gastrointestinal colonization with carbapenemase-producing organisms, and Eazyplex® SuperBug CRE designed to detect carbapenemases and ESBL from bacteria strains or direct from positive blood culture. Our results could be used as proof-of-concept for the development of studies with larger sample size to validate these tests for this indication.

Acknowledgements

We want to thank Dr. Luis Martínez-Martínez to provide us with some strains.

Funding

This work was supported by Ajut a la Recerca “Clínic-LaPedrera” 2016 (PEP:HB-16-JF-VG-C) and grant 2014SGR0653 from the Departament de Universitats, Recerca i Societat de la Informació de la Generalitat de Catalunya; by Plan Nacional de I+D+i 2013-2016, Instituto de Salud Carlos III, Subdirección General de Redes y Centros de Investigación Cooperativa, Ministerio de Economía y Competitividad, Spanish Network for Research in Infectious Diseases (REIPI RD16/0016/0010) and the 2017 call for Strategic Action on Health (PI17/01932) and (PI17/01468), co-financed by European Development Regional Fund "A way to achieve Europe" and operative program Intelligent Growth 2014-2020; and by the Innovative Medicines Initiative (Combacte-Care, grant agreement 115620). I.R. was supported by the Department of Health, Generalitat de Catalunya, grant SLT002/16/00349.

Transparency declarations

None to declare.

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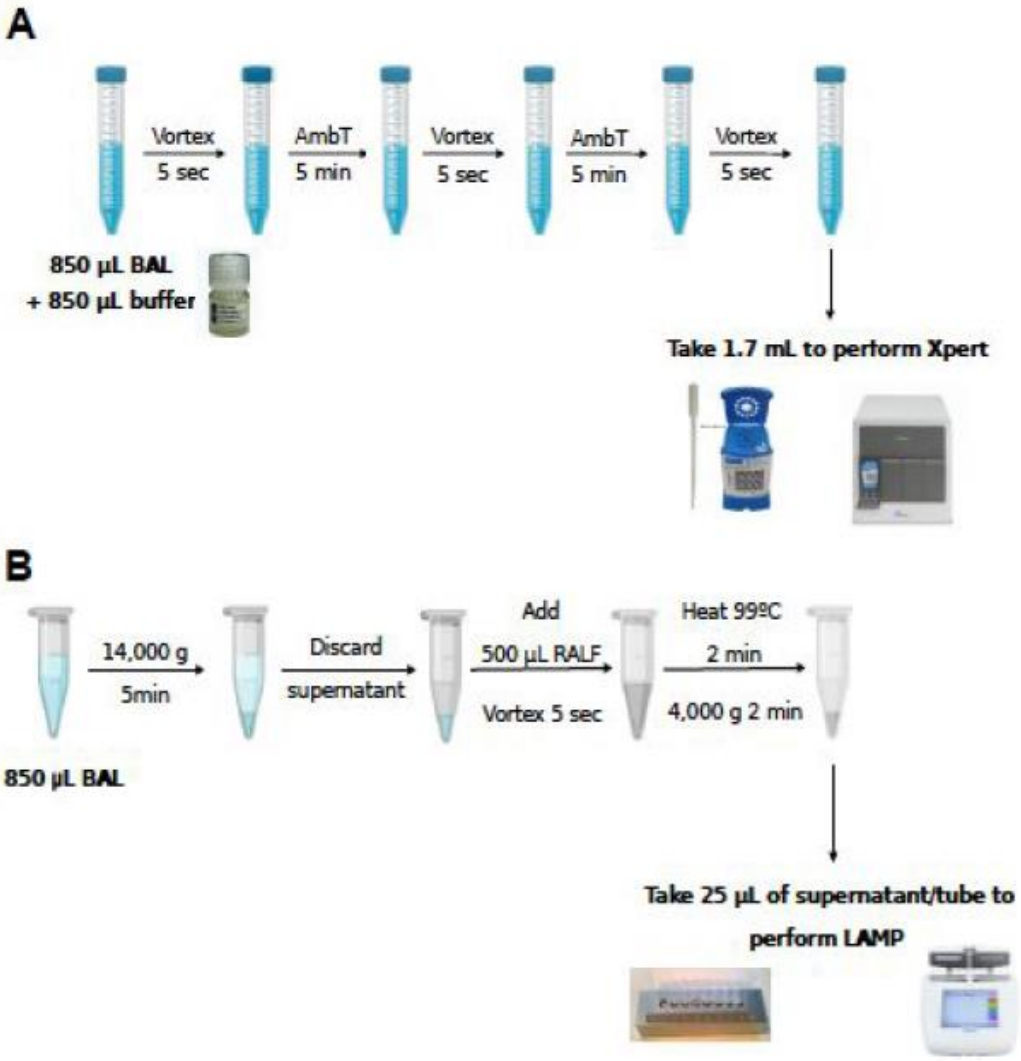
187 **Table 1**. Results of Xpert Carba-R and Eazyplex® SuperBug CRE for each strain and concentration

188 expressed in Ct values and time, respectively.

Figure 1. Sample preparation workflow for Xpert Carba-R (Figure 1A) and Eazyplex® SuperBug CRE (Figure 1B).

BAL: bronchoalveolar lavage; AmbT: ambient temperature; RALF: resuspension and lysis buffer.



195 **Table 1**. Results of Xpert Carba-R and Eazyplex® SuperBug CRE for each strain and concentration
196 expressed in Ct values and time, respectively.

