

## Rapid detection of carbapenemase-producing Enterobacteriaceae from blood cultures

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### Abstract

The biochemical-based Carba NP test has been evaluated to detect carbapenemase-producing Enterobacteriaceae ( $n = 193$ ) directly from spiked blood cultures. It was able to rapidly detect KPC ( $n = 50$ ), IMP ( $n = 27$ ), VIM ( $n = 37$ ), NDM ( $n = 33$ ) and OXA-48-like producers ( $n = 46$ ) with sensitivity and specificity of 97.9% and 100%, respectively. This cost-effective technique may be implemented in any microbiology laboratory and offers a reliable test for an early identification of carbapenemase-producing Enterobacteriaceae directly from blood culture that could be useful for the management of infected patients.

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### Introduction

Since the 2000s, extended spectrum  $\beta$ -lactamase (ESBL) and acquired cephalosporinase (AmpC) -producing isolates have been reported worldwide. These isolates are often responsible for nosocomial infections but during the last 5 years their dissemination in the community (*Escherichia coli*) has been increasingly reported. As these isolates are resistant to almost all  $\beta$ -lactams with the exception of carbapenems, therapeutic options are often extremely limited for treating infected patients and carbapenems have become antibiotics of last resort [1]. It is therefore of ultimate importance to preserve now the clinical efficacy of carbapenems (imipenem, ertapenem, meropenem, doripenem). However, carbapenem-resistant Enterobacteriaceae are being increasingly reported worldwide, mostly as a consequence of carbapenemase genes

acquisition [2]. Actually, carbapenem resistance in Enterobacteriaceae may be a result of either the association of a decrease in bacterial outer-membrane permeability with over-expression of  $\beta$ -lactamases possessing very weak carbapenemase activity, or the expression of carbapenemases [2–4]. A variety of carbapenemases has been reported in Enterobacteriaceae including the Ambler class A  $\beta$ -lactamases of KPC-type, the metallo- $\beta$ -lactamases (MBL) (Ambler class B) of VIM-, IMP-, GIM-, KHM- and NDM-types, and the carbapenem-hydrolysing class D  $\beta$ -lactamases of the OXA-48-type [2]. Spread of carbapenemase producers is a critical issue because carbapenemases usually confer resistance to most  $\beta$ -lactams. In addition, those carbapenemase producers are usually associated with many other non- $\beta$ -lactam resistance determinants, giving rise to multidrug- and even pandrug-resistant isolates [5].

Specific tests may identify carbapenemase activity phenotypically. The modified Hodge test based on the *in vivo* carbapenemase production has also been suggested for detecting carbapenemase producers. This technique is useful to detect KPC and OXA-48-like producers but may lack specificity (high-level AmpC producers) and sensitivity (weak detection of NDM producers) in several instances [6]. Other detection methods based on the inhibitory properties of several molecules

against MBL (e.g. EDTA) or KPC (e.g. boronic acid, clavulanic acid) producers, may allow discrimination between the diverse types of carbapenemases [7,8]. Those techniques require isolation of the bacteria from the infected samples and, at least, an additional 24-h. Several molecular methods such as simplex and multiplex PCRs, DNA hybridization and sequencing are considered as the reference for identification of carbapenemase genes [8,9]. Recently a real-time PCR technique has been used for detecting KPC producers directly from blood cultures [10]. Although interesting, these molecular-based techniques remain expensive and require expertise. More recently, mass spectrometry-based techniques have been developed to detect carbapenemase production on bacterial isolates [11–13]. Although these techniques seem promising, they require a preliminary step of bacterial isolation from the infected samples because no direct application on the clinical specimen has been developed yet. In addition, they require mass spectrometry equipment, which is not currently available in most clinical microbiology laboratories. Additionally, the UV-spectrophotometric assay has been proposed but is time-consuming and requires trained personnel [14]. These techniques are mostly used for research purposes.

Recently, a biochemical test (Carba NP test) based on the colorimetric detection of carbapenemase production has been developed to identify carbapenemase producers from isolated colonies [15]. This Carba NP test has not been evaluated using directly clinical samples. Therefore, the aim of this study was to determine the ability of the Carba NP test to detect carbapenemase-producing Enterobacteriaceae directly from positive blood cultures. This technique used in a routine laboratory may guide the first-line therapy for treating patients with sepsis [16–19].

## Materials and Methods

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### Strain collection

The panel of strains used for spiking blood cultures included carbapenemase producers of KPC-type ( $n = 50$ ) (see Supplementary material, Table S1), VIM-type ( $n = 37$ ) (see Supplementary material, Table S2), IMP-type ( $n = 27$ ) (see Supplementary material, Table S3), NDM-type ( $n = 33$ ) (see Supplementary material, Table S4), OXA-48-type ( $n = 46$ ) (see Supplementary material, Table S5), and non-carbapenemase producers ( $n = 74$ ) (see Supplementary material, Table S6). All strains had previously been characterized for their  $\beta$ -lactamase content at their molecular level.

