Influence of Culture Media on Detection of Carbapenem Hydrolysis by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry

Ana Carolina Ramos,^a Cecília Godoy Carvalhaes,^{a,b} Jhonatha Rodrigo Cordeiro-Moura,^a Anna Carolina Rockstroh,^c Antonia Maria Oliveira Machado,^b Ana Cristina Gales^a

Laboratório Alerta, Division of Infectious Diseases, Department of Internal Medicine, Escola Paulista de Medicina/Universidade Federal de São Paulo, São Paulo, SP, Brazil^a; Laboratório Central, Hospital São Paulo, UNIFESP, São Paulo, SP, Brazil^b; bioMérieux, São Paulo, SP, Brazil^c

In this study, we evaluated the influence of distinct bacterial growth media on detection of carbapenemase hydrolysis by matrix-assisted laser desorption ionization–time of flight mass spectrometry. False-negative results were observed for OXA-25-, OXA-26-, and OXA-72-producing *Acinetobacter baumannii* isolates grown on MacConkey agar medium. The other culture media showed 100% sensitivity and 100% specificity for detecting carbapenemase.

Rapid detection of carbapenemase-producing isolates is important for clinical management of infected patients and implementation of infection control measures. The detection of different classes of carbapenemase activity has been assessed by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (1–4). In this study, we evaluated the influence of distinct growth culture media on detection of carbapenemase activity by MALDI-TOF MS using Vitek MS equipment (bioMérieux, Brazil).

(This study was presented in part as poster D-1532 at the 54th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, 5 to 9 September 2014 [5].)

A total of 61 carbapenemase-producing and 32 non-carbapenemase-producing Gram-negative clinical isolates previously characterized by PCR and DNA sequencing were grown on Mueller-Hinton agar (MHA), blood agar (BA), MacConkey agar (MAC), and chromID CPS agar (CPS) (bioMérieux, Brazil). Sixty-one carbapenemase-producing clinical isolates resistant to at least one carbapenem compound were evaluated, including *Klebsiella pneumoniae* producers of IMP-1 ($n = 6$), KPC-2 ($n = 25$), NDM-1 ($n = 1$), and BKC-1 ($n = 2$), *Enterobacter cloacae* producers of VIM-1 ($n = 1$), IMP-1 ($n = 1$), and KPC-2 ($n = 1$), KPC-2-producing *Escherichia coli* ($n = 1$), *Serratia marcescens* producers of KPC-2 ($n = 13$) and IMP-1 ($n = 1$), SPM-1-producing *Pseudomonas aeruginosa* ($n = 5$), and *Acinetobacter baumannii* producers of OXA-25 ($n = 1$), OXA-26 ($n = 1$), OXA-72 ($n = 1$), and OXA-58 ($n = 1$). In addition, 32 non-carbapenemase-producing bacterial isolates, including *Enterobacteriaceae* ($n = 29$), *P. aeruginosa* ($n = 1$), and *A. baumannii* ($n = 2$) isolates, were tested as negative controls. The MALDI-TOF MS assay was performed by incubating a 1- μ l loop of fresh bacterial colonies from MHA, BA, MAC, or CPS in 100 μ l of buffer-adjusted solution (20 mM Tris-HCl [pH 6.8]) with 0.25 mg/ml ertapenem (ETP) (Merck Sharp & Dohme, NJ) (6). The samples were analyzed after 2 and 4 h of incubation at 37°C. One microliter of the supernatant was spotted onto a MALDI target plate, followed by 1 μ l of α -cyano-4-hydroxycinnamic acid (HCCA) matrix solution (bioMérieux) on each spot, and allowed to dry at room temperature. The mass spectrum was obtained by using the Vitek MS instrument operating in linear positive-ion mode and using SARAMIS version 4.1.2 software (bioMérieux). For each isolate, mass spectra were ac-

quired by accumulating 200 laser shots at 50% to 55% laser power in the m/z range of 400 to 600 Da after instrument calibration using the HCCA matrix peaks ($[M + H]^+ = 189.17$; $[2 M + H]^+ = 379.35$) and ertapenem solution. Carbapenem hydrolysis was considered positive if the ertapenem mass peaks corresponding to intact molecule ($[M + H]^+$) at 475 m/z and its monosodium salt ($[M + Na]^+$) at 497 m/z completely disappeared, as previously described (3,6).

Figure 1 shows the mass peak spectrum observed for a KPC-2-producing *K. pneumoniae* isolate grown on distinct media in a 2-h assay. Independent of the growth medium tested, no false-positive results were observed. MHA, BA, and CPS each showed a sensitivity of 92.4% and specificity of 100% after 2 h of incubation. Only the two BKC-1-producing *K. pneumoniae* isolates and four carbapenem-hydrolyzing class D β -lactamase (CHDL)-producing *A. baumannii* isolates required an extended period of incubation (4 h) for yielding positive results with each of these three media. These results are probably related to the low level of carbapenem hydrolysis activity reported for BKC and most of the CHDL enzymes (7,8). In contrast, in colonies obtained from MAC medium, 9 carbapenemase-producing *K. pneumoniae* (BKC-1 [$n = 2$], IMP-1 [$n = 4$], KPC-2 [$n = 2$], NDM-1 [$n = 1$]) and 4 CHDL-producing *A. baumannii* (OXA-25 [$n = 1$], OXA-26 [$n = 1$], OXA-72 [$n = 1$], and OXA-58 [$n = 1$]) isolates resistant to carbapenems (Table 1) were not detected by Vitek MS after the 2-h incubation period. When the incubation period was extended to 4 h, all carbapenemase-producing *K. pneumoniae* isolates and one OXA-58-producing *A. baumannii* isolate had their carbapenemase activity recognized by Vitek MS. However, the mass peak of

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Address correspondence to Ana Carolina Ramos, anarolramos@msn.com.

