



NitroSpeed-Carba NP Test for Rapid Detection and Differentiation between Different Classes of Carbapenemases in *Enterobacterales*

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ABSTRACT A biochemical test (NitroSpeed-Carba NP test) was developed to identify carbapenemase production in *Enterobacterales* and to discriminate between the different types of clinically significant carbapenemases (Ambler classes A, B, and D). It is based on two main features, namely, the hydrolysis by all β -lactamases, including carbapenemases of the nitrocefin substrate, and the capacity of ertapenem to prevent this hydrolysis for all β -lactamases except carbapenemases. Specific carbapenemase inhibitors of class A (avibactam, vaborbactam), class B (dipicolinic acid), and class D (avibactam) were used to inhibit the nitrocefin hydrolysis and to allow the identification of the carbapenemase types with a turnaround time of ca. 30 min. The test was evaluated with a collection of 248 clinical enterobacterial isolates, including 148 carbapenemase producers and 100 non-carbapenemase producers. Its overall sensitivity and specificity were 100% and 97%, respectively, including detection of all types of OXA-48-like carbapenemases. For the detection of the carbapenemase type, including strains that produce double carbapenemases, the sensitivity was 100%, 97%, and 100% for the detection of classes A, B, and D, respectively. This easy-to-implement test may contribute to optimization of the choice of the β -lactam/ β -lactamase inhibitor combinations for treating infection due to carbapenemase producers.

KEYWORDS carbapenemase, *Enterobacterales*, diagnostics, rapid, Carba NP, UltraSpeed

The growing incidence of multidrug resistance in *Enterobacterales* constitutes a major global health threat (1–3). Carbapenem resistance has been increasingly reported worldwide due to the increased use of carbapenems, which are supposed to be last-line agents retaining activity against multidrug-resistant *Enterobacterales* including those producing extended-spectrum β -lactamase (ESBL) and AmpC β -lactamases (4–6). Resistance to carbapenem among *Enterobacterales* is mediated by various mechanisms, including production of carbapenem-hydrolyzing enzymes (so-called carbapenemases), alteration in outer membrane permeability, and in certain circumstances, overproduction of an AmpC- or ESBL-type enzyme combined with porin loss/modification (7–10). The main clinically important carbapenemases identified in clinical isolates are grouped into three different classes according to their amino acid identity, corresponding to molecular class A (e.g., *Klebsiella pneumoniae* carbapenemase [KPC] enzymes), molecular class B (e.g., New Delhi metallo- β -lactamase [NDM], Verona integron-encoded metallo- β -lactamase [VIM]), and imipenemase [IMP] enzymes), and class D (e.g., OXA-48 and its derivatives) (9, 11).

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Early and accurate detection of carbapenemase-producing organisms (CPOs) in infected or colonized patients is one of the most efficient ways to limit the dissemination of carbapenemase-producing isolates and also to optimally adapt the empirical treatment when needed. Most of the phenotype-based techniques not only suffer from some specificity and sensitivity issues but are also time-consuming (at least 12 to 24 h) and lack guidance regarding the specific carbapenemase being produced; therefore, they are poorly adapted to the clinical need for isolating patients rapidly to prevent nosocomial outbreaks (12, 13). Molecular tests may be more rapid; however, they are often expensive, not easily implementable worldwide, and only detect the most common carbapenemase-encoding genes, missing the so-called “minor” or even unknown ones. The development of rapid carbapenemase detection tests such as the Carba NP test has represented a paradigm shift, since such a technique is not only rapid, specific, and sensitive but also implementable worldwide due to its low cost and ease of implementation. The Carba NP test is based on the biochemical detection of carbapenem (imipenem) hydrolysis (12, 14), meaning it may detect any kind of carbapenemase activity, regardless of its nature. Carbapenemase activity is evidenced through a color change (red to orange or yellow) of the buffer in which bacteria are tested, resulting from its acidification upon imipenem hydrolysis. However, this test suffers from the following two shortcomings: (i) its sensitivity is not optimal for some enzymes exhibiting weak carbapenemase activities, and (ii) it does not distinguish between different classes of carbapenemases.

