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Eazyplex® SuperBug CRE system for the rapid detection of carbapenemase and extended spectrum beta-lactamase genes in gram-negative bacteria

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Abstract

The purpose of this study was to evaluate the performance of the Eazyplex® SuperBug CRE system, which consists of a loop-mediated isothermal amplification (LAMP)-based system for the detection of resistance genes in gram-negative rods. A total of 215 isolates were included in the study after showing resistance to third generation cephalosporins and/or carbapenems. One sample per patient was selected. In 10 cases, the test was performed directly from the positive blood culture. Phenotypic methods for the detection of extended spectrum β-lactamases and carbapenemases were performed. Results were analysed to establish the efficiency of the Eazyplex® SuperBug CRE system. The system correctly detected $bla_{\text{CTX-M}}$ genes and *bla* carbapenemase genes in all the studied strains. Total concordance was observed between the results obtained using the Eazyplex® Superbug CRE system and phenotypic results compatible with the presence of a carbapenemase and/or ESBL.

The eazyplex® SuperBug CRE system has proved to be an easy-to-use tool for the detection of carbapenemases, as well as CTX-M-type ESBLs in only 15 minutes. The system provides information that can improve the antimicrobial therapy results, and it can be useful as an epidemiologic tool in fighting against the spread of multidrug resistant bacteria.

Introduction

Bacterial resistance to antibiotics has become an important threat and it is a major source of concern for public health [1]. Gram-negative rods (especially *Enterobacteriaceae*) have the ability to easily spread among humans and are also able to acquire resistance genes through horizontal gene transfer, mediated mostly by plasmid and transposons [2].

Rapid diagnostic tools that allow resistance genes detection such as the Eazyplex® SuperBug CRE system can help to control the spread of extended spectrum β-lactamase (ESBL) and carbapenemase producers, as well as to better and earlier adjust antimicrobial treatment [3,4], especially when it comes to complicated infections.

The aim of this study was to evaluate the Eazyplex® SuperBug CRE system as a rapid molecular technique for the detection of ESBL and carbapenemase genes among Gram-negative bacteria isolated at the Microbiology Department of Consorcio Hospital General Universitario de Valencia (Spain) during a one-year period.

Materials and methods

The study took place from 1st December 2016 until 30rd November 2017 at the Microbiology Service of Consorcio Hospital General Universitario de Valencia (Spain). A total of 215 microorganisms were included in the study (Table 1) after having been examined according to the laboratory routine testing, using broth microdilution and interpreting the results according to Clinical & Laboratory Standards Institute (CLSI) [5]. Gram-negative rods isolated from clinical samples and active surveillance which were resistant to third generation cephalosporins and/or carbapenems were chosen. The suspicion of ESBL and/or carbapanemase production was inferred from the β-lactams minimal inhibitory concentration (MIC) profiles obtained with the MicroScan system (SIEMENS, West Sacramento, CA, USA) [6]. These 215 isolates were then submitted to the test and at the same time, phenotypic methods for detection of resistance were performed. Only one sample per patient was selected. In ten of the 215 patients included in the study, the test was performed directly from positive blood cultures. They were all at the Intensive Care Unit (ICU) and were already colonized by carbapenemase-producing gram-negative bacteria.

The Eazyplex® SuperBug CRE system (Amplex Biosystems GmbH, Giessen,Germany) [7] is a qualitative genotypic diagnostic test consisting of a freeze-dried, ready-to-use mixture for an isothermal amplification reaction that covers carbapenemase variants of the VIM (-1 to-37), NDM (-1 to -7) and KPC (-2 to -15) families, part of the OXA-48 family (-48,-162,-204 and -244),OXA-181 and the CTX-M ESBL families (CTX-M-1 group and CTX-M-9 group) from gram-negative bacteria. Amplification products that are generated by loop-mediated isothermal amplification (LAMP) are visualized by real-time fluorescence measurement of a fluorescent dye bound to double-stranded DNA using the GENIE II (OptiGene, Horsham, UK) instrument.

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Colonies of the isolates were inoculated with a loop in RALF buffer (provided with the kit) and incubated for 2´at 99ºC, then pipetted in the lyophilized test tubes for amplification reaction in the Eazyplex® Superbug CRE system. Detection of carbapenemase and ESBL genes takes only 15 minutes and it is performed for Gram-negative single bacterial colonies or even directly from positive blood culture bottles.

Special features for positive blood cultures: After performing gram-staining to make sure they had a bloodstream infection caused by gram-negative rods, 25 µL of the blood were pipetted into the tubes provided in the kit, then heated at 99ºC for 2 minutes, and then centrifuged at low speed (4000 rpm) for 1 minute. 25 µL of the lysate is pipetted into each tube of the Eazyplex strip and the test can run as from bacterial colonies.

Phenotypic detection of extended spectrum β-lactamases was performed by the double-disc synergy test and with the ESBL Confirm Kit (ROSCO Diagnostica, Taastrup Denmark). Phenotypic methods for carbapenemase production detection included: β-Carba test (BIORAD), the inhibition profile using ROSCO KITS™ (KPC, MBL and OXA-48 Confirm Kit), Imipenem/Imipenem + EDTA MIC Test Strips for in vitro detection of Metallo β-lactamases (Liofilchem) and inmunocromatography for the detection of OXA-48 production (Letitest). The strains *Escherichia coli* ATCC 25922 (wild type) and *Klebsiella pneumoniae* ATCC BAA-1705 (KPC-2 producer) were used as negative and positive controls, respectively.

