Rapid identification of carbapenemase-producing Enterobacteriaceae, Pseudomonas aeruginosa and Acinetobacter baumannii using a modified Carba NP test

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Abstract

Biochemical tests have been previously developed to identify carbapenemase-producing Enterobacteriaceae, Pseudomonas spp. (Carba NP test) and Acinetobacter spp. (CarbaAcineto NP test). We evaluated a modified Carba NP test to detect carbapenemase production in Enterobacteriaceae, Pseudomonas and Acinetobacter species using a single protocol with rapid results and found good reliability and speed.

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Keywords: Carba NP test, carbapenemase, carbapenems, Gram negative, multidrug-resistant bacteria

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Multidrug-resistant Gram-negative bacteria (GNB) are increasingly being reported worldwide. The spread of carbapenemase-producing Enterobacteriaceae, Pseudomonas and Acinetobacter species have become a global threat. The emergence of resistance to carbapenems makes the treatment for infections caused by these carbapenem-resistant strains very limited [1–3]. Different types of carbapenemase have been reported, such as Ambler class A Klebsiella pneumoniae carbapenemase (KPC) and Guiana extended spectrum (GES) ß-lactamase, Ambler class B metallo-ß-lactamases (MBL) and Ambler class D oxacillinase type [1].

Rapid methods for detecting carbapenemase producers have been described, such as MALDI-TOF MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) carbapenemase assay [4]. Previous studies have described a rapid biochemical carbapenemase detection method based on imipenem hydrolysis, the Carba NP test, for Enterobacteriaceae [5] and Pseudomonas species [6], as well as the CarbaAcineto NP test for Acinetobacter species [7]. Recently, however, several authors have published evaluations of the Carba NP and the CarbaAcineto NP tests; their criticisms focused essentially on the absence of detection of oxacillinase (OXA) type carbapenemases [8–10].

Here we describe a modified Carba NP (MCNP) test which enables the rapid detection of different carbapenemases (KPC, MBL and OXA types) from Enterobacteriaceae, Pseudomonas and Acinetobacter species using a single protocol.

One hundred ten previously characterized GNB, including 69 carbapenemase-producing GNB (Enterobacteriaceae n = 14, Pseudomonas aeruginosa n = 11 and Acinetobacter baumannii n = 44), and 41 non-carbapenemase-producing GNB, including Enterobacteriaceae (n = 24), P. aeruginosa (n = 5) and A. baumannii (n = 12), were tested in two laboratories including Unité de recherche sur les maladies infectieuses et tropicales émergentes (URMITE), Aix-Marseille University, Marseille, France, and Microbial Ecology laboratory, Béjaïa University, Béjaïa, Algeria (Table 1). Carbapenemase activity was assessed using phenotypic and genotypic tests, including the modified Hodge test, MALDI-TOF MS assay, PCR amplification and sequencing [4,11].

The Carba NP and the CarbaAcineto NP tests are straightforward biochemical tests which identify carbapenemase production in GNB by detecting imipenem hydrolysis using phenol red solution as a colour indicator and a bacterial lysis buffer (B-PER II, Bacterial Protein Extraction Reagent) for Enterobacteriaceae and Pseudomonas species (Carba NP test) [5,6] and 5 M NaCl for Acinetobacter species (CarbaAcineto NP test) [7].

