Evaluation of the Amplidiag CarbaR+VRE Kit for Accurate Detection of Carbapenemase-Producing Bacteria

Saoussen Oueslati,^{a,b,c} Delphine Girlich,^{a,b,c} Laurent Dortet,^{a,b,c,d} Thierry Naas^{a,b,c,d}

^aBacteriology-Hygiene Unit, Assistance Publique/Hôpitaux de Paris, Bicêtre Hospital, Le Kremlin-Bicêtre, France

^bEA7361 "Structure, dynamic, function and expression of broad spectrum β -lactamases," Université Paris-Sud, Université Paris-Saclay, LabEx Lermite, Faculty of Medicine, Le Kremlin-Bicêtre, France

^cAssociated French National Reference Center for Antibiotic Resistance: Carbapenemase-producing Enterobacteriaceae, Le Kremlin-Bicêtre, France

^dEvolution and Ecology of Resistance to Antibiotics Unit, Institut Pasteur-APHP-Université Paris-Sud, Paris, France

ABSTRACT As carbapenemase-producing Gram-negative bacilli (CP-GNB) (*Enterobacteriaceae*, *Pseudomonadaceae*, and *Acinetobacter* spp.) are becoming a major public health issue, there is an urgent need for accurate and fast diagnostic tests. The Amplidiag CarbaR+VRE assay is a multiplex nucleic acid-based *in vitro* diagnostic test intended for the detection of CP-GNB and vancomycin-resistant enterococci (VRE) from cultured colonies. We have evaluated its ability to detect carbapenemase genes in 100 well-characterized GNB and in 200 consecutive enterobacterial isolates with reduced susceptibility to carbapenems that were referred to the French National Reference Center for carbapenem resistance. The assay has been validated on purified DNA but also directly on colonies. The Amplidiag CarbaR+VRE assay could detect all KPC, NDM, VIM, IMP, and OXA-48-like variants tested and all acquired carbapenem-hydrolyzing oxacillinases from *Acinetobacter baumannii* (OXA-23, OXA-24/-40, and OXA-58) as well as the overexpressed chromosomally encoded OXA-51-like β -lactamase associated with an upstream inserted *ISAba1*. However, as claimed by the manufacturer, other carbapenemases such as GES-like carbapenemases (GES-2, GES-5, and GES-14), GIM-1, AIM-1, SPM-1, DIM-1, OXA-198 in *Pseudomonas aeruginosa*, or OXA-143-like in *A. baumannii* were not detected. Amplidiag CarbaR+VRE's performance values were high (100% sensitivity and 99% specificity) as it could detect the five major carbapenemases—NDM, VIM, IMP, KPC, and OXA-48—as well as OXA-type carbapenemases from *Acinetobacter* spp. that are currently emerging also among *Proteus mirabilis* and other enterobacterial isolates. It can provide a result directly from colonies growing on Mueller-Hinton (MH) agar or on selective screening medium in less than 2 h. Further evaluations are now necessary to determine the performance values directly on rectal swabs.

KEYWORDS multiplex PCR, real-time detection, screening, confirmation, carbapenemase, molecular methods

Carbapenemase-producing *Enterobacteriaceae* (CPE) and nonfermenters (CPNF; *Pseudomonadaceae* and *Acinetobacter* species) have increasingly been reported worldwide (1, 2). The most clinically relevant carbapenemases belong to either Ambler class A (KPC type); Ambler class B, i.e., metallo- β -lactamases (MBLs) (such as IMP, VIM, and NDM types); or Ambler class D, such as (i) OXA-48-like in *Enterobacteriaceae*; (ii) OXA-23, OXA-24/-40, OXA-58, OXA-143, and the overexpressed intrinsic OXA-51-like enzymes in *Acinetobacter* spp.; and (iii) OXA-198 in *Pseudomonas aeruginosa* (3–6).

