



HAL
open science

Different phenotypic expression of KPC β -lactamase variants and challenges in their detection

Saoussen Oueslati, Linda Tlili, Cynthia Exilie, Sandrine Bernabeu, Bogdan Iorga, Rémy Bonnin, Laurent Dortet, Thierry Naas

► To cite this version:

Saoussen Oueslati, Linda Tlili, Cynthia Exilie, Sandrine Bernabeu, Bogdan Iorga, et al.. Different phenotypic expression of KPC β -lactamase variants and challenges in their detection. *Journal of Antimicrobial Chemotherapy*, 2020, 75 (3), pp.769-771. 10.1093/jac/dkz508 . hal-02840859

HAL Id: hal-02840859

<https://hal.science/hal-02840859>

Submitted on 7 Dec 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Different phenotypic expressions of KPC β -lactamases and challenges in their detection

Saoussen OUESLATI,¹ Linda TLILI,¹ Cynthia EXILIE,¹ Sandrine BERNABEU,^{1,2} Bogdan IORGA,³

Rémy A. BONNIN,^{1,4} Laurent DORTET^{1,2,4} and Thierry NAAS^{1,2,4*}

¹ EA7361 "Structure, dynamic, function and expression of broad spectrum β -lactamases", Faculty of Medicine Université Paris-Sud, LabEx Lermite, Université Paris-Saclay, Le Kremlin-Bicêtre, France

² Bacteriology-Hygiene unit, Assistance Publique/Hôpitaux de Paris, Bicêtre Hospital, Le Kremlin-Bicêtre, France

³ Institut de Chimie des Substances Naturelles, CNRS UPR 2301, Université Paris-Saclay, Labex LERMIT, Gif-sur-Yvette, France.

⁴ Associated French National Reference Center for Antibiotic Resistance: Carbapenemase-producing *Enterobacteriaceae*, Le Kremlin-Bicêtre, France

Running title: Phenotypic expressions of KPC variants (38 characters spaces included)

Words : 1059

Table: 1; Supplemental Figure S1

*Corresponding author: Service de Bactériologie-Hygiène, Hôpital de Bicêtre 78 rue du Général Leclerc, 94275 Le Kremlin-Bicêtre, France.

Tel : +33 1 45 21 20 19 ;

Fax : +33 1 45 21 63 40,

thierry.naas@aphp.fr

25 Sir,

26 Carbapenem-resistance among Enterobacteriaceae has become a major public health
27 issue owing to their rapid worldwide spread.¹ In Enterobacteriaceae this resistance is largely
28 due to the production of carbapenemases, the most prevalent throughout the world being
29 KPC-, VIM-, IMP-, NDM- and OXA-48 type variants. KPC-2, initially described in the USA in
30 2001,² went global in less than a decade likely as a result of being located on a transposon,
31 Tn4401 capable of high frequency transposition, itself inserted on self-conjugative plasmids,
32 and its frequent association with the highly successful *K. pneumoniae* clonal group 258.³
33 Today, more than 44 natural KPC variants have been described, but only a few of them have
34 been characterized in terms of β -lactam hydrolytic properties.⁴ It is crucial to understand the
35 impact of single amino-acid changes on the hydrolytic profile of KPC-variants and the ability
36 of commercially-available diagnostic tests to efficiently detect them.

37 In this work, we have compared the phenotypic expressions of 14 KPC variants either
38 natural or generated by site-directed mutagenesis and evaluated the performance of several
39 diagnostic tests to detect them. Bla_{KPC-2} and bla_{KPC-3} alleles were cloned into pTOPO vector
40 along with its natural ribosomal binding site (RBS) upstream, and electroporated into
41 electrocompetent *E. coli* TOP10 as previously described.⁵ The resulting plasmids were used to
42 construct 11 single bla_{KPC-2} mutants and one single bla_{KPC-3} mutant using the QuikChange II Site-
43 Directed Mutagenesis Kit (Agilent Technologies, Les Ulis, France) (Table 1). Thus among the
44 12 generated mutants (Figure S1A), four were natural variants of KPC-2 [KPC-5 (P104R)⁶, KPC-
45 6 (V240G)⁷, KPC-12 (L168M), and KPC-33 (D179Y)] and one of KPC-3 [KPC-31 (D179Y)]. Six
46 substitutions correspond to natural substitutions that occurred in KPC-2, but that were not
47 reported alone: V240A, G147K, A120L, D92G, W105A and W105G. Finally, the last mutant has
48 a substitution C238S, which is a conserved residue that establishes a di-sulfide bridge with the