### Blood culture preparation

Detection of carbapenemase producers was attempted from spiked blood cultures, the positivity of which was assessed using

the BactAlert blood culture system (bioMérieux, Marcy l'Etoile, France). Blood cultures were made from 10 mL sterile total human blood inoculated with  $1 \times 10^3$  CFU of each strain. The  $1 \times 10^3$  CFU inoculum was prepared by diluting a 0.5 McFarland suspension ( $10^8$  CFU/mL) in sterile water. Then, blood culture bottles (aerobic and anaerobic bottles without charcoal) were incubated until a positivity of the blood culture was detected by the BactAlert system (detection time ranged from 6 to 15 h). As previously described, the final inoculum ranged from  $5 \times 10^7$  to  $5 \times 10^9$  CFU/mL using this protocol [20].

### Detection of carbapenemase activity from blood culture using the Carba NP test

Detection of carbapenemase producers from blood cultures using the Carba NP test was performed as follows: 20 mL brain–heart infusion (BHI) supplemented with 0.12  $\mu$ g/mL imipenem (final concentration) and 70  $\mu$ g/mL ZnSO<sub>4</sub> (final concentration) were inoculated with 20 drops (300  $\mu$ L) of the positive blood culture. Inoculated BHI were then incubated in agitation at 37°C for 3 h. Bacteria were recovered by centrifugation at 4000 g for 15 min. The bacterial pellet was resuspended in 150  $\mu$ L of 20 mM Tris–HCl lysis buffer (B-PERII, Bacterial Protein Extraction Reagent, Thermo Scientific, Pierce, Rockford, IL, USA), and distributed in Microbead tubes (Ultraclean Microbial DNA isolation kit Bead Tubes, MO BIO Laboratories, Carlsbad, CA, USA). Mechanical lysis of bacteria was performed by vigorous agitation of Microbead tubes using a vortex adapter (MO BIO Laboratories) for 30 min at room temperature. This bacterial suspension was centrifuged at 10 000 g at room temperature for 5 min. Thirty microliters of the supernatant, corresponding to the enzymatic bacterial suspension, was mixed in a microwell with 100  $\mu$ L of (i) a diluted phenol red solution containing 0.1 mM ZnSO<sub>4</sub> (Merck Millipore, Guyancourt, France), or (ii) a diluted phenol red solution containing 0.1 mM ZnSO<sub>4</sub> and 3 mg/mL imipenem monohydrate (Sigma-Aldrich, Saint-Quentin Fallavier, France). The diluted phenol red solution used was prepared by taking 2 mL of a phenol red (Merck Millipore) solution 0.5% weight/volume to which 16.6 mL distilled water was added. The pH value was then adjusted to 7.8 by adding drops of 1 M NaOH. Mixtures of the phenol red ( $\pm$  imipenem) solutions and the enzymatic suspension being tested were incubated at 37°C for a maximum of 2 h.

Test results were interpreted by technicians who were blinded to the identity of the samples.

## Results and Discussion

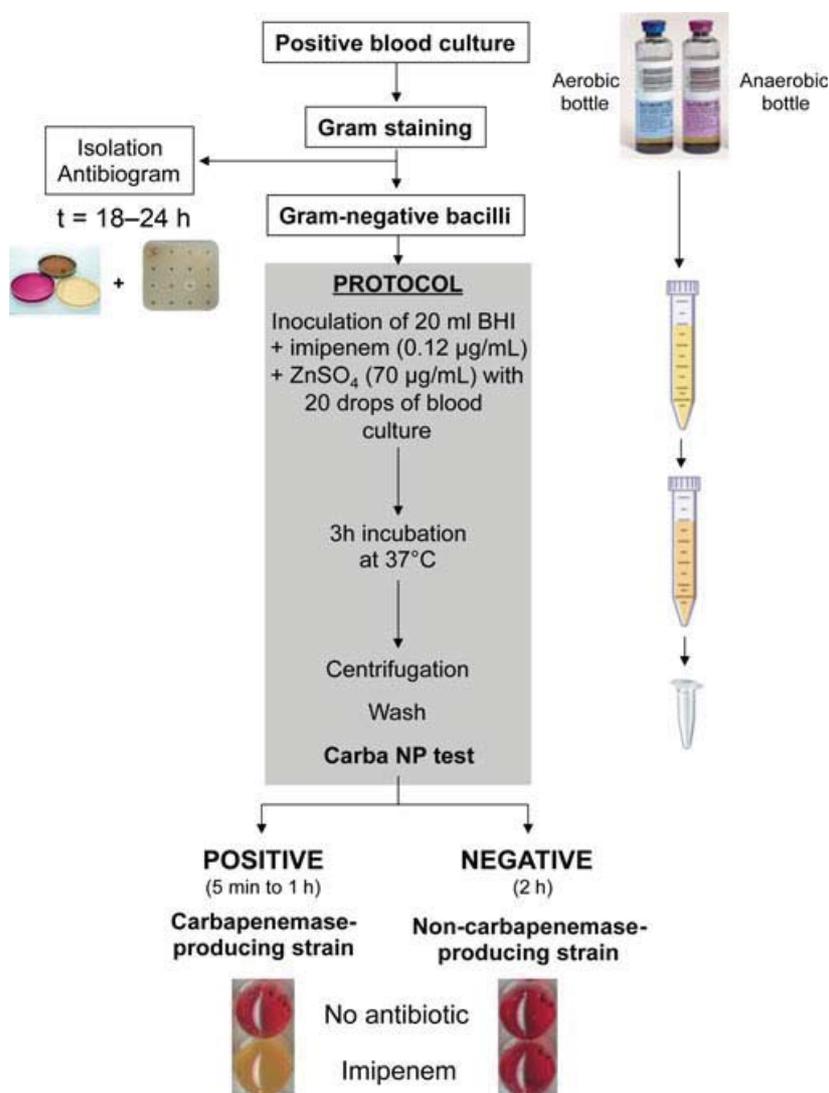
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When considering bacteraemia due to carbapenemase producers, several studies indicate that treatment schemes