Distinguishing between different classes of carbapenemases makes sense today, since novel β -lactamase inhibitors have been recently developed, counteracting, for instance, the activity of the class A carbapenemase KPC and/or class D carbapenemase OXA-48, although class B enzymes (metallo- β -lactamases [MBLs]) remain resistant to it. This is especially true for avibactam, which is a bridged diazabicyclo[3.2.1]octanone non- β -lactam inhibitor, inhibiting efficiently the aforementioned enzymes (15). This inhibitor is coupled with ceftazidime and soon will be with aztreonam, making excellent combinations that are active against KPC and OXA-48 producers but also MBL producers, respectively (16). Furthermore, the recently developed vaborbactam adds very significantly to the current armamentarium of β -lactamase inhibitors (17). Indeed, this first boronic acid inhibitor to be developed very efficiently inhibits classes A and D carbapenem-hydrolyzing β -lactamases but has no activity against OXA-48 and derivatives, while dipicolinic acid that is used only *in vitro* inhibits the activity of class B carbapenemases. Here, we developed a very rapid, simple, sensitive, and specific test for identification of carbapenemase-producing *Enterobacteriales*, combining a capacity to assess which group the carbapenemase belongs to. The principle of this so-called NitroSpeed-Carba NP test relies, on one hand, on the hydrolysis of the nitrocefin substrate by all β -lactamases and, on the other hand, on the capacity of ertapenem (a carbapenem) to prevent this hydrolysis for all β -lactamases lacking carbapenemase activity. Indeed, enzymes lacking carbapenemase activity bind covalently and irreversibly with ertapenem and are no longer able to subsequently hydrolyze nitrocefin. By contrast, carbapenemases significantly hydrolyze ertapenem and are then still able to hydrolyze nitrocefin. This test was further developed by evaluating the capacity of different β -lactamase inhibitors (dipicolinic acid, avibactam, and vaborbactam) to inhibit the hydrolysis process of those carbapenemases.

MATERIALS AND METHODS

A genetically characterized collection of 248 various enterobacterial species from the Medical and Molecular Microbiology unit at the University of Fribourg, Switzerland, were included in this study. Isolates were obtained from various clinical sources (e.g., blood cultures, urine, sputum) and from various countries. The isolates included 144 carbapenemase producers and 100 non-carbapenemase producers. This collection included a large variety of enterobacterial species, including *Escherichia coli*, *K. pneumoniae*, *Klebsiella oxytoca*, *Enterobacter cloacae*, *Enterobacter asburiae*, *Klebsiella aerogenes*, *Enterobacter hormaechei*, *Citrobacter freundii*, *Citrobacter braakii*, *Citrobacter murlinae*, *Citrobacter werkmanii*, *Providencia stuartii*, *Providencia rettgeri*, *Proteus mirabilis*, *Hafnia alvei*, *Morganella morganii*, and *Serratia marcescens*. The carbapenemase types were as follows: class A carbapenemases ($n = 23$; KPC, NmCA, SME-1, GES, FRI, IMI), class B carbapenemases ($n = 66$; VIM, NDM, IMP, GIM), class D carbapenemases

($n = 49$; OXA-48 and its derivatives). Notably, five isolates coproducing the NDM and OXA-48-like carbapenemases and a single isolate coproducing VIM and OXA-48-like enzymes were included (Table 1). In addition, four isolates producing enzymes that are not detected by using the Carba NP test (namely, CTX-M-33, SHV-38, and overexpressed OXY β -lactamase from *K. oxytoca*) were also tested. Previous studies reported that those four enzymes are exhibiting a very low level of carbapenemase activity; therefore, we wanted to check if our test is able to detect such a low level of carbapenemase activity (18–20). Quality control (QC) strains have been included with well-characterized carbapenemases. They included two *E. coli* strains producing NDM-5 and OXA-48-like carbapenemases (strains N679 and N1084, respectively) and two *K. pneumoniae* strains producing KPC-2 and NDM-1 (strains R307 and N1086, respectively). The β -lactamase gene content of those four isolates was determined by whole-genome sequencing. One hundred non-carbapenemase producers were tested that included ESBL and non-ESBL ($n = 50$) producers of plasmid-mediated AmpC enzymes, and isolates combining production of their chromosomally encoded AmpC together with a decreased membrane permeability ($n = 49$) were also included as well as a single β -lactamase-negative *E. coli* reference strain ATCC 25922 (Table 1). The antimicrobial resistance profiles of the strains were determined by disk diffusion according to EUCAST (http://www.eucast.org/clinical_breakpoints/) guidelines. The MICs of ertapenem, meropenem, and imipenem was determined using Etest strips (bioMérieux, La Balme-les-Grottes, France) on Mueller-Hinton agar plates at 37°C. Etest (including QC) was performed according to the manufacturer's instructions, and results were interpreted according to the latest EUCAST breakpoints (Table 1). All strains had previously been characterized for their β -lactamase content and carbapenemase-encoding genes by PCR approaches followed by subsequent DNA sequencing.