In order to use a single protocol to detect the production of carbapenemases in the three types of bacteria...
| Group             | Species          | Carbapenemase or other β-lactamase gene | Test result by: |
|-------------------|------------------|----------------------------------------|-----------------|
|                   |                  |                                        | MHT MALDI-TOF MS| MCNP |
| Enterobacteriaceae| Klebsiella pneumoniae HS50047 | NDM-1 | + | + | + |
|                   | K. pneumoniae 472 | NDM-1 | + | + | + |
|                   | K. pneumoniae U2A 224647 | KPC-3 | + | + | + |
|                   | K. pneumoniae 36047 | KPC-2 | + | + | + |
|                   | K. pneumoniae 41347 | OXA-48 | + | + | + |
|                   | K. pneumoniae 47347 | TEM-1/CTX-M-3/SHV-12/DHA-1 | − | − | − |
|                   | K. pneumoniae 46347 | TEM-1/CTX-M-15 | − | − | − |
|                   | K. pneumoniae 55047 | TEM-1/CTX-M-3 | − | − | − |
|                   | K. pneumoniae 47647 | CTX-M-15 | − | − | − |
|                   | K. pneumoniae 12347 | CTM-4 | − | − | − |
|                   | K. pneumoniae 31847 | CTX-M-15 | − | − | − |
|                   | K. pneumoniae 4747 | CTX-M-15 | − | − | − |
|                   | K. pneumoniae 61347 | CTX-M-15 | − | − | − |
|                   | K. pneumoniae 61547 | CTX-M-15 | − | − | − |
|                   | Escherichia coli H563167 | NDM-1 | + | + | + |
|                   | E. coli 18147 | NDM-5 | + | + | + |
|                   | E. coli 9947 | NDM-5 | + | + | + |
|                   | E. coli 10047 | OXA-48 | + | + | + |
|                   | E. coli 13247 | OXA-48 | + | + | + |
|                   | E. coli 19247 | OXA-48 | + | + | + |
|                   | E. coli 54447 | TEM-1/CTX-M-15/OXA-1 | − | − | − |
|                   | E. coli 46947 | TEM-1/CTX-M-3/OXA-1 | − | − | − |
|                   | E. coli 47247 | TEM-1/CTX-M-3/OXA-1 | − | − | − |
|                   | E. coli 23447 | TEM-1/CTX-M-15/OXA-1 | − | − | − |
|                   | E. coli 61147 | TEM-1/CTX-M-15/OXA-1 | − | − | − |
|                   | E. coli 53647 | TEM-1/CTX-M-15/OXA-1 | − | − | − |
|                   | E. coli 53447 | CTX-M-15/OXA-1 | − | − | − |
|                   | E. coli 54247 | CTX-M-15/OXA-1 | − | − | − |
|                   | E. coli 56047 | TEM-1/CTX-M-15/OXA-1 | − | − | − |
|                   | E. coli 60647 | TEM-1/CTX-M-15/OXA-1 | − | − | − |
|                   | E. coli ATCC 2592247 | — | — | — | — |
|                   | E. coli 76247 | — | — | — | — |
|                   | E. cloaceae 10847 | TEM-1/CTX-M-15/OXA-1 | − | − | − |
|                   | E. cloaceae 57047 | TEM-1/CTX-M-15/OXA-1 | − | − | − |
| Pseudomonas        | P. aeruginosa 45 | VM-2 | + | + | + |
|                   | P. aeruginosa 46 | VM-2 | + | + | + |
|                   | P. aeruginosa 47 | VM-2 | + | + | + |
|                   | P. aeruginosa 48 | VM-2 | + | + | + |
|                   | P. aeruginosa 49 | VM-2 | + | + | + |
|                   | P. aeruginosa 50 | VM-2 | + | + | + |
|                   | P. aeruginosa 51 | VM-2 | + | + | + |
|                   | P. aeruginosa 52 | VM-2 | + | + | + |
|                   | P. aeruginosa 53 | VM-2 | + | + | + |
|                   | P. aeruginosa 54 | VM-2 | + | + | + |
|                   | P. aeruginosa 55 | VM-2 | + | + | + |
|                   | P. aeruginosa 56 | VM-2 | + | + | + |
|                   | P. aeruginosa 57 | VM-2 | + | + | + |
|                   | P. aeruginosa 58 | VM-2 | + | + | + |
|                    | P. aeruginosa UAA 225747 | IMP-1 | + | + | + |
| Acinetobacter      | A. baumannii 4 | OXA-23 | + | + | + |
|                   | A. baumannii 5 | OXA-23 | + | + | + |
|                   | A. baumannii 6 | OXA-23 | + | + | + |
|                   | A. baumannii 7 | OXA-23 | + | + | + |
|                   | A. baumannii 8 | OXA-23 | + | + | + |
|                   | A. baumannii 9 | OXA-23 | + | + | + |
|                   | A. baumannii 10 | OXA-23 | + | + | + |
|                   | A. baumannii 11 | OXA-23 | + | + | + |
|                   | A. baumannii 12 | OXA-23 | + | + | + |
|                   | A. baumannii 13 | OXA-23 | + | + | + |
|                   | A. baumannii 14 | OXA-23 | + | + | + |
|                   | A. baumannii 15 | OXA-23 | + | + | + |
|                   | A. baumannii 16 | OXA-23 | + | + | + |
|                   | A. baumannii 17 | OXA-23 | + | + | + |
|                   | A. baumannii 18 | OXA-23 | + | + | + |
|                   | A. baumannii 19 | OXA-23 | + | + | + |
|                   | A. baumannii 20 | OXA-23 | + | + | + |
|                   | A. baumannii 21 | OXA-23 | + | + | + |
|                   | A. baumannii 22 | OXA-23 | + | + | + |
|                   | A. baumannii 23 | OXA-23 | + | + | + |
|                   | A. baumannii 24 | OXA-23 | + | + | + |
|                   | A. baumannii 25 | OXA-23 | + | + | + |
|                   | A. baumannii 26 | OXA-23 | + | + | + |
|                   | A. baumannii 27 | OXA-23 | + | + | + |
|                   | A. baumannii 28 | OXA-23 | + | + | + |
|                   | A. baumannii 29 | OXA-23 | + | + | + |
|                   | A. baumannii 30 | OXA-23 | + | + | + |

