Published in "Journal of Clinical Microbiology
doi: 10.1128/JCM.00594-14, 2014"
which should be cited to refer to this work.

CarbAcineto NP test for rapid detection of carbapenemase-producers in *Acinetobacter* spp.

Laurent Dortet[1], Laurent Poirel[1,2] Caroline Errera[1], and Patrice Nordmann[1,2]*

[1]INSERM U914 "Emerging Resistance to Antibiotics", Faculté de Médecine Paris Sud, K. -Bicêtre, France, and [2]Medical and Molecular Microbiology, Department of Medecine, Faculty of Science, University of Fribourg, Switzerland

Word count : 2,725 words

Running title: Rapid detection of carbapenemase-producing in *Acinetobacter*

Keywords: Carba NP test, carbapenem resistance, Gram negative

-

Corresponding author: patrice.nordmann@unifr.ch

Medical and Molecular Microbiology Unit, Department of Medicine, Faculty of Science, University of Fribourg

3 rue Albert Gockel

CH-1700 Fribourg, Switzerland

Phone :+41 26 300 9581

Multidrug-resistant *Acinetobacter baumannii* isolates, and particularly carbapenemase-producing isolates, are increasingly reported worldwide. The biochemically-based Carba NP test, extensively validated for detection of carbapenemase producers among Enterobacteriaceae and *Pseudomonas* spp., has been modified for the detection of carbapenemase production in *Acinetobacter* spp. A collection of 151 carbapenemase-producing and 69 non-carbapenemase producing *Acinetobacter* spp. using the Carba NP test and a modified Carba NP protocol (CarbAcineto NP test) set up in this study. The CarbAcineto NP test requires modified lysis conditions and an increased bacterial inoculum as compared to the original Carba NP test. The Carba NP test detects metallo-β-lactamase producers but failed to detect production of other carbapenemase types among *Acinetobacter* spp. By contrast, the newly-designed CarbAcineto NP test, which is rapid and reproducible, detects all types of carbapenemases, with a sensitivity of 94.7% and a specificity of 100%. This cost-effective technique offers a reliable and affordable technique for identification of carbapenemase production in *Acinetobacter* spp., which is a marker of multidrug resistance in those species. Its use will facilitate their recognition and prevent their spread.

Introduction

Acinetobacter spp., and particularly the *A. baumannii-calicoaceticus* complex, are opportunistic pathogens frequently involved in nosocomial outbreaks that mostly occurred in intensive care units (ICU). Those infections range from septicemia to pneumonia and urinary tract infections (1). Due to their ability to develop resistance to new antibiotic rapidly, multidrug-resistant strains belonging to the *A. baumannii-calicoaceticus* complex have been increasingly reported during the last decade (2). Consequently, carbapenems are often considered to be the antibiotic of last resort to treat infections caused by those strains. Therefore, resistance to carbapenems in those species is *per se* considered to define the isolate to be highly resistant.

During the recent years, the spread of carbapenem-resistant *Acinetobacter* spp. became a worldwide issue. In *Acinetobacter* sp., resistance to carbapenems may result from (i) decreased permeability of the outer membrane due to the loss or modification of porins, (ii) rarely modification of penicillin-binding proteins, and (iii) in most cases production of a carbapenemase (2). Those carbapenemases identified in *Acinetobacter* sp. either belong to the Ambler classes A, B, or D. Some class A carbapenemases have been identified, being of the KPC or GES types (3, 4). Whereas KPC-producing isolates have been rarely described (4), dissemination of GES-11 and GES-14-producing isolates was recently reported in the Middle East (5). Metallo-β-lactamases (MBLs) (Ambler class B) of VIM-, IMP-, SIM- and NDM-types have been also reported in *Acinetobacter* spp. (2, 6). Apart from SIM, all those MBLs have been also reported from Enterobacteriaceae and *Pseudomonas* spp. (7). In addition, carbapenem-hydrolyzing Ambler class D β-lactamases (CHDLs) constitute the first source of acquired carbapenem resistance in