The NitroSpeed-Carba NP test. The test was prepared and performed as follows (Fig. 1). For each bacterial isolate, 1 to 2 calibrated 1- μ l loopfuls of tested colonies, grown overnight at 37°C on UriSelect 4 (Bio-Rad, Marnes-la-Coquette, France; reference no. 64694), Mueller-Hinton agar (Bio-Rad; reference no. 64884), or Luria-Bertani agar (Carl Roth GmbH and Co., Karlsruhe; article no. X969.2) plates or primary specimen plating (e.g., blood agar plates) were selected. To extract the enzymes, the sample was resuspended in one of the following: (i) 100 μ l of a Tris-HCl 20 mmol/liter lysis buffer (B-PER II bacterial protein extraction reagent; Thermo Scientific Pierce, Rockford, IL, USA) containing 0.1 mM ZnSO₄ (Carl Roth GmbH; reference no. K301.1), pH 7.5, in tubes 1 and 2; (ii) 100 μ l of a Tris-HCl 20 mmol/liter lysis buffer containing 0.1 mM ZnSO₄ and 25 mM dipicolinic acid (Sigma Aldrich; reference no. P63808), pH 7.5, in tube 3; (iii) 100 μ l of a Tris-HCl 20 mmol/liter lysis buffer containing 0.1 mM ZnSO₄ and avibactam sodium hydrate (0.04 mM [12.2 μ g/ml]; MedChemExpress; reference no. HY-14879B) in tube 4; or (iv) 100 μ l of a Tris-HCl 20 mmol/liter lysis buffer containing 0.1 mM ZnSO₄ and vaborbactam (50 μ g/ml; MedChemExpress; reference no. HY-19930) in tube 5.

Tubes were then vortexed vigorously for 30 to 60 s until complete resuspension. Then, 50 μ l of distilled water was added to tube 1 (control) and 50 μ l of ertapenem sodium (ETP) (80 μ g/ml; Sigma-Aldrich; reference no. SML1238) to tubes 2 to 5. Following incubation at room temperature for 5 min, 50 μ l of 1 g/liter nitrocefim (Toronto Research Chemicals, Canada; reference no. N493815) was added into each tube 1 to 5. The final concentrations of ETP were 20 μ g/ml.

The test was considered as interpretable if one of the following 5 conditions was met (Fig. 1). (i) A color change from yellow to red (hydrolysis of nitrocefim) in tubes 1, 2, and 3 and the absence of any color change (no hydrolysis of nitrocefim) in tube 4 containing ertapenem plus avibactam and tube 5 containing ertapenem plus vaborbactam indicate class A carbapenemase production (e.g., KPC). (ii) A color change from yellow to red (hydrolysis of nitrocefim) in tubes 1, 2, 4, and 5 and the absence of any color change (no hydrolysis of nitrocefim) in tube 3 containing ertapenem and dipicolinic acid indicate production of a class B carbapenemase, such as NDM, VIM, IMP, or GIM. (iii) A color change from yellow to red (hydrolysis of nitrocefim) in tubes 1, 2, 3, and 5 and the absence of any color change (no hydrolysis of nitrocefim) in tube 4 containing ertapenem and avibactam indicate production of a class D carbapenemase, such as OXA-48 and its derivatives. (iv) A color change from yellow to red in tube 1 only containing water and the absence of any color change (no hydrolysis of nitrocefim) in the other tubes 2 to 5 indicate lack of carbapenemase activity but production of a noncarbapenemase β -lactamase, such as TEM-1 or CTX-M-type enzymes. (v) The absence of any color change (no hydrolysis of nitrocefim) in all tubes (1 to 5) indicates lack of β -lactamase activity.

All results are interpreted within 15 min (10 s to 15 min) of incubation at room temperature. The different readers of the tests were blinded from the type of isolates tested.

