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Evaluation of the eazyplex® SuperBug CRE system for rapid detection of carbapenemases and ESBLs in clinical Enterobacteriaceae isolates recovered at two Spanish hospitals

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Objectives: To evaluate the performance of the eazyplex® SuperBug CRE system, a loop-mediated isothermal amplification (LAMP)-based system, for confirming the presence of carbapenemases in addition to CTX-M-type ESBLs in previously genotypically and/or phenotypically characterized clinical Enterobacteriaceae isolates recovered in two centres in Spain.

Methods: A collection of 94 carbapenemase-producing strains previously characterized by conventional PCR and sequencing and a total of 45 prospectively collected isolates with phenotypes compatible with the presence of a carbapenemase were tested with the eazyplex® SuperBug CRE system. In both cases, the presence of an ESBL was also assessed. Results were evaluated to establish the accuracy of this rapid LAMP-based system as well as to determine the concordance between all approaches.

Results: The eazyplex® SuperBug CRE system correctly detected *bla* carbapenemase genes with or without $bla_{CTX\text{-}M}$ genes in 100% of the molecularly characterized strains. Absolute concordance (100%) was also observed in the case of isolates with phenotypes compatible with the presence of a carbapenemase with or without an ESBL inferred by susceptibility patterns and phenotypic inhibitory profiles. Determinations performed with the eazyplex® SuperBug CRE system took 15 min.

Conclusions: The eazyplex® SuperBug CRE system proved to be a powerful tool for the detection of different carbapenemases as well as CTX-M-type ESBLs in Enterobacteriaceae with a rapid resolution time. The test has the high-performance parameters attributable to the sensitivity and specificity already demonstrated by LAMP-based assays. These results assure the usefulness of this test for routine rapid confirmation of carbapenemase-producing Enterobacteriaceae.

Keywords: isothermal amplification, β-lactamases, LAMP

Introduction

The emergence and subsequent wide dissemination of a variety of bacterial species producing acquired carbapenemases encoded by transmissible plasmids are responsible for an unprecedented public healthcare threat of global dimensions.[1,2] Rapid detection of these types of isolates, capable of efficaciously colonizing and/or infecting both community and in-hospital patients, is essential to implement containment measures to prevent and limit the spread of carbapenemase producers.[3] This detection is complex due to the increasing diversity of carbapenemase enzymes with variable expression rendering different phenotypes.[4,5]

The aim of this study was to evaluate the performance of the eazyplex® SuperBug CRE system using: (i) a collection of genotypically characterized carbapenemase-producing Enterobacteriaceae (CPE) isolates; and (ii) a group of contemporary clinical isolates with a carbapenemase-producer phenotype prospectively collected in two Spanish hospitals.

Materials and methods

The eazyplex® SuperBug CRE system (Amplex Biosystems GmbH, Giessen, Germany) 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; excluding OXA-181) and the CTX-M-1 and CTX-M-9 ESBL families 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.[6]

Table 1. Characteristics of the 94 Enterobacteriaceae isolates with characterized carbapenemase-mediated resistance mechanisms with or without ESBL-mediated resistance mechanisms

Resistance mechanisms	Microorganisms (no. of isolates)
KPC-2	*K. pneumoniae* (2), *Enterobacter cloacae* (2), *E. coli* (1)
KPC-3	*K. pneumoniae* (22), *E. cloacae* (1), *E. coli* (2)
KPC-3 + CTX-M-15	*K. pneumoniae* (1)
VIM-1	*K. pneumoniae* (2), *Klebsiella oxytoca* (1), *E. coli* (2), *E. cloacae* (3), *Citrobacter freundii* (1), *Serratia marcescens* (2), *Raoultella ornithinolytica* (1)
VIM-1 + CTX-M-10	*K. oxytoca* (1)
VIM-1 + CTX-M-15	*K. pneumoniae* (1), *K. oxytoca* (1)
VIM-1 + CTX-M-32	*E. coli* (1)
VIM-1 + CTX-M-14	*K. oxytoca* (1)
NDM-1	*Providencia rettgeri* (1)
NDM-5	*E. coli* (1)
OXA-48	*K. pneumoniae* (3), *K. oxytoca* (1), *E. coli* (2), *E. cloacae* (1), *Citrobacter koseri* (1), *Citrobacter braakii* (1)
OXA-48 + CTX-M-15	*K. pneumoniae* (30), *E. coli* (2), *Enterobacter aerogenes* (1), *Citrobacter amalonaticus* (1)
OXA-48 + CTX-M-14	*E. coli* (1)

Detection of carbapenemase and ESBL genes, which takes 15 min, is performed for Gram-negative single bacterial colonies according to the manufacturer's instructions (http://www.hyplex.de).