Acinetobacter spp. Those CHDLs are divided into five different subgroups, namely OXA-23, OXA-40, OXA-51, OXA-58, and OXA-143 (2, 8, 9). As opposed to KPC and MBLs, these CHDLs have been identified only among *Acinetobacter* spp. isolates. OXA-51-like enzymes are intrinsic and chromosomally-encoded in the *A. baumannii-calicoaceticus* complex, possessing a weak carbapenemase activity, and sharing weak amino acid identity with the other known class D ß-lactamases (10-12). Although the $bla_{\text{OXA-51}}$-like genes are usually not expressed in a wild-type isolate, insertion of IS*Aba1* at the 5' end of $bla_{\text{OXA-51}}$-like genes may lead to the overexpression of the corresponding β-lactamase gene (13). On the other hand, genes encoding OXA-23, OXA-40, OXA-58, and OXA-143 CHDLs have been mainly identified onto transferable genetic structures, such as plasmids or transposons that contribute to their dissemination among the *Acinetobacter* genus (2).

Since most of those carbapenemases identified in *Acinetobacter* sp. are located on mobile genetic elements that may be transferred to other clinical relevant species (i.e other *Acinetobacter* species, Enterobacteriaceae, and *P. aeruginosa*), it is critical to identify those carbapenemase-producing *Acinetobacter* and consequently differentiate them from isolates that are carbapenem resistant due to non-transferable mechanisms (i.e. permeability defects, overexpression of efflux pumps). Rapid identification of NDM-producing *A. baumannii-calicoaceticus* complex may help to limit the dissemination of carbapenemase genes not only in *Acinetobacter* genus but also in Enterobacteriaceae through a rapid identification of their potential reservoirs (7).

Due to its intrinsic low permeability, detection of carbapenemase production in *Acinetobacter* genus is considered to be more difficult than in Enterobacteriaceae and

Pseudomonas spp. Several phenotypic techniques have been proposed to detect carbapenemase-producing *Acinetobacter* spp. (14). The modified Hodge test has been largerly used for this purpose. It is based on the *in-vitro* detection of the carbapenemase activity therefore inactivating the antibiotic effect. Although this test is efficient for the detection of IMP and VIM producers, NDM producers and CHDL producers may remain undetected, leading to false-negative results (15). Several techniques using the inhibition properties of EDTA were also proposed for the detection of MBL-producing *Acinetobacter* spp. Those techniques include the combined disk test and the Etest MBL strip (15, 16). However, those techniques are not highly sensitive or specific, and they require an additional period of growth of 24h.

Biochemical detection of carbapenemase production using UV spectrophotometry has also been proposed (17). This technique efficiently detects VIM, IMP, and SIM producers, but NDM and CHDL producers remain difficult to detect (15). Recently, detection of carbapenemase production using MALDI-TOF mass spectrometry has been proposed for *Acinetobacter* spp. (18-20). It is based on the detection of the spectra of imipenem and of its hydrolyzed product, respectively. It shows good sensitivity and specificity, but requires trained microbiologists and expensive equipment. Finally, molecular-based techniques using specific primers are useful to identify carbapenemase genes. Simplex or multiplex PCR methods are available (21), as well as real-time PCR approaches having the advantage to provide a result in 3 hours (22). More recently, a DNA microarray has been developed to detect 91 target sequences associated with antibiotic resistance within 4 h from bacterial culture to result (23). Although those

molecular-based tests are highly sensitive and specific, they fail to detect unknown carbapenemase genes, or those that are not included in the panel of the considered test.