RESULTS AND DISCUSSION

A rapid, sensitive, and specific test has been developed here, not only for ultrarapid and simple detection of carbapenemase-producing *Enterobacterales* but also to identify the different classes of carbapenemases. This test is a derivative of the Nitro-Carba test (21) but presents here the significant advantage of identifying the carbapenemases according to the classes they belong to. The NitroSpeed-Carba NP test allows rapid identification of all types of carbapenemase producers, including isolates producing KPC, OXA-48-types, NDM, VIM, and IMP, with a turnaround time of ca. 30 min (only 10 to 15 min for the final reaction after the addition of nitrocefim). The formerly developed Carba NP test allowed rapid identification of carbapenemase production by detecting acid products resulting from the hydrolysis of imipenem in the presence of a phenol

TABLE 1 Features of clinical enterobacterial isolates, MICs of carbapenems, and results of the NitroSpeed-Carba NP test^a

β-lactamase type (no.)	Species	β-Lactamase(s) (no.)	MIC range (mg/liter)			NitroSpeed-Carba NP result							
			ETP	IPM	MEM	Water	ETP	ETP and DPA	ETP and AVI	ETP and VAB			
Carbapenemase producers (n = 144)													
Class A (n = 23)													
	<i>K. pneumoniae</i>	KPC-2 (5)	6 to >32	8 to >32	4 to >32	+	+	+	-	-			
		KPC-3 (10)	2 to >32	4 to >32	1 to >32	+	+	+	-	-			
		KPC-11 (1)	3	4	2	+	+	+	-	-			
	<i>Escherichia coli</i>	KPC-3 (1)	16	6	4	+	+	+	-	-			
		<i>E. cloacae</i>	KPC-2 (1)	1.5	6	0.75	+	+	+	-	-		
			NmcA (1)	8	>32	8	+	+	+	-	-		
			GES-5 (1)	16	>32	16	+	+	+	-	-		
			FRI-2 (1)	4	4	2	+	+	+	-	-		
			<i>E. asburiae</i>	IMI-2 (1)	16	>32	>32	+	+	+	-	-	
			<i>S. marcescens</i>	SME-1 (1)	4	32	12	+	+	+	-	-	
Class B (n = 66)													
NDM													
	<i>K. pneumoniae</i>	NDM-1 (9)	4 to >32	3 to >32	2 to >32	+	+	-	+	+			
		NDM-2 (1)	>32	>32	>32	+	+	-	+	+			
		NDM-5 (1)	16	>32	>32	+	+	-	+	+			
		NDM-7 (1)	>32	>32	>32	+	+	-	+	+			
	<i>E. coli</i>	NDM-1 (2)	3 to 16	16 to >32	3 to 16	+	+	-	+	+			
		NDM-5 (13)	>32	16 to >32	>32	+	+	-	+	+			
		NDM-7 (1)	>32	>32	>32	+	+	-	+	+			
	<i>Morganella morganii</i>	NDM-1 (1)	>32	>32	16	+	+	-	+	+			
		<i>Proteus mirabilis</i>	NDM-1 (1)	>32	24	>32	+	+	-	+	+		