There is a large heterogeneity of carbapenem resistance mechanisms depending on the bacterial species and its geographical location. Concerning *Enterobacteriaceae*, KPC

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Address correspondence to Thierry Naas,
thierry.naas@aphp.fr.

dominates in the United States, Greece, Italy, and Israel (1, 4). The IMP-1 enzyme is mostly prevalent in Japan, and OXA-48-like carbapenemases are of great concern in the Middle East, in most Mediterranean countries, and in many European countries such as France, where a large variety of enzymes are present (7). In *Pseudomonas* spp. and *Acinetobacter* spp., Ambler class A, B, and D carbapenemases have been detected. In addition to the acquired OXA carbapenemases (mostly OXA-23, OXA-24/40, OXA-58, and OXA-143), the *Acinetobacter baumannii*-*Acinetobacter calcoaceticus* complex possesses a chromosomally encoded OXA-51-like enzyme with weak carbapenemase activity (6). However, transposition of an insertion sequence (IS), mostly IS_{Aba1}, upstream of the *bla*_{OXA-51-like} gene can lead to overexpression of the latter, resulting in carbapenem resistance (5). Currently, in the United States and Europe, acquired OXA carbapenemase production (particularly of OXA-23) is the most prevalent mechanism among *A. baumannii* isolates. At the opposite end, mutations or the loss of expression in OprD porin, even in the absence of any carbapenemase, is the primary mechanism of resistance to imipenem among *P. aeruginosa* species (5). However, in *Pseudomonas* spp. the most prevalent carbapenemases worldwide are of the VIM and IMP types (5).

Simple, rapid, and accurate methods are needed to detect all carbapenemase producers and discriminate them among non-carbapenemase producers in order to implement proper infection control measures to prevent further spread in hospitals. Various tests (combined with algorithms) have been developed for the early detection of carbapenemase producers: (i) algorithms based on disc diffusion (8–11); (ii) combination disc diffusion assays (9); (iii) tests able to detect a carbapenem-hydrolyzing activity, such as the Carba NP test and derivatives (12), matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) protocols (13), the BYG test (14), the carbapenem inactivation method (CIM) test (15), and the β -Carba test (16); (iv) immunochromatographic tests for the rapid detection of KPC, OXA-48-type, and NDM carbapenemases (17, 18); and (v) molecular biology-based techniques that all aim to detect the most widespread carbapenemase-encoding genes from bacterial colonies and/or directly from clinical samples (such as rectal swabs) (19, 20).

Molecular diagnostic assays include DNA amplification-based methods: PCR, loop-mediated isothermal amplification (LAMP), microarrays, and whole-genome sequencing (WGS) (19, 21, 22). These methods detect the presence of a carbapenemase gene likely accounting for carbapenem resistance (19). The most common commercially available molecular assays are (i) the Xpert Carba-R kit (Cepheid, Sunnyvale, CA, USA), which detects KPC, NDM, VIM, IMP-1-like, and OXA-48-like carbapenemases; (ii) Eazyplex SuperBug CRE (Amplex Biosystems GmbH, Giessen, Germany), which detects KPC, NDM, VIM, and OXA-48 carbapenemases; (iii) Eazyplex complete A (Amplex Biosystems), which detects also OXA-23, OXA-40, and OXA-58 from *Acinetobacter* spp.; and (iv) the Check-Direct CPE assay (Check-Points, Wageningen, The Netherlands), which detects KPC, NDM, VIM, and OXA-48. Most of these assays detect at least the four most prevalent carbapenemases, representing more than 95% of the carbapenemases produced by *Enterobacteriaceae* in France (19, 20, 23).

Here, we have evaluated the performance of Amplidiag CarbaR+VRE (Mobidiag Ltd., Espoo, Finland), a qualitative multiplexed nucleic acid-based *in vitro* diagnostic test intended for the detection of carbapenemase-encoding genes in *Enterobacteriaceae* and nonfermenting rods as well as vancomycin (Van) resistance related to ligase genes *vanA* and *vanB* in enterococci (VRE).

(Part of these results have been presented at ECCMID 2017, Vienna, Austria.)