49 C68. MICs were performed using the Etest (bioMérieux, Marcy-l'Etoile, France) and detection
50 tests were performed according to manufacturers' recommendations. Phenotypic profiles
51 and diagnostic testing results are summarized in Table 1. Increased MICs for aztreonam and
52 cephalosporins without any modification of those for carbapenems have been observed with
53 mutants in position 240. On the opposite substitutions at positions 168 and 92 led to low
54 carbapenem MICs, but yet detectable carbapenem hydrolysis (as evidenced using biochemical
55 tests, see Table 1). The mutants P104R and G147K led to increased MICs for aztreonam and
56 cephalosporins, with slight decreased MICs for carbapenems. The mutations on residues 105
57 and 238 resulted in a global decrease in MICs for all β -lactams except amoxicillin, with a
58 stronger impact observed for the C238S mutant. With all of these mutants (including those
59 with highly increased MICs for ceftazidime, such as P104R, V240G and V240A) avibactam was
60 able to restore ceftazidime susceptibility. Interestingly, KPC-31 and KPC-33 with a D179Y
61 substitution, exhibit reduced MICs for all β -lactams, including aztreonam and amoxicillin,
62 except for ceftazidime, for which increased MICs were observed. In addition, these two
63 mutants led to MICs for the combination of ceftazidime/avibactam, that were limit to
64 resistance according to CLSI and EUCAST. These results suggest that avibactam is not capable
65 of inhibiting KPC-31 and KPC-33 efficiently. In order to confirm this hypothesis IC₅₀s were
66 measured with purified proteins.⁵ The IC₅₀ values for KPC-31 (20 μ M) and KPC-33 (42 μ M) were
67 ~100 fold higher than those of KPC-2 (230nM) and KPC-3 (350nM), further supporting that the
68 residue D179 is important in the binding of avibactam.^{8,9,5}

69 Overall, all tested residues, except A120, have a direct impact on the phenotypic
70 expression profile of KPC enzymes. Residues 168 and 179 are located on the Ω loop, which is
71 known to play a crucial role in the substrate selectivity of the enzyme.⁹ Residues 105, 104 and
72 240 delimit the cavity of the active site. Accordingly, hydrolysis spectrum is influenced by the

73 nature of the residues at these positions. Since the two cysteines C238 and C68 (adjacent to
74 the S70) form a disulfide bridge that stabilizes the KPC folding, it was expected to identify a
75 dramatic impact of the C238S substitution on the catalytic activity of the enzyme, as shown
76 for other class A carbapenemases such as SME and GES-5.^{10,11} Finally, although located at the
77 opposite of the active site, residues 92 and 147 have also a crucial impact on KPC activity.
78 Molecular modeling of the G147K mutant, performed with UCSF Chimera software,¹² shows a
79 clash with G143 located on the helix α_6 , which is in vicinity of the active site (Figure S1B). This
80 clash might lead to a switch of this helix that subsequently impact on the shape of the active
81 site.

82 Several diagnostic tests based on imipenem hydrolysis (biochemical and MALDI-
83 TOF-based), Lateral flow immunoassays and molecular techniques (PCR) were evaluated on
84 these different mutants. Unlike molecular tests that were able to detect all mutants,
85 biochemical tests based on imipenem hydrolysis: Carba NP test¹³, Rapidec CARBA NP¹³
86 (bioMérieux, Marcy l'Etoile, France) and β -CARBA¹⁴ (BioRad, Marnes la Coquette, France), and
87 MALDI-TOF -based MBT STAR-Carba IVD¹⁵ (Bruker, Illkirch-Graffenstaden, France) were able
88 to distinguish between KPC variants with carbapenem hydrolytic capacities from those lacking
89 any carbapenemase activity (Table 1). Molecular assays, being an in-house KPC specific PCR
90 or a commercially-available PCR (Xpert Carba, Cepheid, Maurens-Scopont, France) detected
91 accurately all variants. NG-test Carba5 (NG Biotech, Guipry, France) and Resist-4 O.K.N.V. K-
92 Set (Coris, Gembloux – Belgium) lateral flow immunoassays (LFIA)¹⁶ assays were also able to
93 detect all KPC variants except KPC-31 and KPC-33.⁴ As the two latter variants lack
94 carbapenemase activity, the non-detection does not lead to false negative results in terms of
95 true carbapenemase detection. As these 2 variants lead to avibactam resistance, it would be
96 nevertheless important that they are detected, but not classified as carbapenemases.