**TABLE I.** Results of detection of carbapenemase producers directly from positive spiked blood cultures using the Carba NP test

Carbapenemase types	Tested isolates (n)	Carba NP test on positive blood culture				Sensitivity (%)	Specificity (%)
		Positive		Negative			
		n	%	n	%		
KPC	50	50	100	0	0	100	100
IMP	27	27	100	0	0	100	100
VIM	37	37	100	0	0	100	100
NDM	33	33	100	0	0	100	100
OXA-48-like	46	42	91.3	4	8.7	91.3	100
No carbapenemase	74	0	0	74	100	–	–
Total results						97.9	100

n, number of isolates.  
–, not determined.



**FIG. 1.** Strategy for identification of carbapenemase producers directly from spiked blood cultures using the Carba NP test.

including combinations of effective antimicrobials lead to a significant decrease in mortality compared with monotherapy [16–19]. The most commonly used combinations are colistin

and tigecycline together with a carbapenem. As the successful treatment of bacteraemia depends on prompt administration of the appropriate antimicrobial agents, we may speculate that

the use of the Carba NP test directly from positive blood cultures may significantly improve the outcome of infected patients by early implementation of such combined antimicrobial therapies.

When performed directly on spiked blood cultures, this protocol of carbapenemase detection using the Carba NP test allowed the detection of 100% of KPC ( $n = 50$ ), IMP ( $n = 27$ ), VIM ( $n = 37$ ) and NDM ( $n = 33$ ) producers, and 91.3% of the OXA-48 producers (42/46) (Table 1, and see Supplementary material, Tables S1, S2, S3, S4 and S5). Usually, the time required for obtaining positive results after the start of the Carba NP test was 5–30 min for KPC producers, 15–60 min for MBL producers and 30–60 min for OXA-48 producers. Negative results were always obtained for carbapenemase-negative strains (see Supplementary material, Table S6). Overall sensitivity and specificity of the Carba NP test performed on positive blood cultures were 97.9% and 100%, respectively (Table 1). The lower detection sensitivity of OXA-48 producers compared with the other carbapenemase types may be due to their lower hydrolysis activity compared with the other types of carbapenemases.

Recently, real-time PCR was demonstrated to be a reliable technique for rapid detection of KPC producers directly from positive blood cultures [10]. This technique may reduce the time for identification of KPC producers in blood cultures. Compared with this real-time PCR assay, use of the Carba NP test directly from blood culture has multiple advantages. First, the Carba NP test could detect any carbapenemase whereas molecular-based techniques are designed to detect only one or a few carbapenemase genes. Additionally, the Carba NP test is inexpensive (no need for expensive equipment) and could be implemented in low-income countries that are known to be large reservoirs of carbapenemase producers.

Using this test, the time for identification of a carbapenemase producer responsible for a bacteraemia can be reduced from 24–48 h to 3–5 h (Fig. 1). Consequently, this Carba NP test-based protocol for detection of carbapenemase producers directly from blood cultures may be very useful in two main situations: (i) detection of carbapenemase producers in endemic countries and (ii) in an outbreak situation when the first case of carbapenemase producer has been identified.

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## Transparency Declarations

An international patent form for the Carba NP test has been filed on behalf of INSERM Transfert (Paris, France).

## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Results of detection of KPC-producers directly from positive spiked blood cultures using the Carba NP test.

**Table S2.** Results of detection of VIM-producers directly from positive spiked blood cultures using the Carba NP test.

**Table S3.** Results of detection of IMP-producers directly from positive spiked blood cultures using the Carba NP test.

**Table S4.** Results of detection of NDM-producers directly from positive spiked blood cultures using the Carba NP test.

**Table S5.** Results of detection of OXA-48-like-producers directly from positive spiked blood cultures using the Carba NP test.

**Table S6.** Results of detection of non-carbapenemase producers directly from positive spiked blood cultures using the Carba NP test.

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