MATERIALS AND METHODS

Bacterial isolates. A total of 100 collection isolates with characterized β -lactamase content were used in this study. They included 50 *Enterobacteriaceae*, 30 *Pseudomonas* species, and 20 *Acinetobacter baumannii* isolates (see Tables S1, S2, S3, S4, and S5 in the supplemental material). These isolates comprised 79 carbapenemase-producing Gram-negative bacilli (CP-GNB) and 21 non-CP-GNB. The 79 CP-GNB consisted of 41 *Enterobacteriaceae* (9 KPC, 5 NDM, 4 VIM, 3 IMP, and 15 OXA-48-like producers and 5 isolates producing multiple carbapenemases [three NDM plus OXA-48-like, one NDM-1 plus VIM-2, and one VIM-4 plus OXA-48] [Table S2]), 20 *P. aeruginosa* isolates (2 KPC-2, 2 GES-like, 7 VIM-like, 4 IMP-like, 1 GIM-1, 1 AIM-1, 1 SPM-1, 1 DIM-1, and 1 OXA-198) (Table S3), and 18 *Acinetobacter* isolates (4

FIG 1 Experimental procedures. (A) Experimental setup as recommended by the manufacturer. As DNA extraction is open, the QIAcube automated DNA extractor was used. (B) Boiling DNA extraction. (C) One colony was picked three times in each 20- μ l PCR tube. PCR amplification results have been automatically interpreted using the manufacturer's proprietary software. The total duration of the process is also indicated.

ISAb1-OXA-51-like, 3 OXA-23, 2 OXA-24/-40, 2 OXA-58, 2 OXA-143-like, 2 NDM, 1 IMP, 1 VIM and 1 GES-14) (Table S4). Non-CP-GNB ($n = 21$) were 9 *Enterobacteriaceae*, 10 *P. aeruginosa*, and 2 *A. baumannii* isolates. Non-CP-GNB also included 2 OXA-48-like producers that are devoid of carbapenem-hydrolytic activity (OXA-163 and OXA-405) (22–24), which usually give false-positive results with molecular methods (Table S2) (15). A collection of well-characterized *Proteus mirabilis* isolates have been tested subsequently. These isolates comprised 10 carbapenemase producers (8 OXA-23 and 2 OXA-58) and 2 non-carbapenemase producers.

From 20 January to 10 February 2016, 200 consecutive enterobacterial isolates were referred to the French National Reference Center (F-NRC) for Antibiotic Resistance for evaluation. Reidentification of these 200 isolates revealed 198 *Enterobacteriaceae* and 2 *A. baumannii* isolates.

Susceptibility testing. Antimicrobial susceptibilities were determined by the disc diffusion technique on Mueller-Hinton (MH) agar (Bio-Rad, Marnes-La-Coquette, France) and interpreted according to the EUCAST breakpoints as updated in 2016 (<http://www.eucast.org>). MICs were determined using the Etest technique (bioMérieux).

Carbapenemase detection. Carbapenemase activity was evidenced in *Enterobacteriaceae* using the updated version of the Carba NP test (12), and carbapenemase genes were detected using an in-house PCR-sequencing approach directly on colonies (6, 15, 21).

Automated DNA extraction from cultured bacterial colonies. Fresh overnight bacterial cultures (on MH agar) were used for DNA extraction with a QIAamp DNA minikit (Qiagen, Les Ulis, France) and the QIAcube instrument (Qiagen, Courtaboeuf, France). A full loop of colonies was suspended in 180 μ l of tissue lysis (ATL; Qiagen) buffer and processed in the automated extractor according to the protocol suggested by the manufacturer for bacterial pellets. Total extraction time was 1 h. Five microliters of this DNA was used as sample in a 20- μ l PCR mixture.

Whole-cell DNA extracted by boiling. One colony of each bacterial strain was suspended in 100 μ l of distilled water. After 10 min at 100°C on a dry heating block (Thermo Fisher), the suspensions were centrifuged (1 min at 10,000 rpm). Total extraction time was 11 min. Five microliters of supernatant was used in a 20- μ l PCR assay mixture.

Direct amplification. One colony was pricked three times with the tip of three loops of 1 μ l each, and bacteria of each tip were dispersed directly into each PCR tube containing 20 μ l of PCR mix (see Tables 2 and S5).