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FIG 1 Analysis of ETP hydrolysis in a KPC-2-producing *Klebsiella pneumoniae* isolate by Vitek MS in a 2-h assay. Colony growth on chromID CPS agar (A) MacConkey agar (B), blood agar (C), and Mueller-Hinton agar (D). It is important to note that this strain was detected as a carbapenemase producer in the experiments shown in panels A, C, and D, since the ertapenem-intact molecule mass peak ($[M + H]^+ = 475$ g/mol) was not detected. However, when the bacterial colonies were obtained from MacConkey agar, the mass peak at 475 m/z was detected (black arrow in panel B).

the intact ertapenem molecule remained visible when three (4.9%) CHDL-producing *A. baumannii* isolates (OXA-25 [$n = 1$], OXA-26 [$n = 1$], and OXA-72 [$n = 1$]) grown on the MAC medium were tested, even after the 4-h incubation period. The positive and negative predictive values (PPV and NPV, respectively) were 100% for MHA, BA, and CPS medium using the 4-h incubation period, independent of the bacterial species tested. When MAC medium was evaluated, the PPV and NPV were 100% and 91%, respectively. The PPV and NPV according to specific bacterial groups are presented in Table 2.

A faster determination of the resistance mechanism may play a key role in the initial choice of antimicrobial therapy and implementation of infection control measures. MALDI-TOF hydrolysis has been a useful tool for detecting carbapenemase producers. However, other mechanisms of carbapenem resistance, such as

outer membrane protein alterations associated with AmpC or extended-spectrum β -lactamase (ESBL) production, cannot be assessed by this methodology.

In the present study, we observed that false-negative results were more frequently yielded for OXA-25-, OXA-26-, and OXA-72-producing *A. baumannii* isolates grown on MAC medium, even when the incubation period was extended to 4 h. Anderson et al. observed a poorer performance when testing colonies cultivated on MAC rather than blood agar medium than when testing *Pseudomonas* spp. by MALDI-TOF MS (9). These authors suggested that factors, such as growth conditions and the presence of exogenous proteins, can hinder analysis of mass spectrometry.

The detection of carbapenem hydrolysis by Vitek MS using the proposed protocol was successfully achieved from bacterial colonies grown on all media except MacConkey agar. Due to the occurrence of false-negative results, we recommend that clinical mi-

TABLE 1 Carbapenem susceptibility profiles of isolates grown on MAC medium that showed false-negative results by MALDI-TOF assay

Bacterial species	Enzyme produced	No. of isolates	MIC range (μ g/ml)		
			Ertapenem	Imipenem	Meropenem
<i>K. pneumoniae</i>	BKC-1	2	64	4–8	8–16
	IMP-1	4	>128	128	64
	KPC-2	2	16–128	8–32	16–32
	NDM-1	1	128	64	32
<i>A. baumannii</i>	OXA-25	1		128	256
	OXA26	1		128	256
	OXA-72	1		64	128
	OXA-58	1		16	32

TABLE 2 Positive and negative predictive values of MALDI-TOF assay regarding culture media and incubation periods

Bacterial species	<i>n</i>	MHA, BA, CPS				MAC			
		2 h IP ^a		4 h IP		2 h IP		4 h IP	
		PPV (%)	NPV (%)	PPV (%)	NPV (%)	PPV (%)	NPV (%)	PPV (%)	NPV (%)
All isolates	93	100	89	100	100	100	71	100	91.4
<i>Enterobacteriaceae</i>	81	100	94	100	100	100	76.3	100	100
<i>A. baumannii</i>	6	100	50	100	100	— ^b	33	100	40
<i>P. aeruginosa</i>	6	100	100	100	100	100	100	100	100

^a IP, incubation period.

^b —, not calculated (denominator was zero).

crobiology laboratories not perform the hydrolysis assay by MALDI-TOF from bacterial colonies grown on MacConkey agar. It is worth mentioning that colonies grown on MacConkey agar were not recognized as carbapenemase producers by other rapid tests, such as Carba NP (10). We also emphasize the importance of increasing the incubation time for up to 4 h before reporting a negative result, mainly when *Acinetobacter* sp. isolates are tested, due to possible production of CHDL enzymes. In this study, only *Acinetobacter* sp. producers of weak carbapenemases (CHDL) were tested. For those isolates, an extended incubation period was necessary; however, it might not be required for *Acinetobacter* sp. producers of stronger carbapenemases.

Carbapenemase detection by MALDI-TOF MS can easily be incorporated into routine clinical microbiology if kits for bacterial extraction/lysis become cheaper and if software for automatic interpretation of results become commercially available. However, susceptibility testing will still be necessary to provide information regarding the activity of other antimicrobials and mechanisms of resistance.

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