One of the most promising technique for the rapid and accurate detection of any carbapenemase producer is the Carba NP test. This test is based on the biochemical detection of the hydrolysis of the β-lactam ring of imipenem (24, 25). Although the Carba NP test has been extensively validated worldwide for the detection of carbapenemase producers among Enterobacteriaceae and *Pseudomonas* spp. (24, 26), it has not been validated for the detection of carbapenemase-producing *Acinetobacter* spp. The imipenem hydrolysis rates actually obtained with those CHDLs are too low to be detected by the Carba NP test in its original version. The aim of this study was to further evaluate the Carba NP test for detection of carbapenemase producers in *Acinetobacter* spp. and to settle a modified version of the Carba NP test, the CarbAcineto NP test, for an optimal detection of this activity in *Acinetobacter* spp.

MATERIALS AND METHODS

Strain collection. A total of 220 strains were used to evaluate the performance of the CarbAcineto NP test. They were from various clinical origins (blood culture, urine, sputum) and of worldwide origin. Those strains had previously been characterized for their carbapenemase content at the molecular level (Tables 1 and 2). This strain collection included the most frequently acquired carbapenemases identified in human *A. baumannii* clinical isolates (Table 1). Those carbapenemases were OXA-23 (n = 68), OXA-24/-40-like (n = 19), OXA-58-like (n = 26), OXA-143-like (n = 3), GES (n = 8), IMP (n = 2), VIM (n = 1), SIM (n = 1), and NDM (n = 14). Nine isolates co-produced two

carbapenemases; GES-11 + OXA-23 (n = 6) and NDM-1 + OXA-23 (n = 3). Negative controls (n = 69) included (i) wild-type strains (n = 51), (ii) narrow-spectrum ß-lactamase producers (n = 3) and (iii) extended-spectrum ß-lactamases producers (n = 12) (Table 2). Three strains overexpressing their chromosome-encoded *bla*$_{\text{OXA-51}}$-like carbapenemase genes were also tested (Table 2).

Susceptibility testing. Susceptibility testing was performed by determining MIC values using the Etest® (bioMérieux; La Balmes-les-Grottes, France) on Mueller-Hinton agar plates at 37°C and results were recorded according to US guidelines (CLSI), as updated in 2013. The breakpoints for imipenem ad meropenem were susceptible (S) ≤ 4 and resistant (R) ≥ 16 µg/ml.

Carba NP test. The Carba NP test is based on the colorimetric and pH-based detection of hydrolysis of the β-lactam ring of imipenem. The updated protocol of the Carba NP test was performed on bacterial isolates grown on Trypticase Soy (TS) agar (bioMérieux) as previously described (25).

CarbAcineto NP test. The CarbAcineto NP test was adapted from the updated version of the Carba NP test used for the detection of carbapenemase-producing Enterobacteriaceae and *Pseudomonas* spp. (25), in order to be used for *Acinetobacter* spp. Compared to this updated version, the lysis buffer was replaced by a 5M NaCl solution avoiding any buffer effect, and the bacterial inoculum was doubled from third to half of a calibrated loop (10 µl) to a full calibrated loop in order to increase the enzyme quantity. Briefly, a full calibrated loop (10 µl) of the tested strain was recovered from TS agar plates and resuspended in two 1.5 ml eppendorf tubes (A and B) containing 100 µl NaCl 5M. In tube A (internal control), 100 µl of the revealing solution containing a pH

indicator (phenol red) was added. In tube B (test tube), 100 µl of an extemporaneously prepared revelation solution supplemented with imipenem at 6 mg/ml was added. Tubes A and B were incubated at 37°C for a maximum of 2 h. Optical reading of the color change of each tube was performed. The carbapenemase activity was detected by a color change of phenol red solution (red to yellow/orange) in tube B resulting from the hydrolysis of imipenem into a carboxylic derivative leading to a decrease of the pH value. Results of the CarbAcineto NP test were interpreted as follows: (i) tube A red and tube B remaining red, non carbapenemase-producing isolate, (ii) tube A red and tube B turning yellow/orange, carbapenemase-producing strain and (iii) tube A and tube B both turning yellow/orange, non-interpretable result. The phenol red revealing solution was prepared as previously described (24). The NaCl 5M solution was prepared from a dilution of NaCl powder (Sigma-Aldrich, Saint Quentin Fallavier, France) in distilled water.