Real-time PCR assay (Amplidiag CarbaR+VRE assay). The Amplidiag CarbaR+VRE multiplex amplification was performed to detect carbapenemase (and vancomycin resistance) genes using a fluorophore-labeled TaqMan probe (Fig. 1). The markers detected by the assay are listed in Table S6. Amplidiag CarbaR+VRE comprises required test reagents for PCR and analysis software. The sample material is DNA extracted from a bacterial culture. Assays are performed in 3 reactions per sample,

TABLE 1 Global performance of the Amplidiag CarbaR+VRE assay^a

Test parameter	% for isolate group (95% CI)			
	Prospective (all isolates) (n = 200)	<i>Enterobacteriaceae</i> (n = 50)	<i>Pseudomonas</i> spp. (n = 30)	<i>A. baumannii</i> (n = 20)
Sensitivity	100 (95.4–100)	100 (85.6–100)	100 (71.7–100)	100 (74.7–100)
Specificity	100 (95.3–100)	88.9 (50.7–99.4)	100 (65.6–100)	100 (19.8–100)
PPV	100 (95.4–100)	NA	NA	NA
NPV	100 (95.3–100)	NA	NA	NA

^aAbbreviations: NA, not applicable; PPV, positive predictive value; NPV, negative predictive value.

according to the fluorophores indicated in Table S6. The Amplidiag CarbaR+VRE kit obtained CE marking for *in vitro* diagnostic kits (CE-IVD) on 8 December 2015. The recommended DNA extraction method for pure culture samples to be used in conjunction with the Amplidiag CarbaR+VRE assay is the “Generic 2.0.1” protocol of NucliSENS easyMAG (bioMérieux) or the Amplidiag Easy system (provided by Mobidiag). Other extraction methods could be used with thorough validation by the users (such as for the QIAcube instrument). Concerning PCR instrumentation, the kit is validated on the Rotorgene (Qiagen), CFX96 (Bio-Rad), and ABI7500 (Applied Biosystems) systems.

The assay is divided into three multiplex PCRs, each of which amplifies a specific group of target genes: (i) *Acinetobacter* oxacillinase genes (*bla*_{OXA-23}, *bla*_{OXA-40}, *bla*_{OXA-58}, and *bla*_{OXA-51} with upstream *ISAb1*); (ii) *bla*_{OXA-48-like}, *bla*_{NDM}, *bla*_{VIM}, *bla*_{IMP}, and *bla*_{KPC}; and (iii) *vanA* and *vanB*. Each mix contained 10 μ l of 2 $\times$ PCR master mix, 5 μ l of assay mix, and 5 μ l of DNA (or a bacterial suspension in water for direct amplification assay on one colony pricked three times). The real-time PCR Rotorgene 6000 amplification-detection system (Qiagen) was used. The total run time was 85 min, for 45 cycles of amplifications (recommended by the manufacturer), and 60 min when reducing the number of cycles to 30 (for time optimization), and analysis was available immediately after the run. Results were automatically interpreted using the proprietary analyzer software that analyzes the quantitative PCR (qPCR) raw data obtained in each experiment. To prevent cross talk, the users have to calibrate the system with a DNA calibrator as recommended. The results were represented in several ways: a plate, a graph, and a table panel. The plate panel contains positive or negative symbols and possible warnings and errors related to the analyzed well. The graph panel contains normalized fluorescence intensity and cycle numbers. The table panel contains all results (positive, negative, quantification cycle [*C_q*] values, warnings, and errors). The software considers that the sample is positive when the *C_q* is lower than 35.

Statistical analysis. The sensitivity, specificity, positive predictive value, and negative predictive value of the Amplidiag CarbaR+VRE assay were calculated with their respective 95% confidence intervals (95% CIs) using the free software vassarStats (website for statistical computation) at <http://vassarstats.net/>. The gold standard was PCR followed by sequencing.

RESULTS AND DISCUSSION

The Amplidiag CarbaR+VRE has been tested on a collection of 100 characterized isolates expressing various β -lactamases and on 200 consecutive enterobacterial isolates with reduced susceptibility to at least one carbapenem (imipenem, meropenem, or ertapenem) according to EUCAST guidelines as updated in 2016 (<http://www.eucast.org>) and referred to the Associated French National Reference Center (F-NRC) for Antimicrobial Resistance dedicated to carbapenem-resistant *Enterobacteriaceae* from 20 January to 10 February 2016. The markers detected by this assay are *bla*_{KPC-like}, *bla*_{NDM-like}, *bla*_{VIM-like}, *bla*_{IMP-like}, and *bla*_{OXA-48-like}; *Acinetobacter*-related OXA genes, including *bla*_{OXA-23}, *bla*_{OXA-24/-40}, *bla*_{OXA-58}, and *bla*_{OXA-51} with upstream *ISAb1*; and *vanA* and *vanB* for enterococci. The last parameter was not evaluated here.