97 Whether, carbapenems might be used to treat infections with KPC-31 or KPC-33-producers, is
98 still debatable, but according to EUCAST guidelines the answer is Yes, as the MICs for
99 imipenem are in the susceptibility range.

100 Overall, our results demonstrate that KPC variants are not equal, probably selected by
101 different antibiotic usages. This is the case for KPC-5 that leads to high level cefixime
102 resistance. Some KPC variants cannot be considered as carbapenemases anymore. This is the
103 case for KPC-31 and KPC-33 that have lost their carbapenemase activity in a similar manner to
104 KPC-14 and KPC-28 variants.⁵ These peculiar variants are challenging for their detection, and
105 will require complementary approaches combining molecular or LFIA together with
106 carbapenem hydrolysis detection systems.

107

108 **Funding**

109 This work was supported by the Assistance Publique – Hôpitaux de Paris, by a grant from the
110 Université Paris-Sud (EA 7361), and by the LabEx LERMIT with a grant from the French National
111 Research Agency (ANR-10-LABX-33) and a grant from the French National Research Agency
112 (ANR-17-ASTR-0018).

113 **Transparency declarations**

114 LD is co-inventor of the Carba NP Test, which patent has been licensed to bioMérieux (La
115 Balme les Grottes, France). All other authors: none to declare.

116

117 **References**

118 1. Eichenberger EM, Thaden JT. Epidemiology and Mechanisms of Resistance of Extensively
119 Drug Resistant Gram-Negative Bacteria. *Antibiotics* (Basel) 2019; **8**(2). pii: E37. doi:
120 10.3390/antibiotics8020037.

- 121 2. Yigit H, Queenan AM, Anderson GJ, *et al.* Novel Carbapenem-Hydrolyzing β -Lactamase, KPC-
122 1, from a Carbapenem-Resistant Strain of *Klebsiella pneumoniae*. *Antimicrob Agents*
123 *Chemother* 2001; **45**: 1151-61.
- 124 3. Peirano G, Bradford PA, Kazmierczak KM, *et al.* Importance of Clonal Complex 258 and
125 IncFK2-like Plasmids among a Global Collection of *Klebsiella pneumoniae* with blaKPC.
126 *Antimicrob Agents Chemother* 2017; **61**: e02610-16.
- 127 4. Naas T, Oueslati S, Bonnin RA, *et al.* Beta-lactamase database (BLDB) – structure and
128 function. *Journal of Enzyme Inhibition and Medicinal Chemistry* 2017; **32**: 917-9.
- 129 5. Wolter DJ, Kurpiel PM, Woodford N, *et al.* Phenotypic and Enzymatic Comparative Analysis
130 of the Novel KPC Variant KPC-5 and Its Evolutionary Variants, KPC-2 and KPC-4. *Antimicrob*
131 *Agents Chemother* 2009; **53**: 557-62.
- 132 6. Lamoureaux TL, Frase H, Antunes NT, *et al.* Antibiotic Resistance and Substrate Profiles of
133 the Class A Carbapenemase KPC-6. *Antimicrob Agents Chemother* 2012; **56**: 6006-8.
- 134 7. Oueslati S, Iorga BI, Tlili L, *et al.* Unravelling ceftazidime/avibactam resistance of KPC-28, a
135 KPC-2 variant lacking carbapenemase activity. *J Antimicrob Chemother* 2019; **74**: 2239-46.
- 136 8. Livermore DM, Warner M, Jamrozny D, *et al.* In Vitro Selection of Ceftazidime-Avibactam
137 Resistance in Enterobacteriaceae with KPC-3 Carbapenemase. *Antimicrob Agents*
138 *Chemother* 2015; **59**: 5324–30.
- 139 9. Barnes MD, Winkler ML, Taracila MA, *et al.* *Klebsiella pneumoniae* Carbapenemase-2 (KPC-
140 2), Substitutions at Ambler Position Asp179, and Resistance to Ceftazidime-Avibactam:
141 Unique Antibiotic-Resistant Phenotypes Emerge from β -Lactamase Protein Engineering
142 *mBio* 2017; **8**: e00528-17.