			<i>E. cloacae</i>	NDM-1 (1)	16	8	4	+	+	-	+	+	
			<i>E. aerogenes</i>	NDM-5 (2)	>32	>32	>32	+	+	-	+	+	
			<i>Providencia stuartii</i>	NDM-1 (1)	>32	>32	>32	+	+	-	+	+	
			<i>Providencia rettgeri</i>	NDM-5 (1)	1	>32	>32	+	+	-	+	+	
			VIM	<i>K. pneumoniae</i>	VIM-1 (4)	0.75 to >32	0.5 to >32	0.5 to >32	+	+	-	+	+
					VIM-4 (1)	16	8	16	+	+	-	+	+
	<i>E. coli</i>	VIM-19 (2)	2 to 16	1.5 to 8	1 to 4	+	+	-	+	+			
		VIM-1 (1)	0.25	1	0.5	+	+	-	+	+			
	<i>E. aerogenes</i>	VIM-19 (2)	1 to >32	24 to 32	2 to >32	+	+	-	+	+			
		VIM-1 (1)	16	16	16	+	+	-	+	+			
	<i>E. cloacae</i>	VIM-1 (1)	16	>32	16	+	+	-	+	+			
		VIM-4 (1)	8	24	8	+	+	-	+	+			
	<i>E. hormaechei</i>	VIM-1 + CTX-Mgp1 (1)	16	>32	>32	+	+	±	+	+			
		<i>Proteus mirabilis</i>	VIM-1 (2)	6 to 8	>32	2 to 16	+	+	-	+	+		
			VIM-4 (1)	8	16	8	+	+	-	+	+		
			<i>C. freundii</i>	VIM-2 (1)	6	3	1	+	+	-	+	+	
VIM-2 + OXA-10 + TEM-1 (1)			8	16	16	+	+	±	+	+			
IMP													
	<i>K. pneumoniae</i>	IMP-1 (5)	4 to >32	3 to >32	3 to >32	+	+	-	+	+			
		IMP-4 (1)	>32	>32	>32	+	+	-	+	+			
		IMP-8 (1)	0.75	0.5	0.38	+	+	-	+	+			
	<i>E. coli</i>	IMP-1 (1)	1	1	0.75	+	+	-	+	+			
		IMP-8 (1)	16	24	4	+	+	-	+	+			
	<i>S. marcescens</i>	IMP-1 (1)	>32	>32	>32	+	+	-	+	+			
		IMP-11 (1)	1.5	1.5	1	+	+	-	+	+			
		GIM	<i>E. cloacae</i>	GIM-1 (1)	>32	4	12	+	+	-	+	+	
Class D (n = 49)													
	<i>K. pneumoniae</i>	OXA-48 (10)	0.38 to >32	0.25 to >32	0.38 to >32	+	+	+	-	+			
		OXA-162 (1)	0.25	0.38	0.75	+	+	+	-	+			
		OXA-181 (2)	>32	1.5	0.38	+	+	+	-	+			
		OXA-232 (1)	>32	24	>32	+	+	+	-	+			
		OXA-204 (1)	0.38	0.25	0.125	+	+	+	-	+			
	<i>E. cloacae</i>	OXA-48 (2)	0.5 to 16	0.5 to 1	1.5	+	+	+	-	+			
		OXA-181 (1)	0.5	0.38	0.25	+	+	+	-	+			
		<i>C. freundii</i>	OXA-162 (1)	0.38	3	0.5	+	+	+	-	+		
	<i>E. coli</i>	OXA-48 (12)	0.38 to >32	0.5 to 3	0.125 to 1	+	+	+	-	+			
		OXA-204 (1)	1.5	0.75	0.38	+	+	+	-	+			
		OXA-244 (17)	0.125 to >32	0.19 to 1	0.047 to 1	+	+	+	-	+			
		Double carbapenemases (n = 6)	<i>K. pneumoniae</i>	NDM-1 + OXA-232 (1)	>32	>32	>32	+	+	+	+	+	
	NDM-1 + OXA-181 (3)	>32		>32	>32	+	+	+	+	+			
	NDM-1 + OXA-48 (1)	>32		>32	>32	+	+	+	+	+			
	<i>E. coli</i>	OXA-48 + VIM-1 (1)	>32	>32	>32	+	+	+	+	+			
Non-carbapenemase producers (n = 100)													

