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Brief report

Rapid detection of KPC-producing *Enterobacterales* by using a modified Carba NP test with imipenem/relebactam

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ABSTRACT

Introduction: Here, we propose a novel modified Carba NP test for detecting KPC-producing *Enterobacterales* using imipenem/relebactam.
Material and methods: The test performance was evaluated in a random selection of 160 previously molecularly characterized clinical isolates carrying the 110 bla_{KPC}, 1 bla_{GES}, 12 bla_{VIM}, 4 bla_{IMP}, 3 bla_{NDM} and 42 $bla_{OXA\text{-}48\text{-}like}$ genes. The proposed method relies on the detection of imipenem hydrolysis in an imipenem/relebactam antibiotic solution and subsequent visual interpretation by color change.
Results: All class A producing *Enterobacterales* (111/111) were detected using imipenem/relebactam as no visual appreciation of color change was perceived due to a nule hydrolysis of imipenem in the antibiotic solution. Overall, the assay showed 100% sensitivity (111/111) and specificity (69/69) for detecting class A KPC-producing *Enterobacterales.*
Discussion: The biochemical assay provides very reliable results for detecting KPC-producing *Enterobacterales*, with a turnaround time of less than 1 hour, minimum handling and no specialized equipment required.

Detección rápida de *Enterobacterales* productoras de KPC mediante una modificación del test Carba NP utilizando imipenem/relebactam

Palabras clave:
Enterobacterales productoras de carbapenemasas
Imipenem/relebactam
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RESUMEN

Introducción: En este manuscrito proponemos un novedoso ensayo basado en el Carba NP test para la detección de *Enterobacterales* productoras de KPC utilizando imipenem/relebactam.
Material y métodos: La evaluación se realizó en una selección aleatoria de 160 aislados clínicos previamente caracterizados molecularmente que portaban los genes 110 bla_{KPC}, 1 bla_{GES}, 12 bla_{VIM}, 4 bla_{IMP}, 3 bla_{NDM} y 42 $bla_{OXA\text{-}48\text{-}like}$. El método propuesto se basa en la detección de la hidrólisis de imipenem en una solución de imipenem/relebactam y posteriormente su interpretación mediante un cambio de color.
Resultados: El ensayo tiene una sensibilidad (111/111) y una especificidad (69/69) del 100% para detectar *Enterobacterales* productoras de KPC, de clase A.
Discusión: Este ensayo bioquímico proporciona resultados muy fiables para la detección de *Enterobacterales* productoras de KPC, con un tiempo de respuesta inferior a 1 h, además de una manipulación mínima y sin necesidad de ningún equipamiento especializado.

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Introduction

The global rise in the incidence of carbapenemase-producing *Enterobacterales* (CPE) is alarming and poses a challenge to health services worldwide.[1] This is especially worrying in relation to *Klebsiella pneumoniae* carbapenemase (KPC)-producing bacteria, the most frequently occurring type of CPE worldwide.[2] Limited therapeutic options are available for CPE, and only "second line" drugs such as polymyxins, tigecycline, aminoglycosides and fosfomycin are generally used. Double carbapenem therapy can also be considered, as can combination therapies, which are associated with better outcomes for high-risk patients.[3] Novel β-lactam inhibitors are being developed with the aim of restoring the activity of β-lactam antibiotics against CPE. Relebactam (MSD, USA) inhibits class A and C β-lactamases and is currently under clinical development in combination with imipenem-cilastatin. Imipenem/relebactam has recently undergone testing in phase 3 clinical trials to treat patients with complicated intra-abdominal infections, complicated urinary tract infections and hospital-acquired/ventilator-associated bacterial pneumonia, with promising results. Biochemical methods have been used to detect carbapenem hydrolysis as a surrogate test for susceptibility.[4–7] EUCAST guidelines for carbapenemase detection also recommend biochemical assays as useful methods in clinical practice. These methods measure the color change in the medium due to the presence of a pH indicator, which shifts when the carbapenem antibiotic is hydrolysed as a consequence of acidification. Here, we propose a modified Carba NP test for detecting class A KPC-producing *Enterobacterales*. We focused on KPC-producing isolates (because of their high prevalence) and used imipenem/relebactam as the reaction substrate. This approach makes use of the capacity of relebactam to inhibit imipenem hydrolysis and thus confirm the presence of class A carbapenemase, the only class inhibited by this compound.