Retrospective evaluation of a collection of well-characterized *Enterobacteriaceae*, *P. aeruginosa*, and *A. baumannii* isolates. During this retrospective evaluation, the Amplidiag CarbaR+VRE assay was used as recommended by the manufacturer, on QIAcube-extracted DNA recovered from fresh overnight bacterial colonies.

In *Enterobacteriaceae*, the Amplidiag CarbaR+VRE assay detected all KPC, NDM, VIM, IMP, and OXA-48 producers. In addition, strains that produce an OXA-48 variant with carbapenemase activity, including OXA-162, -181, -204, -232, and -244, were also accurately detected (see Table S2 in the supplemental material). In multiple carbapenemase producers, all resistance determinants were correctly identified (Table 1). None of the non-carbapenemase producers gave positive PCR results, except one *Serratia marcescens* isolate that produced OXA-405, an OXA-48 variant that lacked carbapenemase activity but gained efficient expanded-spectrum cephalosporin hydrolytic activity due to a 4-amino-acid deletion in the active site (Table 1) (24). Consequently, and as

already observed with several other molecular assays (23), this OXA-405-producing *S. marcescens* isolate was considered a false positive. Surprisingly, as opposed to most of the other molecular assays (23), OXA-163, another OXA-48 variant with a slightly different 4-amino-acid deletion in the active site but also lacking carbapenem-hydrolytic activity, was not detected and thus, this isolate was correctly identified as a non-carbapenemase producer (Table S2). Using this strain collection ($n = 50$), the overall sensitivity and specificity were 100% (95% confidence interval [95% CI] = 85.6% to 99.9%) and 88.9% (95% CI = 50.7% to 99.4%) for the detection of carbapenemase-producing *Enterobacteriaceae* (CPE), respectively (Table 1).

Concerning *Pseudomonadaceae*, all KPC, VIM, and IMP producers were correctly detected (Table S3). As claimed by the manufacturer, other carbapenemases such as GES-like carbapenemases (GES-2 and GES-5), GIM-1, AIM-1, SPM-1, DIM-1, and OXA-198 were not detected (Table S3). All non-carbapenemase producers gave a negative result. Excluding strains producing rare carbapenemases (7/30) that are not targeted by the assay, the overall sensitivity and specificity were 100% (95% CI = 71.7% to 100%) and 100% (95% CI = 65.6% to 100%) for the detection of carbapenemase-producing *Pseudomonadaceae*, respectively (Table 1).

The F-NRC for multidrug-resistant (MDR) *P. aeruginosa* isolates showed that between 2013 and 2016 most of the carbapenem resistance was due to OprD mutations (76%) and only 24% was due to carbapenemases. VIM (72.8%), KPC (0.4%), and IMP (15.6%) enzymes would have been identified with this assay, while DIM (0.2%) and GES-carbapenemases (11%) would have been missed (http://www.cnr-resistance-antibiotiques.fr/ressources/pages/Rapport_CNR_2016.pdf). Thus, 88.8% of the currently spreading carbapenemases in *P. aeruginosa* in France would be identified with the Amplidiag CarbaR+VRE assay.