RESULTS

Using the updated protocol of the Carba NP test set up for detection of the carbapenemase activity in Enterobacteriaceae and *Pseudomonas* spp., positive results were obtained only for MBL-producing *Acinetobacter* spp. (Table 1). The Carba NP test failed to detect *Acinetobacter* spp. isolates producing carbapenemase of the GES- and/or OXA-types (Table 1). This test did not detect any carbapenemase activity among strains being resistant to carbapenems due to the overexpression of their intrinsic OXA-51-like enzyme (Table 2). Accordingly, the specificity and sensitivity of the Carba NP test for the detection of *Acinetobacter* spp. isolates producing an acquired carbapenemase were found to be 100% and 11.9% only, respectively.

On the other hand, the CarbAcineto NP test allowed to detect all isolates producing an acquired carbapenemase, with the exception of some GES-type producers (Table 1, Figure 1B). Isolates being carbapenem-resistant due to overexpression of their chromosome-encoded OXA-51-like β-lactamase (Table 2, Figure 1C) or to non-carbapenemase-mediated mechanisms such as combined mechanisms of resistance (outer-membrane permeability defect +/- associated with overproduction of cephalosporinase and/or ESBLs) also remained negative with this test (Table 2, Figure 1A). In most cases, positive results gave frank color change from red to yellow (Figure B). For MBL producers, a positive result was always obtained in less than 15 minutes. For ca. 13% (9/68) of the OXA-23 producers and ca. 23% (6/22) of the OXA-58-like producers, the color in the test tube (tube B) turned from red to orange but only after 2 hours of incubation (Figure 1B). However, this color change was easy to detect when looking at the upper part of the tubes after vortexing (Figure 1B). Among the 211 tested strains of *Acinetobacter* spp., only two gave non-interpretable results (Table 2, Figure 1D). The specificity and sensitivity of the CarbAcineto NP test were therefore estimated to be 100% and 94.7%, respectively.

DISCUSSION

The Carba NP test, initially set up for detection of carbapenemase-producing Enterobacteriaceae and *Pseudomonas* spp., efficiently detects MBL-producing *Acinetobacter* spp. However, it fails to detect OXA-type carbapenemases which are the most frequently identified carbapenemases among *Acinetobacter* spp. Since those CHDLs found in *Acinetobacter* spp. (OXA-23, OXA-24/-40-like, OXA-58-like, OXA-143-like) possess a weaker carbapenemase activity as compared to those usually

measured in Enterobacteriaceae (mostly KPC, MBLs, and OXA-48) and *Pseudomonas* spp. (mostly MBLs), we made the hypothesis that the buffer used in the Carba NP test may buffer at a too high level and may counteract the detection of any weak carbapenemase activity.

Specificity and sensitivity of the CarbAcineto NP test were found to be 100% and 94.7%, respectively. The CarbAcineto NP test efficiently detected the OXA-type carbapenemase producers leading to a significant improvement of the sensitivity (94.7% for the CarbAcineto NP test *vs* 11.9% for the Carba NP test). The better detection of CHDLs is due to two independent factors. First, the bacterial inoculum used in the CarbAcineto NP test was doubled compared to the Carba NP test, leading to an increased amount of enzyme released in the revealing solution. Second, the lysis buffer used for the Carba NP test (B-PER II, Bacterial Protein Extraction Reagent, Pierce, Thermo Scientific, Villebon-sur-Yvette, France) has been replaced by an hyperosmotic solution of NaCl 5M. Use of this latter buffer provides two main advantages. It does not buffer enough to interfer negatively against slight pH changes, and its hyperosmotic properties lead to an efficient lysis of the bacteria. Water has also been tested to substitute the B-PER II lysis buffer, but the color changes were less clear and the test failed to detect nineteen carbapenemase producers (nine OXA-23 and six OXA-58-like) giving 'orange' results (data not shown). This lack of detection might be explained by the weak lysis of the bacteria in water compared to the hyperosmotic solution of NaCl, resulting in lower enzyme release. Recently, a derivative version of the Carba NP test was developed. This test used water instead of B-PER buffer and bromothymol blue instead of red phenol as pH indicator. (27) The authors claimed that the use of water instead of B-PER buffer led

