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Evaluation of a rapid immunochromatographic test for detection of KPC in clinical isolates of *Enterobacteriaceae* and *Pseudomonas* species

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ABSTRACT

The KPC K-SeT immunochromatographic test (Coris BioConcept®, Gembloux, Belgium) has been widely used for detection of KPC in *Enterobacteriaceae* with reported sensitivities and specificities of 100%. However, to our knowledge, there are no reports of its use in KPC-positive *Pseudomonas* species. We evaluated the KPC K-SeT test in 36 clinical isolates of *Enterobacteriaceae* (21 KPC-positive and 15 KPC-negative) and 20 *Pseudomonas* species (5 KPC-positive and 15 KPC-negative) using conventional PCR for carbapenemase genes as the reference method. The KPC K-SeT test detected 25 out of 26 KPC-positive isolates (96.1%). The undetected isolate was 1 *P. aeruginosa* bearing the mutation D179Y in the omega loop region of KPC-2 carbapenemase. This mutation was already reported in *Enterobacteriaceae* as conferring resistance to ceftazidime-avibactam. To our knowledge, this is the first report of evaluation of KPC K-SeT test in KPC-positive *P. aeruginosa* isolates.

1. Introduction

The emergence and dissemination of carbapenemase-producing bacteria are a major public health concern worldwide. Rapid identification of these bacteria is critical for infection control and patient treatment. Moreover, due to the specific spectrum of action of novel antimicrobials, a correct characterization of the resistance mechanism is required. KPC is the most frequent carbapenemase detected worldwide and in Chile accounts for up to 78% of the carbapenemases detected, followed by NDM and VIM with 15% and 6%, respectively (Public Health Institute of, 2018). Ideally, detection of KPC must be rapid, easy, and of low cost. The KPC K-SeT (Coris BioConcept®, Gembloux, Belgium) is an immunochromatographic (IC) test intended for the detection of KPC carbapenemase in *Enterobacteriaceae* directly from colonies in agar plates, with reported sensitivities and specificities of 100% (Glupczynski et al., 2016; Greissl et al., 2019; Meunier et al., 2016). The KPC K-SeT test is not validated for *Pseudomonas* species, and to our knowledge, there are no reports of its use in this genus. In contrast, the multiplex RESIST-4 O.K.N.V. test (Coris BioConcept®, Gembloux, Belgium) intended for the detection of OXA-48, KPC, NDM, and VIM is validated for *Pseudomonas* species, but there are few reports evaluating isolates of this genus. Glupczynski et al. (2019) reported 100% sensitivity for the RESIST-4 O.K.N.V. test, but only 1 KPC-positive *P. aeruginosa* isolate was included. Kolenda et al. (2018) also reported 100% sensitivity, and the species tested included 9 VIM-positive *P. aeruginosa* isolates. We evaluated the performance of the KPC K-SeT test to detect KPC in 56 clinical isolates of *Enterobacteriaceae* ($n = 36$) and *Pseudomonas* species ($n = 20$) using conventional PCR for carbapenemase genes as the reference method.

2. Materials and methods

2.1. Bacterial isolates

Fifty-six isolates of gram-negative bacilli with decreased susceptibility to carbapenems and additionally a positive PCR for carbapenemases were selected to validate the KPC K-SeT test. Isolates were obtained from the Laboratory of Microbiology of the Pontificia Universidad Católica de Chile between March and December 2018; 30 isolates were from colonizations (surveillance) and 26 from clinical infections. Only 1 isolate per patient was included. The isolation sites were 30 rectal swabs (surveillance), 7 respiratory specimens, 6 wounds, 6 urine cultures, 5 blood cultures, 1 peritoneal fluid, and 1 uretral fluid. The species distribution was as follows: 12 *Klebsiella pneumoniae*, 1 *Klebsiella oxytoca*, 1 *Klebsiella* sp., 4 *Escherichia coli*, 5 *Citrobacter* sp., 13 *Enterobacter cloacae*, 16 *Pseudomonas aeruginosa*, 3 *Pseudomonas putida*, and 1 *Pseudomonas alcaligenes*.