Among *A. baumannii* isolates, the Amplidiag CarbaR+VRE assay could detect all OXA-23-like, OXA-24/-40-like, and OXA-58-like enzymes as well as overexpression of the chromosomally encoded OXA-51-like β -lactamases, due to the presence of *ISAbal* upstream of the *bla*_{OXA-51-like} genes (Table S4). As claimed by the manufacturer, carbapenemases of GES type (e.g., GES-14) and of the OXA-143 group (e.g., OXA-143 and OXA-243) were not detected (Table S4). Of note, carbapenem-resistant *A. baumannii* isolates have become increasingly frequently isolated in Brazil, and those producing OXA-23 and OXA-143-like enzymes are the most prevalent (25). Excluding strains producing a rare carbapenemase (3/20) that is not targeted by the assay, the overall sensitivity and specificity were 100% (95% CI = 74.7% to 100%) and 100% (95% CI = 19.8% to 100%) for the detection of carbapenemase-producing *A. baumannii*, respectively (Table S4). During 2013 to 2016, the F-NRC for *A. baumannii* revealed that most of the carbapenem resistance was due to OXA-23 producers (84.4%), followed by producers of OXA-40 (5.4%), OXA-58 (2.2%), NDM (3.6%), and *ISAbal*-OXA-51 (4.4%) (http://www.cnr-resistance-antibiotiques.fr/ressources/pages/Rapport_CNR_2016.pdf). Thus, using the Amplidiag CarbaR+VRE assay, all the carbapenemase-producing *A. baumannii* isolates would have been identified.

One of the limitations of the Amplidiag CarbaR+VRE assay is that it gives only a “positive” or “negative” answer for *Acinetobacter* oxacillinases with carbapenemase activity (AcOXA) without any distinction between the variants OXA-23, OXA-40, OXA-58, and *ISAbal*-OXA-51. Moreover, if the *bla*_{OXA-51} gene is not located downstream of an *ISAbal* sequence, then it is not detected. However, infection control measures with imipenem-resistant *Acinetobacter* (carbapenem-resistant *A. baumannii* [CRAB]) would be the same whatever the resistance mechanism involved (26).

Prospective evaluation of enterobacterial isolates with reduced susceptibility to at least one carbapenem. From 20 January to 10 February 2016, 200 consecutive isolates with reduced susceptibility to at least one carbapenem (imipenem, meropenem, and/or ertapenem) were referred to the F-NRC for Antibiotic Resistance for evaluation. Reidentification of these 200 isolates revealed 198 *Enterobacteriaceae* and 2 *A. baumannii* isolates. They corresponded to 101 non-CP-GNB and 99 CP-GNB as revealed by the Carba NP test and subsequent in-house PCR analysis. The Amplidiag CarbaR+VRE assay performed on QIAcube-extracted whole DNA could detect all NDM

($n = 12$), VIM ($n = 2$), IMP ($n = 1$), OXA-48 ($n = 83$), and NDM plus OXA-48 ($n = 1$) isolates, including several variants of these enzymes (NDM-1, NDM-4, IMP-10, VIM-1, VIM-19, OXA-181, OXA-204, and OXA-244). In addition, both *bla*_{OXA-48} and *bla*_{NDM} genes were correctly detected by the Amplidiag CarbaR+VRE assay in one *Klebsiella pneumoniae* isolate that coexpressed the two enzymes. Among the non-carbapenemase producers, 8 gave positive PCR results for OXA-48, VIM, and/or NDM with threshold cycle (C_T) values ranging from 35 to 40. These isolates were considered contaminations during DNA extraction. Indeed, C_T s of true-positive isolates were below 20. Repeated QIAcube DNA extraction and PCRs directly done on colonies of these 8 isolates grown on MH medium gave a PCR-negative result. *K. pneumoniae* was the most prevalent species ($n = 73$), including 53.4% that were carbapenemase producers (29 OXA-48, 9 NDM, and 1 VIM-19). Among *Escherichia coli* isolates, 67.5% (27/40) produced a carbapenemase. The percentage of carbapenemase producers was lower in *Enterobacter* spp., with only 21.8% (12/55) of the isolates, which is in line with the French epidemiology (6). The other isolates were 7 *Klebsiella oxytoca* (5 producing OXA-48), 1 VIM-1-producing *Raoultella ornithinolytica*, 2 *Morganella morganii* (1 producing NDM-1), 4 *Serratia marcescens* (1 producing OXA-48 and 1 producing IMP-10), and 14 *Citrobacter* (12 producing OXA-48) isolates. Noticeably, the 2 *Hafnia alvei* and the 2 *A. baumannii* isolates were non-CP-GNB. The performances of the Amplidiag CarbaR+VRE assay in the prospective study of 200 isolates referred to the F-NRC were 100% (95% CI = 95.4% to 100%) for sensitivity, 100% (95% CI = 95.3% to 100%) for specificity, 100% (95% CI = 95.4% to 100%) for positive predictive value, and 100% (95% CI = 95.3% to 100%) for negative predictive value, respectively (Table 1).