to the detection of all carbapenemase-producing *Acinetobacter* spp. However, in their study, only fourteen OXA-23 and six OXA-58-like producers were tested, which is not sufficient to assess the reliability of that test, considering that only 13.2% of the OXA-23 producers and 23.1% of the OXA-58-like producers could be detected when using water instead of NaCl. As previously observed using the Carba NP test with *P. aeruginosa* strains, the GES-type carbapenemase producers were not detected using the CarbAcineto NP test (26). The GES-type carbapenemases are point mutant analogues of the ESBL GES-1 and possess an extended but very weak carbapenemase activity that could explain this lack of detection. This lack of detection of GES-type carbapenemases in *P. aeruginosa* may be also due to a weaker release or production of GES-type carbapenemases in *P. aeruginosa* (26), since a GES-5-producing *Enterobacter cloacae* isolate was identified with the Carba NP test (24).

Although the number of tested strains was low (3 isolates), the CarbAcineto NP test gave negative results when testing carbapenem-resistant *Acinetobacter* spp. overexpressing their chromosome-encoded OXA-51-like β-lactamase (Table 2). In fact, lack of detection of OXA-51-like overproducers may be interesting when considering the usefulness of the CarbAcineto NP test. Indeed, those chromosomally-encoded resistance mechanisms are not supposed to be transferable to other organisms, by contrast to plasmid-encoded mechanisms, which therefore constitutes a much less important clinical issue. Thus, clearly distinguishing acquired from non-acquired carbapenemase producers provides an added-value to the test.

In conclusion, the CarbAcineto NP test offers a rapid and cost-effective solution for detecting acquired carbapenemase producers among the *Acinetobacter* species. It

might contribute to prevent the spread of those multidrug-resistant strains, but also of several carbapenemase genes, considering for example that *bla*$_{\text{NDM-1}}$-like genes that initially spread among *Acinetobacter* spp. then targeted also Enterobacteriaceae and *Pseudomonas* spp. (7). Use of the CarbAcineto NP test will be interesting in particularly for ICU patients for whom multidrug-resistant isolates belonging to the *A. baumannii-calicoaceticus* complex are a common source of severe infections. It particularly makes sense testing for cartbapenemase production considering that acquisition of a carbapenemase in *A. baumannii* is a marker of multidrug resistance.

Finally, by using both the Carba NP and CarbAcineto NP tests, any microbiology laboratory worldwide may have the opportunity to efficiently identify one of the most important clinical resistance trait of modern microbiology, i.e. carbapenem resistance-mediated mechanisms leading to multidrug- or pandrug resistance in clinically-significant Gram negatives.

Acknowledgments

We are grateful to Prof. Y. Chong and Prof. K. Lee for providing us with the SIM-1-producing isolate, and to Prof. D.M. Livermore for the gift of the OXA-25- and OXA-26-producing isolates.

Funding

This work was funded by a grant from the INSERM (UMR914).

Conflict of interest

An international patent form for the Carba NP test (that included further developments such as the CarbAcineto NP test) has been filled on behalf of INSERM Transfert (Paris, France).