The susceptibility to carbapenems was determined through agar dilution method using the breakpoints suggested by the Clinical

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Table 1
Primers used for carbapenemase detection.

Gene	Primer sequence (5′ →3′)	Product length (bp)
bla_{KPC}	KPC F: TGTCACTGTATCGCCGTC KPC R: CTCAGTGCTCTACAGAAAACC	1010
bla_{KPC} (sequencing)	KPC-UP: GCTACACCTAGCTCCACCTTC KPC-DW: ACAGTGGTTGGTAATCCATGC	968
bla_{GES}	Ges F: ATGCGCTTCATTCACGCAC Ges R: CTATTTGTCCGTGCTCAGG	864
bla_{IMI}	IMI A: ATAGCCATCCTTGTTTAGCTC IMI B: TCTGCGATTACTTTATCCTC	818
bla_{VIM}	VIM S: CCGATGGTGTTTGGTCGCAT VIM AS: GAATGCGCAGCACCAGGAT	391
bla_{IMP}	IMP S: AAAGA TACTGAAAAGTTAGT IMP AS: TCYCCAAYTT CACTRTGACT	446
bla_{NDM}	NDM1 F: GGTGACTCACGCGCATCAGG NDM1 R: CCAGCCATTGGCGGCGAAAG	154
bla_{OXA-48}	OXA-48A: TTGGTGGCATCGATTATCGG OXA-48B: GAGCACTTCTTTTGTGATGGC	744

Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 2018). We considered a decrease in carbapenem susceptibility when MIC ≥1 of ertapenem and MIC ≥2 of imipenem and meropenem for *Enterobacteriaceae*, and MIC ≥4 of imipenem and meropenem for *Pseudomonas* species isolates. Conventional PCR targeting the carbapenemase genes bla_{KPC}, bla_{OXA-48}, bla_{NDM}, bla_{IMP}, bla_{IMI}, bla_{GES}, and bla_{VIM} was performed as previously described (Wozniak et al., 2012). DNA extraction was performed using MagNA Pure System (Roche®). Primers used are detailed in Table 1. PCRs had 25 μL, and reaction mix used was Go Taq Green Mastermix (Promega®, Madison, USA). Strains bearing the different carbapenemases were used as positive controls. Technicians who performed PCR were blinded for previous results.

According to PCR, 26 isolates were KPC positive (21 *Enterobacteriaceae* and 5 *P. aeruginosa*) and 30 isolates were KPC negative (15 *Enterobacteriaceae* and 15 *P. aeruginosa*) and harbored other carbapenemases: 5 NDM, 1 IMP, 23 VIM, and a metallo-beta-lactamase determined phenotypically through meropenem-EDTA disk method (Yong et al., 2002). The PCR results for carbapenemase genes were used as reference gold standard.

2.2. Carba-NP test and KPC K-SeT test

All 56 isolates were simultaneously analyzed through Carba-NP test and KPC K-SeT test. Carba-NP test used was an "in-house" method performed according to Clinical and Laboratory Standards Institute, 2015.

All of the isolates were analyzed for the presence KPC using KPC K-SeT test (Coris BioConcept®, Gembloux, Belgium) according to manufacturer's instructions. In brief, isolates were grown on Mueller–Hinton agar for 24 h at 37 °C. One bacterial colony was resuspended in LY-A buffer, and 3 drops were delivered in the sample well of the test cassette. Results were read after 15 min. Negative results display only 1 band (quality control), and positive results display 2 bands. Technicians who performed the IC test were blinded for previous results.