When these results were extrapolated to the global French epidemiology (6) the Amplidiag CarbaR+VRE assay would have been able to identify 99.57% (3,306/3,320) of the CPE, missing only 13 IMI producers and one FRI-1 producer (27) (Table 1) and falsely detecting the unique OXA-405-producing *S. marcescens* as a carbapenemase producer (24).

Detection of OXA carbapenemases in *P. mirabilis*. The OXA-23 and OXA-58 carbapenemases, which are highly prevalent in carbapenem-resistant *Acinetobacter* spp., are still considered to be extremely rare in *Enterobacteriaceae*. Consequently, the molecular tests that are commercially available for the detection of CPE usually do not include *bla*_{OXA-23} and *bla*_{OXA-58} genes in their targeted genes (19, 20). However, OXA-23- and OXA-58-producing *P. mirabilis* isolates have been recently described not only in Europe (28–31) but also in other enterobacterial isolates from different countries and continents: Turkey (32), Africa (33), and India (34). It has been suggested (30) that their prevalence might be underestimated due to detection difficulties related to (i) the very weak hydrolysis activity of these carbapenemases and (ii) the intrinsic decreased susceptibility to imipenem (but not ertapenem) of *P. mirabilis*. To assess whether the Amplidiag CarbaR+VRE assay might detect these strains, we have tested 10 OXA-producing (8 OXA-23 and 2 OXA-58) *P. mirabilis* isolates and two negative controls (Table S1). All OXA producers were correctly identified. The Amplidiag CarbaR+VRE assay is the first assay able to detect the five most common carbapenemases and the OXA carbapenemases preferentially found in *A. baumannii*. The use of this assay would allow identification of these “*Acinetobacter*-related” enzymes not only in *P. mirabilis* but also in other enterobacterial isolates (32–34), allowing evaluation of the current spread of these resistance determinants and assessment of the risk of further dissemination of these enzymes in *Enterobacteriaceae*.

Performance of the Amplidiag CarbaR+VRE assay directly on bacterial colonies. The Amplidiag CarbaR+VRE assay allowed detection of the presence of a carbapenemase within a 3-hour period, using the recommended protocol of the manufacturer (Fig. 1). To see whether this time could be reduced, the Amplidiag CarbaR+VRE PCR was directly performed on colonies grown on Muller-Hinton (MH) medium (representing bacteria from a suspicious antibiogram) and on ChromID Carba Smart medium (a medium that is now classically used to screen for CPE).

TABLE 2 Results of the Amplidiag CarbaR+VRE assay on bacterial colonies recovered from ChromID Carba Smart medium directly from colonies versus DNA extraction (QIAamp DNA extraction or boiling extract)

		Amplidiag Carba-R+VRE result ^a		
Species and strain	β -Lactamase content ^c	QIAamp DNA extraction	Direct from colonies ^b	Boiling extract
<i>Enterobacteriaceae</i>				
<i>Klebsiella ozaenae</i> N15	KPC-3 + OXA-9 + TEM-1	KPC	KPC + OXA-48 + AcOXA	KPC
<i>E. coli</i> 2 A1	OXA-48 + CTX-M-15	OXA-48	OXA-48 + AcOXA	OXA-48
<i>K. pneumoniae</i> N38	OXA-204 + CMY-4	OXA-48	NDM + OXA-48 + AcOXA	OXA-48
<i>P. mirabilis</i>				
GUI	OXA-23	AcOXA	IMP + AcOXA	AcOXA
VAC	OXA-23	AcOXA	KPC + IMP + AcOXA	AcOXA
MOR	OXA-23	AcOXA	KPC + IMP + AcOXA	AcOXA
VAG	None		KPC + IMP + OXA-48	
<i>P. aeruginosa</i>				
A1	KPC-2	KPC	KPC	KPC
C4	VIM-4	VIM	VIM	VIM
D1	IMP-1	IMP	IMP	IMP
E7	GIM-1			
N100	OXA-198			

^aAcOXA, *Acinetobacter* oxacillinases with carbapenemase activity (OXA-23-like, OXA-24/-40-like, OXA-58-like, and ISAba plus OXA-51-like).^bDiscrepancies compared to the manufacturer-recommended protocol (i.e., QIAamp DNA extraction) are highlighted in gray.^cCarbapenemases are highlighted in bold.