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Bacterial species	Resistance mechanism	Concentration (ufc/mL)	Xpert Carba-R (Target Ct)	Eazyplex® SuperBug CRE (Target min:sec)
Klebsiella pneumoniae 16-516	NDM CTXM-1	10^4	NDM 26.3	NDM 9:45; CTXM-1 10:00
		10^3	NDM 28.4	NDM 14:15; CTXM-1 15:30
		10^2	NDM 29.3	NDM NEG; CTXM-1 NEG
K. pneumoniae 16-377	NDM CTXM-1	10^4	NDM 26.6	NDM 11:45; CTXM-1 10:15
		10^3	NDM 30.5	NDM 17:00; CTXM-1 13:30
		10^2	NDM 35.4	NDM NEG; CTXM-1 16:30
Escherichia coli DVR22	NDM CTXM-1	10^4	NDM 24.2	NDM 8:30; CTXM-1 10:00
		10^3	NDM 27.5	NDM 13:30; CTXM-1 11:00
		10^2	NDM 30.5	NDM NEG; CTXM-1 22:15
E. coli 16-543	NDM CTXM1	10^4	NDM 25.7	NDM 8:15; CTXM-1 6:00
		10^3	NDM 27.2	NDM 9:30; CTXM-1 8:30
		10^2	NDM 30.6	NDM 15:15; CTXM-1 10:00
K. pneumoniae HR4	NDM OXA-48 CTXM1	10^4	NDM 27.0; OXA-48 26.7	NDM 12:00; OXA-48 17:15; CTXM-1 11:45
		10^3	NDM 31.4; OXA-48 31.2	NDM NEG; OXA-48 NEG; CTXM-1 12:00
		10^2	NDM 33.4; OXA-48 33.4	NDM NEG; OXA-48 NEG; CTXM-1 19:00
K. pneumoniae 16-420	OXA-48	10^4	OXA-48 28.2	OXA-48 11:30
		10^3	OXA-48 29.8	OXA-48 18:00
		10^2	OXA-48 30.4	NEG
K. pneumoniae 45-425	OXA-48 CTXM1	10^4	OXA-48 26.3	OXA-48 10:45; CTXM1 9:45
		10^3	OXA-48 30.5	OXA-48 13:15; CTXM1 11:30
		10^2	OXA-48 33.1	NEG
E. coli 16-476	OXA-48	10^4	OXA-48 27.0	OXA-48 12:30
		10^3	OXA-48 28.3	OXA-48 20:00
		10^2	OXA-48 31.3	NEG
K. pneumoniae 66134	OXA-48 CTXM1	10^4	OXA-48 29.7	OXA-48 13:30; CTXM1 10:30
		10^3	OXA-48 31.7	OXA-48 17:00; CTXM1 13:30
		10^2	OXA-48 35.3	OXA-48 NEG; CTXM1 NEG
E. coli 65273	OXA-48	10^4	OXA-48 29.4	OXA-48 13:45
		10^3	OXA-48 29.9	OXA-48 14:30
		10^2	OXA-48 30.3	OXA-48 NEG

E. coli 15-288	OXA-181 CTXM1	10^4	OXA-48 28.0	OXA-181 14:15; CTXM1 12:00
		10^3	OXA-48 31.0	OXA-181 23:30; CTXM1 NEG
		10^2	OXA-48 33.4	OXA-181 NEG; CTXM1 NEG
K. pneumoniae MB545	KPC	10^4	KPC 28.3	KPC 19:30
		10^3	KPC 31.1	NEG
		10^2	KPC 35.3	NEG
K. pneumoniae 45872	KPC	10^4	KPC 28.5	KPC 17:15
		10^3	KPC 32.6	KPC 21:00
		10^2	KPC 35.9	NEG
E. coli 182046440	KPC	10^4	KPC 28.6	KPC 16:45
		10^3	KPC 31.2	KPC 22:50
		10^2	KPC 35.5	NEG
Enterobacter asburiae	KPC CTXM1	10^4	KPC 28.6	KPC 18:15; CTXM-1 13:15
		10^3	KPC 30.4	KPC 18:45; CTXM-1 14:00
		10^2	KPC 33.1	KPC NEG; CTXM-1 NEG
K. pneumoniae 19-684	KPC CTXM1	10^4	KPC 28.1	KPC 16:00; CTXM-1 11:00
		10^3	KPC 31.0	KPC 21:00; CTXM-1 11:45
		10^2	KPC 34.8	KPC NEG; CTXM-1 NEG
K. pneumoniae 18-87	VIM	10^4	VIM 22.3	VIM 13:00
		10^3	VIM 28.3	VIM 19:45
		10^2	VIM 31.1	NEG
E. coli 18-38	VIM	10^4	VIM 24.1	VIM 9:30
		10^3	VIM 26.7	VIM 12:15
		10^2	VIM 30.6	VIM 19:45
K. pneumoniae MC-32-145	VIM	10^4	VIM 25.1	VIM 15:30
		10^3	VIM 28.9	VIM 18:00
		10^2	VIM 31.3	VIM 19:30
Enterobacter cloacae MC-13-3	VIM CTXM9	10^4	VIM 27.2	VIM 12:15; CTXM9 14:15
		10^3	VIM 32.5	VIM 16:15; CTXM9 18:30
		10^2	VIM 36.7	VIM NEG; CTXM9 19:45
E. coli MC-4-19	VIM CTXM9	10^4	VIM 25.8	VIM 12:45; CTXM9 17:30
		10^3	VIM 29.9	VIM 18:20; CTXM9 18:15
		10^2	VIM 36.8	VIM NEG; CTXM9 NEG

198
199