2.3. bla_{KPC} gene sequencing

A 968-bp fragment spanning the entire bla_{KPC} gene was PCR amplified using primers KPC-UP and KPC-DW (Shen et al., 2009) (Table 1). The PCR product was purified with QIAquick purification kit (Qiagen®, Hilden, Germany). Purified DNA was sequenced using Big Dye Terminator v3.1 Cycle Sequencing kit on an ABI 310 sequencer (Applied Biosystems®, Foster City, CA, USA) using both primers. Chromatograms were compared to wild-type bla_{KPC-2} gene sequence using Sequencher software.

3. Results

The 30 KPC-negative isolates yielded negative results with the KPC K-SeT test (Table 2). The test was able to detect 25 out of 26 KPC-positive isolates (according to PCR results) (Table 2).The undetected KPC-positive isolate was *P. aeruginosa* no. 847. The bla_{KPC} gene of this isolate was sequenced and corresponded to KPC-2 type but had a point mutation in G532T. This substitution produced the aminoacid change D179Y located in the omega loop of the enzyme which is crucial for enzymatic activity. Isolate no. 847 was analyzed through a second IC test, the NG-Test CARBA 5 (NG Biotech®; Guipry, France), and it was also negative. Overall sensitivity and specificity of the KPC K-SeT test were 96.1% and 100%, respectively. Sensitivity for *Enterobacteriaceae* was 100%, in accordance with reported results (Glupczynski et al., 2016; Greissl et al., 2019; Meunier et al., 2016). However, sensitivity for *Pseudomonas* species was 80%.

All of the isolates were Carba-NP positive, except for *P. aeruginosa* isolate no. 847, which had a negative Carba-NP test.

4. Discussion

To our knowledge, this is the first report of evaluation of KPC K-SeT test in KPC-positive *P. aeruginosa*. In previous studies, false negatives of this test were reported only for KPC-6 and KPC-8 types in *Enterobacteriaceae* (Ramos et al., 2017). Here we report a false negative of KPC K-SeT test with KPC-2 carbapenemase. The mutation D179Y has been described previously in KPC-2 variants of *K. pneumoniae* because it confers resistance to ceftazidime-avibactam (Compain and Arthur, 2017). In fact, this isolate has a ceftazidime-avibactam MIC of >256 μg/mL as

Table 2
Results of the KPC K-SeT test.*a

KPC (no. of isolates)	Species	No. of isolates	CarbaNP positive	KPC K-SeT positive	Other carbapenemases detected[a]
KPC positive[a] ($N = 26$)	*K. pneumoniae*	12	12/12	12/12	N/D
	K. oxytoca	1	1/1	1/1	N/D
	Klebsiella spp.	1	1/1	1/1	N/D
	E. coli	4	4/4	4/4	N/D
	Citrobacter spp.	2	2/2	2/2	N/D
	E. cloacae	1	1/1	1/1	N/D
	P. aeruginosa	5	4/5	4/5	N/D
KPC negative[a] ($N = 30$)	*Citrobacter* spp.	3	3/3	0/3	3 NDM
	E. cloacae	12	12/12	0/12	10 VIM; 2 NDM
	P. aeruginosa	11	11/11	0/11	10 VIM; 1 IMP
	P. putida	3	3/3	0/3	3 VIM
	P. alcaligenes	1	1/1	0/1	1 MBL

MBL = metallo-beta-lactamase; N/D = not detected.
[a] Conventional PCR was used to determine the presence of KPC and other carbapenemases.

determined through E-test (Biomerieux®). However, this patient had not been exposed to ceftazidime/avibactam before because this antibiotic combination is not available for clinical use in Chile yet. The omega loop region of the wild-type KPC enzyme is formed through a salt bridge between glutamic acid 179 and arginine 164 (Winkler et al., 2015). Disruption of the salt bridge could have a profound impact in tridimensional structure and could modify the epitopes targeted by the IC test. This could explain how a single mutation could hinder antibody binding. Besides this, D179Y mutation could negatively affect carbapenemase activity as evidenced through a negative Carba-NP test.

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