REFERENCES

1. **Bergogne-Berezin E, Towner KJ.** 1996. *Acinetobacter* spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. Clin. Microbiol. Rev. **9:**148-165.
2. **Poirel L, Nordmann P.** 2006. Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology. Clin. Microbiol. Infect. **12:**826-836.
3. **Bonnin RA, Rotimi VO, Al Hubail M, Gasiorowski E, Al Sweih N, Nordmann P, Poirel L.** 2013. Wide dissemination of GES-type carbapenemases in *Acinetobacter baumannii* isolates in Kuwait. Antimicrob. Agents Chemother. **57:**183-188.
4. **Robledo IE, Aquino EE, Sante MI, Santana JL, Otero DM, Leon CF, Vazquez GJ.** 2010. Detection of KPC in *Acinetobacter* spp. in Puerto Rico. Antimicrob. Agents Chemother. **54:**1354-1357.
5. **Moubareck C, Bremont S, Conroy MC, Courvalin P, Lambert T.** 2009. GES-11, a novel integron-associated GES variant in *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **53:**3579-3581.
6. **Bonnin RA, Poirel L, Naas T, Pirs M, Seme K, Schrenzel J, Nordmann P.** 2012. Dissemination of New Delhi metallo-β-lactamase-1-producing *Acinetobacter baumannii* in Europe. Clin. Microbiol. Infect. **18:**E362-365.

7. **Bonnin RA, Poirel L, Nordmann P.** 2014. New Delhi Metallo-ß-lactamase-producing *Acinetobacter baumannii*: a novel paradigm for spreading antibiotic resistance genes. Future Microbiol. **9:**33-41.

8. **Higgins PG, Poirel L, Lehmann M, Nordmann P, Seifert H.** 2009. OXA-143, a novel carbapenem-hydrolyzing class D β-lactamase in *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **53:**5035-5038.

9. **Mugnier PD, Poirel L, Naas T, Nordmann P.** 2010. Worldwide dissemination of the bla_{OXA-23} carbapenemase gene of *Acinetobacter baumannii*. Emerg. Infect. Dis. **16:**35-40.

10. **Brown S, Young HK, Amyes SG.** 2005. Characterisation of OXA-51, a novel class D carbapenemase found in genetically unrelated clinical strains of *Acinetobacter baumannii* from Argentina. Clin. Microbiol. Infect. **11:**15-23.

11. **Evans BA, Brown S, Hamouda A, Findlay J, Amyes SG.** 2007. Eleven novel OXA-51-like enzymes from clinical isolates of *Acinetobacter baumannii*. Clin. Microbiol. Infect. **13:**1137-1138.

12. **Héritier C, Poirel L, Fournier PE, Claverie JM, Raoult D, Nordmann P.** 2005. Characterization of the naturally occurring oxacillinase of *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **49:**4174-4179.

13. **Turton JF, Ward ME, Woodford N, Kaufmann ME, Pike R, Livermore DM, Pitt TL.** 2006. The role of IS*Aba1* in expression of OXA carbapenemase genes in *Acinetobacter baumannii*. FEMS Microbiol. Lett. **258:**72-77.

14. **Bonnin RA, Nordmann P, Poirel L.** 2013. Screening and deciphering antibiotic resistance in *Acinetobacter baumannii*: a state of the art. Expert Rev. Anti Infect. Ther. **11:**571-583.

15. **Bonnin RA, Naas T, Poirel L, Nordmann P.** 2012. Phenotypic, biochemical, and molecular techniques for detection of metallo-β-lactamase NDM in *Acinetobacter baumannii*. J. Clin. Microbiol. **50:**1419-1421.

16. **Franklin C, Liolios L, Peleg AY.** 2006. Phenotypic detection of carbapenem-susceptible metallo-β-lactamase-producing gram-negative bacilli in the clinical laboratory. J. Clin. Microbiol. **44:**3139-3144.

17. **Bernabeu S, Poirel L, Nordmann P.** 2012. Spectrophotometry-based detection of carbapenemase producers among *Enterobacteriaceae*. Diagn. Microbiol. Infect. Dis. **74:**88-90.