(Continued on next page)

TABLE 1 (Continued)

β-lactamase type (no.)	Species	β-Lactamase(s) (no.)	MIC range (mg/liter)			NitroSpeed-Carba NP result				
			ETP	IPM	MEM	Water	ETP	ETP and DPA	ETP and AVI	ETP and VAB
ESBL producers (n = 50)	<i>E. coli</i>	OXA-1 (5)	0.003 to 0.125	0.19 to 0.25	0.016 to 0.023	+	-	-	-	-
		TEM (3)	0.032	0.125	0.047	+	-	-	-	-
		CTX-M-15 (8)	1.5 to >32	0.38 to 1	0.75 to 0.19	+	-	-	-	-
	<i>E. cloacae</i>	CTX-M-3 (2)	0.125	0.25	0.125	+	-	-	-	-
		TEM-3 (2)	0.125	0.125	0.125	+	-	-	-	-
		CTX-M-3 + TEM-1 (1)	0.38	0.125	0.25	+	-	-	-	-
		CTX-M-3 (1)	0.38	0.12	0.25	+	-	-	-	-
		OXA-163 (1)	2	0.5	0.5	+	-	-	-	-
	<i>E. aerogenes</i>	CTX-M-3 (2)	0.125	0.125	0.125	+	-	-	-	-
	<i>K. pneumoniae</i>	CTX-M-3 (2)	0.125	0.38	0.125	+	-	-	-	-
		CTX-M-15 (9)	0.064 to 16	0.25 to 8	0.75 to 32	+	-	-	-	-
		SHV-5 (2)	0.125	0.125	0.125	+	-	-	-	-
		SHV-11 (1)	0.047	0.19	0.023	+	-	-	-	-
		CTX-M-1 (3)	0.125 to 0.25	0.125 to 0.25	0.12 to 0.25	+	-	-	-	-
	<i>K. oxytoca</i>	CTX-M-3 + TEM-1 (2)	0.38	0.125	0.38	+	-	-	-	-
		CTX-M-3 + TEM-1 (1)	0.125	0.125	0.38	+	-	-	-	-
	<i>C. freundii</i>	CTX-M-1 (1)	0.047	0.25	0.016	+	-	-	-	-
		CTX-M-3 (3)	0.047	0.38 to 2	0.032 to 0.047	+	-	-	-	-
		CTX-M-15 + TEM-1 (1)	0.38	0.25	0.19	+	-	-	-	-
	Non-ESBL producers (n = 49)	<i>K. pneumoniae</i>	DHA (10)	0.75 to >32	0.5 to >32	0.19 to >32	+	-	-	-
<i>E. coli</i>			CMY + CTX-M-15 (1)	0.125	0.125	0.25	+	-	-	-
<i>Proteus mirabilis</i>		CMY-2 like (1)	0.094	0.38	0.064	+	-	-	-	-
		CMY + LAT (1)	0.5	0.5	0.125	+	-	-	-	-
		CMY (1)	0.38	0.125	0.125	+	+	-	-	-
		ACC-1 (1)	0.125	0.38	0.125	+	+	-	-	-
		ACC-2 (4)	0.032 to 0.38	0.38 to 0.5	0.047 to 0.094	+	-	-	-	-
<i>Hafnia alvei</i>		Overexpressed AmpC cephalosporinase (1)	12	0.25	1	+	-	-	-	-
		<i>C. freundii</i>	WT (3)	0.012 to 0.064	0.38	0.032 to 0.047	+	-	-	-
<i>C. braakii</i>		AmpC cephalosporinase (2)	0.094 to 12	0.38 to 2	0.38 to 6	+	-	-	-	-
		ACC-2 (1)	0.008	0.19	0.032	+	-	-	-	-
<i>C. murliniae</i>		WT (1)	0.016	0.38	0.064	+	-	-	-	-
		AmpC cephalosporinase (2)	0.006	0.25	0.016	+	-	-	-	-
<i>C. werkmanii</i>		AmpC cephalosporinase (1)	0.006	0.25	0.016	+	-	-	-	-
		AmpC cephalosporinase (1)	0.008	0.05	0.023	+	-	-	-	-
<i>Morganella morganii</i>		WT (1)	0.023	1.5	0.125	+	-	-	-	-
		Overexpressed AmpC (1)	0.64	32	0.25	+	-	-	-	-
<i>E. aerogenes</i>		Natural cephalosporinase (1)	0.23	1.5	0.125	+	-	-	-	-
		Overexpressed AmpC cephalosporinase (1)	32	16	4	+	-	-	-	-
<i>E. cloacae</i>		WT (1)	0.012	0.064	0.12	+	-	-	-	-
	Overexpressed AmpC cephalosporinase (8)	0.38 to >32	0.38 to 2	0.094 to 4	+	-	-	-	-	
	Natural cephalosporinase (2)	0.125	0.38	0.12	+	-	-	-	-	
Negative control (n = 1)	<i>E. coli</i>	CMY (2)	1 to >32	0.38 to >32	0.125 to >32	+	-	-	-	-
		ATCC 25922 (1)	0.003	0.125	0.008	-	-	-	-	-

^aWT, wild type; ETP, ertapenem; IPM, Imipenem; MEM, meropenem; DPA, dipicolinic acid; AVI, avibactam; VAB, vaborbactam; +, red; -, yellow; ±, reddish yellow.