For bacterial colonies grown on MH medium and directly suspended in the Amplidiag CarbaR+VRE PCR mix, correct results were obtained for all tested strains (Table S5) compared to the manufacturer-recommended protocol (i.e., DNA extraction using the QIAamp DNA extraction kit), with C_T values below 20. Thus, in addition to the avoidance of DNA extraction steps, this protocol optimization also allowed the reduction of the total number of cycles to 30 (instead of 45 in the original protocol), leading to a time-to-result of less than 1 h 10 min instead of 3 h (Fig. 1).

When applied on bacterial colonies grown on ChromID Carba Smart medium, the Amplidiag CarbaR+VRE yielded variable results (Table 2). Although *P. aeruginosa* isolates gave correct results, several false-positive results were obtained with *Enterobacteriaceae* (Table 2). Assuming that the pigmentation of enterobacterial isolates on ChromID Carba Smart medium might interfere with direct PCR, a fast (11-min) and cheap whole-DNA boiling extraction was performed. By directly applying 5 μ l of this boiling extracted DNA to the Amplidiag CarbaR+VRE PCR mix, correct results were then obtained for all tested *Enterobacteriaceae* (Table 2). Thus, the time-to-result was slightly increased to 1 h 11 min, which is still compatible with a short time-to-result. In addition, this low-cost DNA extraction technique leads to a drastic decrease in the global cost of the test. However, the presence of vancomycin (Van) primers in the present PCR mix is not appropriate for a study on colonies, because carbapenemase genes will be searched for in Gram-negative bacteria and vancomycin resistance genes will be searched for in Gram-positive bacteria. This PCR mix has been developed for an application to complex clinical samples such as rectal swabs or blood cultures.

Conclusion. Our study demonstrated that the Amplidiag CarbaR+VRE assay is well adapted to the French epidemiology of CP-GNB, which reflects the epidemiology of many European countries. Overall, and when excluding the nontargeted less-prevalent carbapenemases in CP-GNB (10/100), the Amplidiag CarbaR+VRE performances reached 100% (95% CI = 91.1% to 99.9%) and 99% (95% CI = 74.1% to 99.8%) sensitivity and specificity, respectively. The main advantage of this test is that it contains a large panel of targeted resistance determinants. Indeed, it detects the main carbapenemases encountered in *Enterobacteriaceae* (KPC, NDM, VIM, IMP, and OXA-48-like), but it also identifies the most widespread OXA carbapenemases usually identified in *A. baumannii* (OXA-23-like, OXA-24/-40-like, and OXA-58-like). This last panel might be increasingly important in the future with the recent reports of OXA-23- and OXA-58-producing *P. mirabilis* and *E. coli* (28–33).

As for most molecular assays, mutation and/or polymorphisms in the primer/probe binding region of the targeted gene may lead to false-negative results, and thus, new emerging variants may not be detected. Regular testing of novel variants is required to assess sensitivity and specificity of this assay in a constantly evolving carbapenemase field. The main drawback of this assay compared to other molecular techniques (23) is that it requires pure cultures and DNA extraction steps before analysis. The DNA extraction step significantly increases the global cost of the test since ready-to-use kits are recommended by the manufacturer. However, we demonstrated that a rapid and very cheap DNA extraction by boiling might be accurate enough to recover DNA extract and obtain adequate results. In addition, we also showed that the Amplidiag CarbaR+VRE assay might be directly performed on bacterial colonies grown on MH agar, avoiding any additional DNA extraction step. Of note, our results suggested that at least the boiling DNA extraction step is crucial for bacterial colonies recovered from chromogenic media. Other limitations of the current test are that it is not validated directly on stool samples and that it lacks random access. Thus, the use of the Amplidiag CarbaR+VRE assay for the investigation of an outbreak will require at least 24 or 48 h for the culture, and the global cost of this assay will be approximately 15 euros, but the assay might efficiently confirm the presence of carbapenemase production and identify its determinant if targeted.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/JCM.01092-17>.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

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