18. **Alvarez-Buylla A, Picazo JJ, Culebras E.** 2013. Optimized method for *Acinetobacter* species carbapenemase detection and identification by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J. Clin. Microbiol. **51:**1589-1592.

19. **Kempf M, Bakour S, Flaudrops C, Berrazeg M, Brunel JM, Drissi M, Mesli E, Touati A, Rolain JM.** 2012. Rapid detection of carbapenem resistance in *Acinetobacter baumannii* using matrix-assisted laser desorption ionization-time of flight mass spectrometry. PLoS One **7:**e31676.

20. **Lee W, Chung HS, Lee Y, Yong D, Jeong SH, Lee K, Chong Y.** 2013. Comparison of matrix-assisted laser desorption ionization-time-of-flight mass spectrometry assay with conventional methods for detection of IMP-6, VIM-2,

NDM-1, SIM-1, KPC-1, OXA-23, and OXA-51 carbapenemase-producing *Acinetobacter* spp., *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. Diagn. Microbiol. Infect. Dis. **77:**227-230.

21. **Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S, Amyes SG, Livermore DM.** 2006. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. Int. J. Antimicrob. Agents **27:**351-353.

22. **Huang XZ, Cash DM, Chahine MA, Nikolich MP, Craft DW.** 2012. Development and validation of a multiplex TaqMan real-time PCR for rapid detection of genes encoding four types of class D carbapenemase in *Acinetobacter baumannii*. J. Med. Microbiol. **61:**1532-1537.

23. **Dally S, Lemuth K, Kaase M, Rupp S, Knabbe C, Weile J.** 2013. DNA microarray for genotyping antibiotic resistance determinants in *Acinetobacter baumannii* clinical isolates. Antimicrob. Agents Chemother. **57:**4761-4768.

24. **Nordmann P, Poirel L, Dortet L.** 2012. Rapid detection of carbapenemase-producing *Enterobacteriaceae*. Emerg. Infect. Dis. **18:**1503-1507.

25. **Dortet L, Bréchard L, Poirel L, Nordmann P.** 2014. Impact of the isolation medium for detection of carbapenemase-producing *Enterobacteriaceae* using an updated version of the Carba NP test. J. Med. Microbiol. In press.

26. **Dortet L, Poirel L, Nordmann P.** 2012. Rapid detection of carbapenemase-producing *Pseudomonas* spp. J. Clin. Microbiol. **50:**3773-3776.

27. **Pires J, Novais A, Peixe L.** 2013. Blue-CARBA: an easy biochemical test to detect diverse carbapenemase producers directly from bacterial cultures. J. Clin. Microbiol. **51:**4281-4283.

Figure legend.

Figure 1. Representative results obtained using the CarbAcineto NP test on *Acinetobacter* spp. isolates. **A.** Representative results obtained using the CarbAcineto NP test on wild-type (WT), narrow-spectrum β-lactamase-producing (OXA-21, RTG-4) and extended-spectrum β-lactamase-producing *Acinetobacter* spp. (PER-1, VEB-1, SHV-5). **B.** Representative results obtained using the CarbAcineto NP test on acquired carbapenemase producing *Acinetobacter* spp. isolates. Carbapenemase of Ambler class A (GES-types), Ambler class B (IMP-type, NDM-type) and Ambler class D (OXA-23, OXA-24/-40, OXA-58 and OXA-143) are represented. **C.** Representative result obtained using the CarbAcineto test on a carbapenem-resistant *Acinetobacter* spp. overexpressing its chromosome-encoded OXA-51 β-lactamase. **D.** Representative result of a non interpretable result using the CarbAcineto NP on a wild-type *Acinetobacter* spp. isolate.

Table 1. Results of the Carba NP and CarbAcineto NP tests when performed on *Acinetobacter* spp. producing an acquired carbapenemase.