Material and methods

Bacterial isolates

The proposed method was applied to a random selection of 172 carbapenemase-producing clinical isolates previously molecularly characterized using the Xpert Carba-R Assay (Cepheid, Sunnyvale, USA). The isolates carried 110 bla_{KPC}, 1 bla_{GES}, 12 bla_{VIM}, 4 bla_{IMP}, 3 bla_{NDM} and 42 $bla_{OXA\text{-}48\text{-}like}$ genes.[8] In the case of the GES-producing isolate, WGS was performed to determine the underlying mechanism of resistance. The molecular results were used as gold standard. As negative controls clinical isolates, *Enterobacter cloacae* ($n = 2$), *Citrobacter freundii* ($n = 2$), and *Serratia marcescens* ($n = 2$), were used. Also, reference strains as *K. pneumoniae* ATCC 700613 and *E. coli* ATCC 35218 were tested. Species identification was confirmed by MALDI-TOF MS. The CPE comprised 120 *K. pneumoniae*, 3 *Klebsiella oxytoca*, 12 *Escherichia coli*, 18 *Enterobacter cloacae*, 17 *Citrobacter freundii* and 2 *Serratia marcescens. E. coli* ATCC 25922 was used as a negative control, and a PCR-confirmed KPC-producing *E. coli* was used as a positive control in every assay.

Biochemical assay

The proposed method relies on the detection of imipenem hydrolysis in an imipenem/relebactam antibiotic combination by using a modified Carba NP test,[7] a buffer developed by the authors of this paper[9] and subsequent visual interpretation of the results. The test was performed as follows: 1 μl loop of the different bacterial isolates were resuspended in a tube containing 100 μl of imipenem (3 mg/ml) dissolved in 0.5% phenol red solution, 10 mM NH_4HCO_3, 10 mM $ZnSO_4$ and 1% of SDS (pH = 8) and in a second tube containing imipenem/relebactam (3 mg/ml/4 mg/ml) dissolved in the same buffer. Imipenem hydrolysis was also measured as an internal control. The suspensions were incubated at room temperature for 5 min and then incubated at 37 °C under agitation for another 30 min. The test results were interpreted by technicians who were blinded to sample identification. Class A carbapenemases were identified when the imipenem solution turned yellow or orange-yellow and the imipenem/relebactam solution remained red, due to the impossibility of the enzyme to hydrolyze imipenem in the presence of relebactam (Fig. 1).

Results

After incubation of the bacterial isolates with imipenem for 30 min at 37 °C, we observed a color change from red to yellow in all carbapenemase-producing isolates. The test showed 100% sensitivity and specificity for detecting carbapenemase activity, as assessed by comparison with molecular-based methods. Regarding imipenem hydrolysis in the class A carbapenemases ($n = 111$), the color change was obvious after incubation for 15 min at room temperature, but was clear as early as after 5 min for most isolates ($n = 103$). In the imipenem/relebactam combination, all isolates yielded a red color, irrespective of the incubation time, demonstrating the inhibitory capacity of relebactam and the susceptibility of the isolates to the imipenem/relebactam combination. Regarding the class B carbapenemases ($n = 19$), for most isolates ($n = 10$) the color change in the tube containing imipenem was clear after incubation for 5 min at room temperature and very obvious after incubation for 15 min at 37 °C. For class D carbapenemases ($n = 42$), the color change in the tube containing imipenem was not as evident after incubation for 30 min at 37 °C. None of the isolates belonging to class B and D carbapenemases yielded a red color in the tube containing imipenem/relebactam after incubation for 30 min, demonstrating the inability of relebactam to inhibit these carbapenemases. These results are consistent with those of the molecular methods, yielding 100% sensitivity (111/111) and specificity (69/69) for detection of class A producing *Enterobacterales* susceptible to imipenem/relebactam.

Carbapenemase non-producing isolates did not hydrolyze imipenem, as demonstrated by the absence of color change in the imipenem solution, thus in the imipenem/relebactam.

Discussion

We report here a novel approach for the rapid detection of class A carbapenemases using a modified Carba NP test with imipenem/relebactam as a substrate for the hydrolysis reaction. The study findings support previous data concerning the use of modified Carba NP tests for detecting carbapenemase activity in *Enterobacterales*.[4–7]

There are currently few or no therapeutic options available for treating infections caused by KPC-producing *Enterobacterales,* which are usually multidrug resistant, especially since the emergence of ceftazidime-avibactam resistance.[10–11] The early and accurate detection of these isolates is therefore extremely important in regard to prescribing targeted treatments.

The study findings showed that this novel Carba-NP-based method is an excellent tool for detecting KPC-producing *Enterobacterales*, providing results with a turnaround time of less than 1 h. The method yielded 100% sensitivity and specificity (111/111; 69/69), as indicated by comparison with the molecular characterization of the isolates. The findings also revealed that the incubation buffer strongly affected imipenem hydrolysis. Low pH incubation buffers (e.g. NH_4-citrate, pH 6) have yielded false-negative results in isolates with OXA-type enzymes.[9,12] Conversely, in the present