TABLE 1. Test results for Enterobacteriaceae, Pseudomonas aeruginosa and Acinetobacter baumannii strains
(Enterobacteriaceae, Pseudomonas and Acinetobacter) and to accelerate the speed with which results are produced, the lysis buffer and pH of the colour indicator solution used in the Carba NP and CarbAcineto NP tests were changed.

In the MCNP test, the lysis buffers used for the Carba NP test and CarbAcineto NP test, B-PER II, Bacterial Protein Extraction Reagent and NaCl 5 M, respectively, were replaced by cetyl trimethyl ammonium bromide (CTAB) 0.02%, and the pH value of the phenol red solution was adjusted to 7.5 (instead of 7.8). In addition, two steps used in the previous protocols [5,6], centrifugation and incubation at room temperature for 30 minutes, were eliminated in our method. These modifications simplify the lysis step and produce results more quickly.

The MCNP test was performed as follows. One inoculation loop (10 μL) of the tested strain, directly recovered from a Mueller Hinton agar plate (bioMérieux, Marcy l’Étoile, France), was resuspended in 200 μL of 0.02% CTAB (Sigma-Aldrich Chimie, Saint-Quentin-Fallavier, France) and vortexed for 1 to 2 minutes. Subsequently, 100 μL of the bacterial suspension was mixed with 100 μL of diluted phenol red solution (2 mL of phenol red (Sigma-Aldrich) solution 0.5% (wt/vol) with 16.6 mL of distilled water) containing 0.1 mM ZnSO4 (pH 7.5) in the first tube, tube 1, used as negative control, and a diluted phenol red

**TABLE 1. Continued**

| Group | Species          | Carbapenemase or other β-lactamase gene | Test result by: |
|-------|------------------|-----------------------------------------|-----------------|
|       |                  |                                         | MHT  | MALDI-TOF MS | MCNP |
| A. baumannii<sup>a</sup> | NDM-1           | +                                       | +    | +            | +    |
| A. baumannii<sup>a</sup> | NDM-1           | +                                       | +    | +            | +    |
| A. baumannii<sup>a</sup> | NDM-1           | +                                       | +    | +            | +    |
| A. baumannii<sup>a</sup> | OXA-23/NDM-1    | +                                       | +    | +            | +    |
| A. baumannii<sup>a</sup> | OXA-23/NDM-1    | +                                       | +    | +            | +    |
| A. baumannii<sup>a</sup> | OXA-23/NDM-1    | +                                       | +    | +            | +    |
| A. baumannii<sup>a</sup> | OXA-23/NDM-1    | +                                       | +    | +            | +    |
| A. baumannii<sup>a</sup> | TEM-128         | --                                      | --   | --           | --   |
| A. baumannii<sup>a</sup> | TEM-128         | --                                      | --   | --           | --   |
| A. baumannii<sup>a</sup> | TEM-128         | --                                      | --   | --           | --   |
| A. baumannii<sup>a</sup> | TEM-128         | --                                      | --   | --           | --   |
| A. baumannii<sup>a</sup> | TEM-128         | --                                      | --   | --           | --   |
| A. baumannii<sup>a</sup> | TEM-128         | --                                      | --   | --           | --   |
| A. baumannii<sup>a</sup> | TEM-128         | --                                      | --   | --           | --   |
| A. baumannii<sup>a</sup> | TEM-128         | --                                      | --   | --           | --   |
| A. baumannii<sup>a</sup> | TEM-128         | --                                      | --   | --           | --   |
| A. baumannii<sup>a</sup> | TEM-128         | --                                      | --   | --           | --   |
| A. baumannii<sup>a</sup> | TEM-128         | --                                      | --   | --           | --   |
| A. baumannii<sup>a</sup> | TEM-128         | --                                      | --   | --           | --   |
| A. baumannii<sup>a</sup> | AYE<sup>b</sup> | VEβ-1                                   | --   | --           | --   |
| A. baumannii SDF<sup>c</sup> |               |                                         | --   | --           | --   |