Carbapenemase	Species	n	MIC (µg/ml)		Carba NP test	CarbAcineto NP test
			IMP	MER		
Ambler class A carbapenemases						
GES-type						
GES-11	*A. baumannii*	3	2	4	-	-
GES-14	*A. baumannii*	5	24 - 32	16 - 32	-	-
Ambler class B carbapenemases						
NDM-type						
NDM-1	*A. baumannii*	13	>32	>32	+	+
NDM-2	*A. baumannii*	1	>32	>32	+	+
IMP-type						
IMP-1	*A. baumannii*	1	4	6	+	+
IMP-4	*A. baumannii*	1	24	16	+	+
VIM-type						
VIM-4	*A. genomospecies* 16	1	>32	>32	+	+
SIM-type						
SIM-1	*A. baumannii*	1	>32	>32	+	+
Ambler class D carbapenemases						
OXA-23 group						
OXA-23	*A. baumannii*	68	24 - >32	8 - >32	-	+
OXA-40 group						
OXA-24/OXA-40	*A. baumannii*	8	>32	>32	-	+
OXA-25	*A. baumannii*	1	>32	>32	-	+
OXA-26	*A. baumannii*	1	>32	>32	-	+
OXA-72	*A. baumannii*	9	>32	>32	-	+
OXA-58 group						
OXA-58	*A. baumannii*	24	16 - >32	8 - >32	-	+
OXA-58	*A. haemolyticus*	1	>32	8	-	+
OXA-97	*A. baumannii*	1	>32	>32	-	+
OXA-143 group						
OXA-143	*A. baumannii*	2	>32	>32	-	+
OXA-293	*A. baumannii*	1	>32	>32	-	+
Multiple carbapenemases						
GES-11 + OXA-23	*A. baumannii*	6	32	>32	-	+
NDM-1 + OXA-23	*A. baumannii*	3	>32	>32	+	+

IMP, imipenem ; MER, meropenem
A. baumannii means *A. baumannii-calcoaceticus* complex

Table 2. Results of the Carba NP and CarbAcineto NP tests when testing *Acinetobacter* spp. strains that do not possess acquired carbapenemases.

Acquired β-lactamase	Species	n	MIC (µg/ml)		Carba NP test	CarbAcineto NP test
			IMP	MER		
None						
None	*A. baumannii*	36	0.19 - 2	0.12 - 3	-	-
None	*A. junii*	3	0.05 - 0.09	0.05 - 0.09	-	-
None	*A. lwolffi*	4	0.09 - 0.19	0.09 - 0.19	-	-
None	*A. ursingii*	2	0.06 - 0.09	0.06 - 0.09	-	-
None	*A. johnsonii*	4	0.06 - 0.09	0.06 - 0.09	-	-
None	*A. baumannii*	1	0.5	0.75	**NI**	-
None	*A. johnsonii*	1	0.09	0.09	**NI**	-
Narrow-spectrum β-lactamase						
SCO-1	*A. baumannii*	1	0.38	0.5	-	-
RTG-4	*A. baumannii*	1	0.38	0.38	-	-
OXA-21	*A. baumannii*	1	0.75	0.5	-	-
Extended-spectrum β-lactamase						
SHV-5	*A. baumannii*	1	6	4	-	-
PER-1	*A. baumannii*	1	1.5	1.5	-	-
VEB-1	*A. baumannii*	9	0.38 - 1	0.5 - 1.5	-	-
GES-12	*A. baumannii*	1	32	32	-	-
Overexpressed chromosome-encoded OXA-51-like β-lactamase						
IS*Aba1* + OXA-51	*A. baumannii*	2	2 - 3	3	-	-
IS*Aba1* + OXA-66	*A. baumannii*	1	4	6	-	-

IMP, imipenem ; MER, meropenem
NI, not interpretable
A. baumannii means *A. baumannii-calcoaceticus* complex