Results obtained using the eazyplex® Superbug CRE system were compared to those obtained with phenotypic methods, and later classified as concordant or discordant.

Results

The most frequently detected extended spectrum β-lactamase was CTX-M-1, which appeared in 167 cases (alone or together with a carbapenemase), whereas CTX-M-9 was only found in 7 cases (5 of them together with a carbapenemase).

The most frequently detected carbapenemase in this period of time was OXA-48 (153), followed by NDM Metallo-β-lactamase (33), both mostly found in *Klebsiella pneumoniae*. KPC production was only found in one *Enterobacter cloacae* isolate and in one *Klebsiella pneumoniae* isolate. Regarding non-*Enterobacteriaceae*, among the 20 isolates of *Pseudomonas aeruginosa* that were included in the study, all of them harboured VIM Metallo-β-lactamase, and in one case there was co-production of ESBL+ Metallo-β-lactamase.

Among the 153 isolates that harboured bla_{OXA-48}, 137 of them were bla_{CTXM} producers (CTXM-1 group and CTXM-9 group). bla_{OXA-48} and bla_{NDM} co-production was detected in 14 cases, all of them co-producing ESBL as well.

Concordance of 100% was observed between the results obtained using the Eazyplex® Superbug CRE system and the phenotypic methods, including those obtained directly from positive blood cultures: all of them were positive for bla_{OXA-48} and bla_{CTXM-1}, identified as *Klebsiella pneumoniae* and were later concordant with the MIC profiles and phenotypic methods. The double-disc synergy test was sensitive enough to confirm the presence of an ESBL when co-expressed with a carbapenemase. Furthermore, the ROSCO KITS™ was able to detect co-production of carbapenemases.

In table 1 gram-negative bacteria included in the study are described and classified according to the ESBL and/or carbapenemase-encoding gene and it also the results inferred from the phenotypical methods.

Table 1. Resistance genes detected in 215 Gram-negative bacteria isolates

Microorganism (total no of isolates)	Genes detected by the Eazyplex® Superbug CRE system	Phenotypically detected resistance mechanism
Enterobacter cloacae (3)	bla_{KPC} (1) bla_{OXA-48} (2)	ESBL OXA-48
Escherichia coli (6)	bla_{CTXM-1} (2) bla_{CTXM-9} (1) bla_{OXA-48}+bla_{CTXM-1} (2) bla_{OXA-48}+bla_{CTXM-9} (1)	ESBL ESBL OXA-48+ESBL OXA-48+ESBL
Klebsiella pneumoniae (182)	bla_{CTXM-1} (15) bla_{CTXM-9} (3) bla_{OXA-48} (14) bla_{OXA-48}+bla_{CTXM-1} (113) bla_{OXA-48}+bla_{CTXM-9} (4) bla_{OXA-48}+bla_{NDM}+bla_{CTXM-1} (14) bla_{NDM} (3) bla_{NDM}+bla_{CTXM-1} (15) bla_{KPC}+bla_{CTXM-1} (1)	ESBL ESBL OXA-48 OXA-48+ESBL OXA-48+ESBL OXA-48+ Metallo-β-lactamase +ESBL Metallo-β-lactamase Metallo-β-lactamase +ESBL Class A carbapenemase+ESBL
Klebsiella oxytoca (2)	bla_{OXA-48}+bla_{NDM}+bla_{CTXM-1} (1) bla_{OXA-48}+bla_{CTXM-1} (1)	OXA-48+ Metallo-β-lactamase +ESBL OXA-48 +ESBL
Pseudomonas aeruginosa (20)	bla_{VIM} (19) bla_{VIM}+bla_{CTXM-1} (1)	Metallo-β-lactamase Metallo-β-lactamase + ESBL
Proteus mirabilis (1)	bla_{CTXM-1} (1)	ESBL
Serratia marcescens (1)	bla_{OXA-48}+bla_{CTXM-1} (1)	OXA-48+ ESBL

Discussion

After analysing the phenotypic detection of both carbapenemase and ESBL production, kappa coefficient (κ) of 1 was observed when comparing the results with the Eazyplex® SuperBug CRE system results, as shown in table 1**.** It is important to mention the correct detection of both ESBL and carbapenemase co-production, as well as carbapenemase co-production, which can sometimes be challenging to identify when only using phenotypic methods.

Preliminary results from positive blood cultures are promising (no previous studies about this specific subject have been performed), but further studies are assured, as only ten samples with these characteristics were studied.

Results with non-*Enterobacteriaceae* microorganisms were also evaluated favourably, as testing with *Pseudomonas aeruginosa* was successful in all cases. One anaerobe, *Bacteroides fragilis,* was also tested yielding a concordant ESBL-positive result.

One limitation of the test would be that it only covers the most spread resistance genes for ESBL and carbapenemase and would not work in case an isolate carried an infrequent resistance gene. However, it succeeded to cover the genes in all our isolates.

To summarize, patients infected by multidrug resistant Gram-negative bacteria have higher mortality rates and thus require an effective and quickly adjusted treatment. The use of rapid methods for the detection of resistant microorganisms is really important (especially if it can be done directly from positive blood cultures), as it provides information that can improve antibiotic therapy results as well as the patient's outcome. On the other hand, the Eazyplex® Superbug CRE system could also be used as a tool for epidemiology issues for each Healthcare centre, and thus implement the proper hygiene measures in order to avoid the spread of these threatening multidrug resistant bacteria.

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Conflict of interest

The authors have declared no conflict of interest.

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