red as a pH indicator (12). Although the Carba NP test requires an additional 30-min extraction step, the NitroSpeed-Carba NP is performed by the direct use of a bacterial cell suspension in the lysis solution without an incubation period. The detection of some OXA-type carbapenemases by using the Carba NP test may be challenging due to (i) their weak carbapenemase activity, (ii) the low level of expression of the corresponding genes, and (iii) in some cases, a limited copy number of the corresponding gene (e.g., a single copy of the carbapenemase gene may be inserted into the chromosome). Therefore, PCR-based methods often remain useful for the identification of those carbapenem-hydrolyzing class D β-lactamases (22). However, these methods usually require an additional step of bacterial culture (additional 24 h) and then 3 to 8 h to be performed and require specific, expensive equipment and a significant degree of expertise, apart from the high costs associated.

For the detection of carbapenemase production, the sensitivity and specificity of the NitroSpeed-Carba NP test were 100% (144/144) and 97% (97/100), respectively. Ertap-

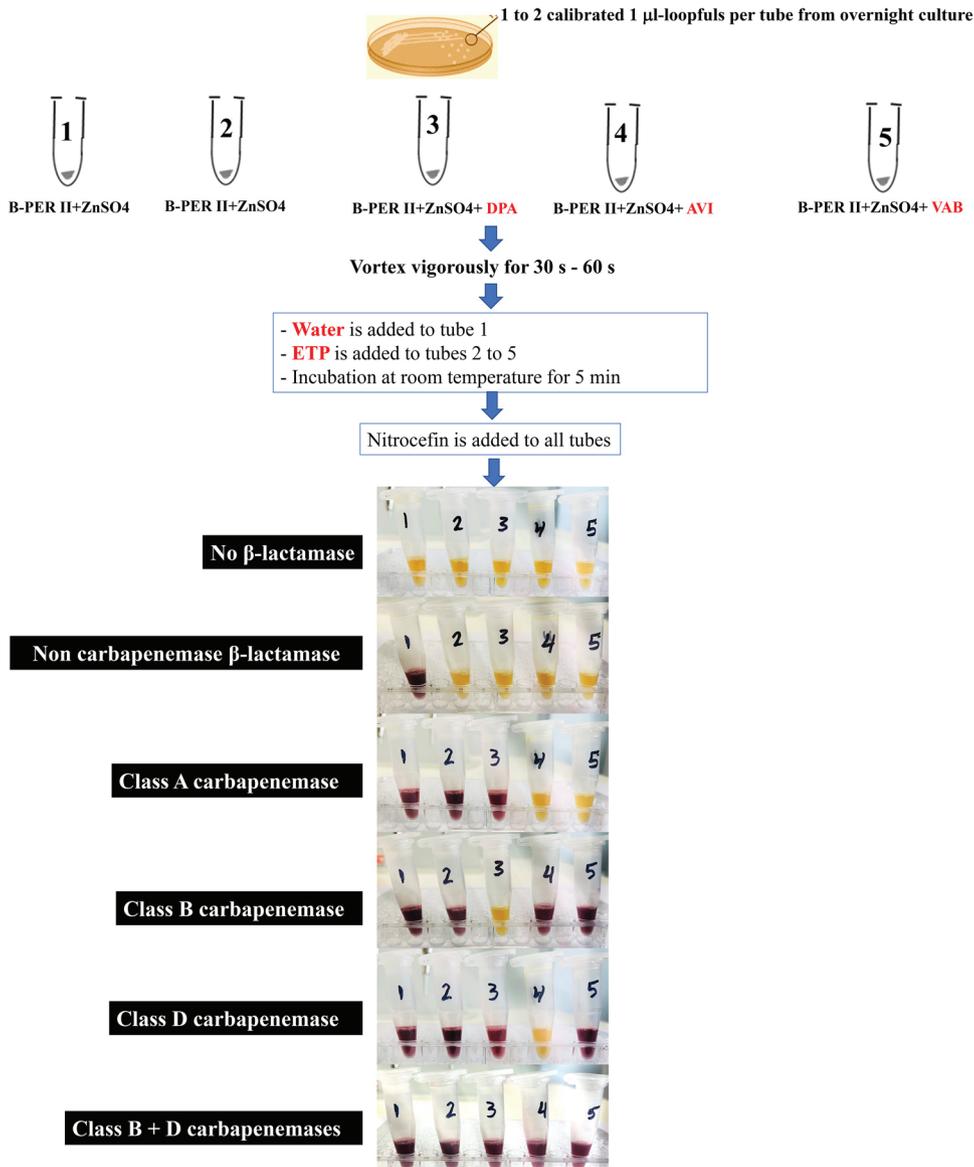


FIG 1 Detailed protocol of the NitroSpeed-Carba NP test.