In this study, all tested isolates were Enterobacteriaceae recovered at the microbiology laboratories of the Ramón y Cajal University Hospital (Madrid, Spain) and the Clínic University Hospital (Barcelona, Spain). A collection (2009–13) of 94 strains with carbapenemase genes previously characterized at the molecular level, with or without concomitant ESBL genetic determinants, was included (Table 1). Molecular typing was performed by conventional PCR assays and sequencing according to previously published studies.[7–9] Additionally, a total of 45 prospectively collected routine clinical isolates (February–June 2014) with phenotypic resistance compatible with the presence of a carbapenemase, with or without an ESBL, were also tested (Table 2). In this latter group, the first approach towards the possible presence of a carbapenemase was inferred from the β-lactam MIC profiles obtained with the MicroScan System (Siemens, West Sacramento, CA, USA) or the Phoenix System (BD Diagnostic Systems, Sparks, MD, USA) considering the updated EUCAST clinical breakpoints and the corresponding guidelines for detection of these organisms.[10,11] These isolates were then submitted to the eazyplex® SuperBug CRE system test and, simultaneously, to assessment of the inhibition-based profile using the ROSCO KPC/Metallo-β-lactamase and OXA-48 Confirm Kit (ROSCO Diagnostica, Taastrup, Denmark) and the modified Hodge test (MHT).[12] Detection of ESBLs was performed by the double-disc synergy test.[13] *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC BAA-1705 (KPC-2 producer) were used as negative and positive controls, respectively. Genetic relatedness among the most numerous *K. pneumoniae* subgroups was previously determined.[9,14]

Results and discussion

Considering the collection of genotypically characterized CPE isolates, 100% agreement was observed between PCR and sequencing and the eazyplex® SuperBug CRE system results (Table 1). Again, when analysing contemporary isolates, 100% concordant results were found between both the inferred phenotype and the eazyplex® SuperBug CRE system results (Table 2). Moreover, full consistency was observed with the carbapenemase inhibition profile given by specific ROSCO tablets and the MHT when combining both test results. Furthermore, the double-disc synergy test was sensitive enough to confirm the presence of an ESBL when coexpressed with a carbapenemase. Cycle threshold (Ct) values

Table 2. Contemporary clinical Enterobacteriaceae isolates: compared performance between the eazyplex® SuperBug CRE system and the inferred resistance phenotype

Phenotypically inferred resistance mechanisms (no. of isolates)	Microorganisms (no. of isolates)	Resistance mechanisms identified by the eazyplex® SuperBug CRE system (no. of isolates)
Class A carbapenemase (1)	*E. cloacae* (1)	KPC (1)
MBL (9)	*K. pneumoniae* (2), *K. oxytoca* (1), *E. cloacae* (4), *E. aerogenes* (1), *C. freundii* (1)	VIM (9)
MBL + ESBL (2)	*E. coli* (1), *E. cloacae* (1)	VIM + CTX-M-1-group (2)
OXA-48 family (8)	*K. pneumoniae* (3), *E. coli* (3), *C. freundii* (1), *R. ornithinolytica* (1)	OXA-48 (8)
OXA-48 family + ESBL (22)	*K. pneumoniae* (21), *E. coli* (1)	OXA-48 + CTX-M-1-group (22)
OXA-48 family + ESBL (3)	*Kluyvera ascorbata* (2), *E. coli* (1)	OXA-48 + CTX-M-9-group (3)

MBL, metallo-β-lactamase.

(minutes:seconds) for the genes detected by the eazyplex® SuperBug CRE system ranged from 4:00 to 7:00 for the VIM group, from 4:45 to 8:00 for the OXA-48 group, from 5:15 to 9:45 for the KPC group, from 3:45 to 7:30 for the CTX-M group and from 4:00 to 4:45 for the NDM group; the range was 6:00–10:45 for the internal control amplification. A limitation of our study was the lack of isolates expressing more than one carbapenemase simultaneously, which made this aspect untestable, although this epidemiological feature is still infrequent in Spain.[14–16] Interestingly, high-risk clones currently circulating in Spain were observed. Overall, the KPC-3-producing *K. pneumoniae* isolates included ST384, ST659 and ST454, while ST20, ST11 and ST971 were less represented. Moreover, among the *K. pneumoniae* isolates harbouring OXA-48+CTX-M-1-group, two dominant clones were represented: ST11 and ST405.

Infected patients with CPE have increased mortality rates and thus require timely and rapidly efficacious treatment.[17] Moreover, colonization with these types of isolates requires rapid detection to prevent and limit their dissemination in the hospital environment. It has been recently stressed that the control of their spread is still possible and mainly relies on the use of rapid diagnostic techniques and strict implementation of hygiene measures.[3] The availability of a rapid test for confirming the presence of such resistance determinants is also essential to expedite therapeutic decision making. However, heterogeneous expression of carbapenemases and complex phenotypes may hamper laboratory detection by conventional phenotypic confirmatory tests including automated antimicrobial susceptibility systems, even with updated carbapenem breakpoint values. Moreover, in many cases, long response times with these methods as well as with conventional PCR techniques may not be clinically useful, particularly in the case of severely ill patients. The eazyplex® SuperBug CRE system demonstrated high versatility and accuracy for the rapid detection of isolates harbouring various carbapenemase resistance determinants. It is of note that the ample spectrum of genes and gene variants included in this system reflects those mostly prevalent worldwide. Other rapid tests have also been implemented, including colorimetric assays such as the Carba NP test and the Blue-Carba test (ROSCO Diagnostica).[18,19] Although they have been coupled with the use of β-lactamase inhibitors for the discrimination of carbapenemase types, they still have lower discriminative capacity than that observed in our study.[20,21] Moreover, false negative results have been observed with such colorimetric tests with some mucoid isolates, especially isolates with low carbapenemase activity, particularly OXA-48-like producers.[21]

In summary, the eazyplex® SuperBug CRE system represents a promising platform with optimal sensitivity for the rapid detection of many of the most prevalent carbapenemase and ESBL genes among clinical CPE and should be considered for routine use. Future cost-effectiveness studies should also be performed to demonstrate the system's usefulness for rapid implementation of control measures and therapeutic decision making.

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Transparency declarations

None to declare.

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