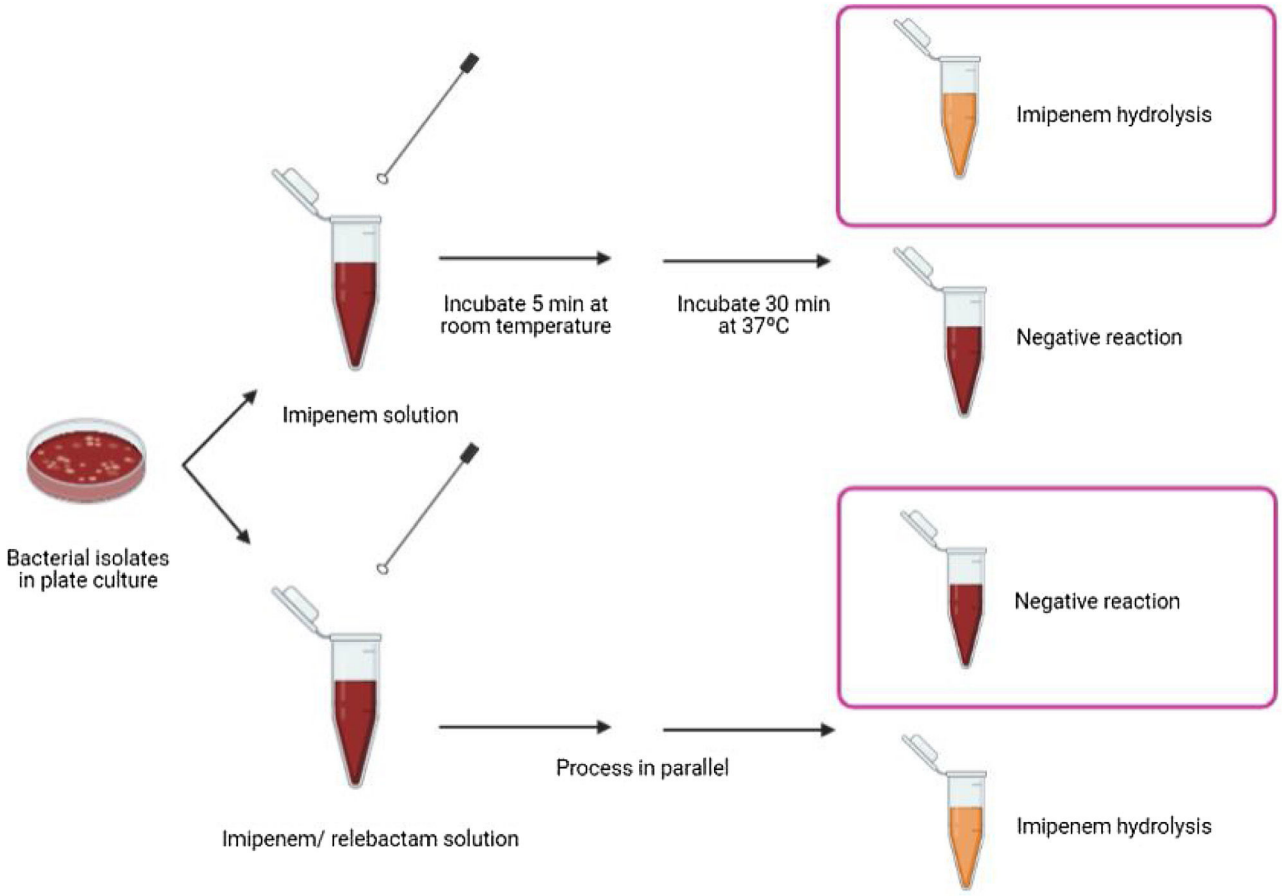

Fig. 1. Class A carbapenemases detection in *Enterobacterales* by using a modified Carba NP test with imipenem/relebactam. Representative results obtained using a modified Carba NP test with imipenem/relebactam. Class A carbapenemases hydrolyze imipenem providing a color change in the imipenem solution whereas they are incapable of hydrolysing imipenem when incubated with relebactam, providing no color change in the antibiotic solution.

study the isolates showed high reactivity with NH_4HCO_3 buffer (pH 8) and shorter reaction times. Inclusion of SDS in the incubation buffer facilitated the reactivity in hypermucoviscous *K. pneumoniae*.

In addition to the KPC-producing isolates, we tested one GES-producing *K. oxytoca*, in which the negative hydrolysis of imipenem/relebactam appeared consistent with the results obtained with the KPC-producing isolates. However, further isolates must be tested to confirm the results. The biochemical assay exclusively detects the presence of carbapenemase enzymes, but does not detect carbapenem resistance due to other resistance mechanisms such as OmpK35 disruption and/or mutated OmpK36, which have been described as chromosomal resistance in these isolates.[13]

This in-house method is rapid, simple and inexpensive, making it very useful for the identification of KPC-producing *Enterobacterales* in clinical microbiological laboratories. The rapid identification of these multidrug resistant bacteria will help in the early administration of targeted treatments such as imipenem/relebactam, which have produced excellent outcomes.[14,15] Other commercial and in-house developed methods have a turnaround time of 2 h, but the proposed modified Carba-NP test provides results in less than 1 h, with minimum handling and no need for specialized equipment. The method could therefore be easily implemented in microbiologial laboratories, even in low-resource settings.

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Conflict of interests

The authors declare having no conflict of interest.

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