**FIG. 1.** Modified Carba NP test results for Enterobacteriaceae, Pseudomonas and Acinetobacter species. (A) Escherichia coli ATCC 25922. (B) NDM-5-positive E. coli. (C) KPC-2-positive Klebsiella pneumoniae 360. (D) IMP-1-positive Pseudomonas aeruginosa UAA 2257. (E) OXA-23-positive Acinetobacter baumannii. (F) OXA-24-positive A. baumannii. (G) NDM-1-positive A. baumannii. (a) Tube containing phenol red solution 0.1 mM ZnSO₄ (pH 7.5) and cetyl trimethyl ammonium bromide (CTAB) 0.02%. (b) Tube containing phenol red solution 0.1 mM ZnSO₄ (pH 7.5) supplemented with 6 mg/mL of imipenem and CTAB 0.02%.

**MALDI-TOF MS.** matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; **MCNP.** modified Carba NP test; **MHT.**modified Hodge test.

<sup>a</sup>Strains tested in Microbial Ecology Laboratory, Béjaia University, Béjaia, Algeria.

<sup>b</sup>Strains tested in Unité de recherche sur les maladies infectieuses et tropicales émergentes (URMITE), Aix-Marseille University, Marseille, France.

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solution containing 0.1 mM ZnSO₄ (pH 7.5) supplemented with 6 mg/mL of commercially available imipenem (Tienam 500; Merck Sharp & Dohme, Paris, France) in the second tube, tube 2. Tubes 1 and 2 were vortexed, then incubated at 37°C for a maximum of 2 hours.

Carbapenemase activity was revealed when the test and negative control solutions, respectively, were yellow vs. red or orange vs. red. In contrast, both solutions remained red in the case of noncarbapenemase producers (Fig. 1).

The results showed that the MCNP method detected all carbapenemases produced by carbapenem-resistant strains with 100% sensitivity and 100% specificity. Positive results were observed at different times for the different carbapenemases types (MBL, KPC and OXA-48 at 10 to 30 minutes vs. 1 to 2 hours for OXA type). The most interesting aspect of this method is that the colour changed from red to orange or yellow (positive result) even before incubation in some cases (NDM-5-producing Escherichia coli, NDM-1-producing Klebsiella pneumoniae (Kpnasey) and imipenem-producing P. aeruginosa UAA2257). Moreover, a higher inoculums (two inoculation loops (10 μL)) is recommended for Acinetobacter species tests.

Currently, the MCNP test is routinely used in Timone Hospital, Marseille, France. It was performed when antibiotic susceptibility testing revealed a resistance to ertapenem and susceptibility or resistance to imipenem. The suspicion of carbapenemase producers, in particular OXA-48, was based on this phenotype. Between November 2014 and May 2015, a total of 233 strains were tested. Among them, 35 positives strains with carbapenemase producers were detected (Table 2). These positives strains were isolated from 25 different patients. These results confirm the efficiency of the MCNP test with high sensitivity, given the detection of all strains producing OXA-48-type carbapenemases. Also, two carbapenemase-producing A. baumannii were detected, thus confirming the advantages of the MCNP test.

In conclusion, the advantages of the MCNP test are the detection of different carbapenemase types from Enterobacteriaceae, Pseudomonas and Acinetobacter species using a single protocol, as well as the short time to results, particularly in the case of MBL-producing Enterobacteriaceae and Pseudomonas species. In addition, the effectiveness of this test on a large series of bacteria may allow us to identify the production