enem was found to prevent nitrocefin hydrolysis by ESBLs, AmpC β-lactamases, and coproduced ESBL and AmpC β-lactamases (negativity of the NitroSpeed-Carba NP test), therefore, permitting the differentiation between carbapenem-resistant *Enterobacteriales* producing or not producing carbapenemases. The only three strains in our collection that gave false-positive results were producers of ACC- or CMY-type AmpC enzymes that are known to have very slight carbapenemase activity. The NitroSpeed-Carba NP test showed excellent results for detecting any OXA-48-type producer, including the difficult-to-detect OXA-244 producers (23, 24). The NitroSpeed-Carba NP test allowed categorization of the different carbapenemase classes. The sensitivity of the NitroSpeed-Carba NP test was 100% (23/23), 97% (64/66), and 100% (49/49) for the detection of class A, B, and D carbapenemases, respectively. Those values were obtained even by including strains that produced two types of unrelated carbapenemases, for which the precise identification of the carbapenemase is not possible. Noteworthy, the test could detect producers of two carbapenemases, such as those producing NDM and OXA-48-type or VIM and OXA-48-type enzymes (sensitivity, 100% [6/6]). In addition, the NitroSpeed-Carba NP test was positive for strains producing

enzymes with very-low-level carbapenemase activity (sensitivity, 100% [4/4]), such as CTX-M-33 (18), SHV-38 (19), and overexpressed β -lactamase OXY in *K. oxytoca*, with corresponding carbapenemase activities not detected using the Carba NP test (20). Detection of all carbapenemase producers was possible using the NitroSpeed-Carba NP test, regardless of the respective levels of carbapenemase activity. It was observed that the positivity of the NitroCarba NP test was immediate (less than 1 min) when testing enzymes with high carbapenemase activity (such as KPC-2), whereas it was a bit delayed (for instance, 15-min incubation) for enzymes showing low-level carbapenemase activity (such as CTX-M-33). Moreover, the NitroSpeed-Carba NP test gave a negative result when testing an OXA-163 producer, that latter OXA-48-like β -lactamase possessing a unique hydrolytic profile mirroring that of class A ESBLs but almost lacking any carbapenemase activity (25).

The NitroSpeed-Carba NP test will be further compared to the β -Carba test, which is known to be less sensitive than the Carba NP test for detecting carbapenemase activity (14). The rapid immunochromatography-based detection of carbapenemase producers is another technique that can detect carbapenemases (KPC, IMP, VIM, OXA-48 like enzymes) (26). However, that latter technique cannot detect several carbapenemases, such as GES, NMC-A, SME, FRI, or IMI enzymes, whereas the NitroSpeed-Carba NP can detect any type of carbapenemase including some potentially novel ones.

In conclusion, the NitroSpeed-Carba NP test is an easy-to-perform and reliable test for rapid identification of carbapenemase-producing *Enterobacteriales*, with excellent sensitivity and specificity. Use of this test that additionally presents the advantage to rapidly identify the carbapenemase type does not require any specific equipment. Given its user-friendliness, simplicity, and short turnaround time to result, the NitroSpeed-Carba NP test would be suitable for any microbiology laboratory, in particular if a commercial test is developed.

Finally, use of this test may contribute to the choice of β -lactamase/ β -lactamase inhibitor combinations for treating infections due to carbapenemase producers. Indeed, ceftazidime-avibactam may be proposed for treating infections due to KPC or OXA-48 producers but not due to metallo-carbapenemase producers, whereas the spectrum of activity of meropenem-vaborbactam includes KPC producers only. Finally, since resistance to ceftazidime-avibactam and meropenem-vaborbactam may occur among *Enterobacteriales* (16), the NitroSpeed-Carba NP test may provide the opportunity to easily monitor the occurrence of those multidrug-resistant isolates.

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