### TABLE 2. Results of MCNP test applied for carbapenem-resistant strains isolated in La Timone Hospital, Marseille, France

| Date       | Sample source | Strain              | Antibiotic susceptibility testing results | Carbapenemases gene detected |
|------------|---------------|---------------------|------------------------------------------|-----------------------------|
| 10/11/2014 | Urine         | Klebsiella pneumonia | ETP: R, IMP: R | + | OXA-48 |
| 20/11/2014 | Rectal swab   | E. coli             | ETP: R, IMP: R | + | OXA-48 |
| 25/11/2014 | Bronchoalveolar lavage fluid | K. pneumonia | ETP: R, IMP: R | + | OXA-48 |
| 05/12/2014 | Bronchoalveolar lavage fluid | K. pneumonia | ETP: R, IMP: R | + | OXA-48 |
| 09/12/2014 | Recal swab    | K. pneumonia         | ETP: R, IMP: R | + | OXA-48 |
| 11/12/2014 | Urine         | Enterobacter cloacae | ETP: R, IMP: R | + | OXA-48 |
| 21/12/2014 | Stools        | K. pneumonia         | ETP: R, IMP: R | + | OXA-48 |
| 31/12/2014 | Spittle       | K. pneumonia         | ETP: R, IMP: R | + | OXA-48 |
| 12/01/2015 | Urine         | E. coli             | ETP: R, IMP: R | + | OXA-48 |
| 24/01/2015 | Arial swab    | K. pneumonia         | ETP: R, IMP: R | + | OXA-48 |
| 02/02/2015 | Rectal swab   | K. pneumonia         | ETP: R, IMP: R | + | OXA-48 |
| 06/02/2015 | Urine         | E. coli             | ETP: R, IMP: R | + | OXA-48 |
| 17/02/2015 | Urine         | E. coli             | ETP: R, IMP: R | + | OXA-48 |
| 19/02/2015 | Spittle       | K. pneumonia         | ETP: R, IMP: R | + | OXA-48 |
| 20/02/2015 | Rectal swab   | K. pneumonia         | ETP: R, IMP: R | + | OXA-48 |
| 23/02/2015 | Urine         | K. pneumonia         | ETP: R, IMP: R | + | OXA-48 |
| 04/03/2015 | Rectal swab   | K. pneumonia         | ETP: R, IMP: R | + | OXA-48 |
| 16/03/2015 | Rectal swab   | K. pneumonia         | ETP: R, IMP: R | + | OXA-48 |
| 23/03/2015 | Bronchial aspirate | K. pneumonia | ETP: R, IMP: R | + | OXA-48 |
| 30/03/2015 | Arial swab    | K. pneumonia         | ETP: R, IMP: R | + | OXA-48 |
| 31/03/2015 | Urine         | K. pneumonia         | ETP: R, IMP: R | + | OXA-48 |
| 13/04/2015 | Sinus         | Seratia marcescens   | ETP: R, IMP: R | + | OXA-48 |
| 13/04/2015 | Blood culture | E. cloacae          | ETP: R, IMP: R | + | OXA-48 |
| 18/04/2015 | Blood culture | E. coli             | ETP: R, IMP: R | + | OXA-48 |
| 18/04/2015 | Rectal swab   | K. pneumonia         | ETP: R, IMP: R | + | OXA-48 |
| 18/04/2015 | Blood culture | E. coli             | ETP: R, IMP: R | + | OXA-48 |
| 04/05/2015 | Urine         | K. pneumonia         | ETP: R, IMP: R | + | OXA-48 |
| 06/05/2015 | Urine         | K. pneumonia         | ETP: R, IMP: R | + | OXA-48 |
| 07/05/2015 | Bronchial aspirate | K. pneumonia | ETP: R, IMP: R | + | OXA-48 |
| 08/05/2015 | Blood culture | Acinetobacter baumannii | ETP: R, IMP: R | + | OXA-23 |
| 11/05/2015 | Urine         | A. baumannii        | ETP: R, IMP: R | + | OXA-23 |
| 18/05/2015 | Rectal swab   | K. pneumonia         | ETP: R, IMP: R | + | OXA-48 |

ETP, ertapenem; IMP, imipenem; MCNP, modified Carba NP test; NT, not tested; R, resistant; S, susceptible; I, Intermediate.
of carbapenemase enzymes even before identification of the bacterial strain.

Interestingly, as well as using this test in developed countries such as France (URMITE laboratories, La Timone Hospital), given the simplicity and the low cost of the MCNP test, it could be used by any laboratory, including laboratories in developing countries. In Algeria, this test has been used in the Microbial Ecology Laboratory of Béjaia University since May 2014, and it will soon be introduced to laboratories in Algerian hospitals.

Conflict of interest

None declared.

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