- 143 10. Sougakoff W, L'Hermite G, Pernet L, *et al.* Structure of the imipenem-hydrolyzing class A
144 beta-lactamase SME-1 from *Serratia marcescens*. *Acta Crystallogr D Biol Crystallogr* 2002;
145 **58**:267-74.
- 146 11. Smith CA, Nossoni Z, Toth M, *et al.* Role of the Conserved Disulfide Bridge in Class A
147 Carbapenemases. *J Biol Chem.* 2016; **291**:22196-206.
- 148 12. Pettersen EF, Goddard TD, Huang CC, *et al.* UCSF Chimera-A visualization system for
149 exploratory research and analysis. *J Comput Chem* 2004; **25**: 1605-12.
- 150 13. Dortet L, Agathine A, Naas T, *et al.* Evaluation of the RAPIDEC® CARBA NP, the Rapid CARB
151 Screen® and the Carba NP test for biochemical detection of carbapenemase-producing
152 Enterobacteriaceae. *J Antimicrob Chemother* 2015; **70**: 3014-22.
- 153 14. Bernabeu S, Dortet L, Naas T. Evaluation of the β-CARBA™ test, a colorimetric test for the
154 rapid detection of carbapenemase activity in Gram-negative bacilli. *J Antimicrob*
155 *Chemother* 2017; **72**: 1646-58.
- 156 15. Dortet L, Tandé D, de Briel D, *et al.* MALDI-TOF for the rapid detection of carbapenemase-
157 producing Enterobacteriaceae: comparison of the commercialized MBT STAR®-Carba IVD
158 Kit with two in-house MALDI-TOF techniques and the RAPIDEC® CARBA NP. *J Antimicrob*
159 *Chemother* 2018; **73**: 2352-59.
- 160 16. Boutal H, Vogel A, Bernabeu S, *et al.* A multiplex lateral flow immunoassay for the rapid
161 identification of NDM-, KPC-, IMP- and VIM-type and OXA-48-like carbapenemase-
162 producing Enterobacteriaceae. *J Antimicrob Chemother* 2018; **73**: 909-15.

163

Table 1. MIC profiles of *E. coli* TOP10 expressing single KPC mutants and performances of various diagnostic tests.

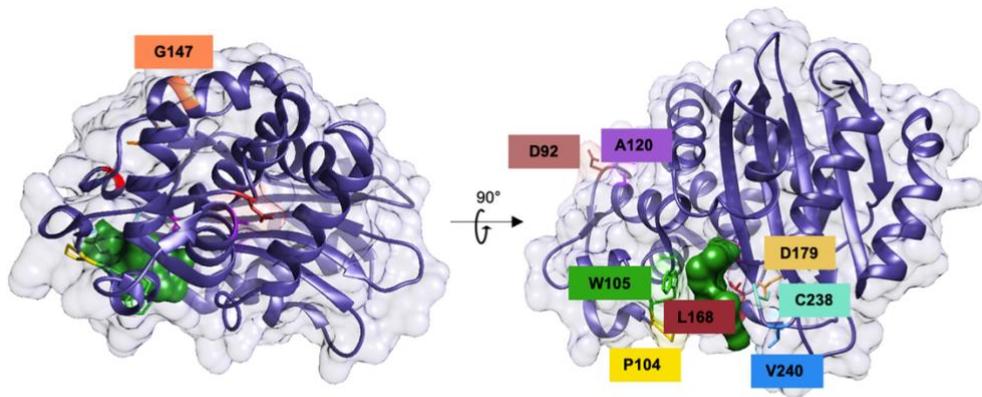
AA changes	Variant	MICs (mg/L)											Carbapenem hydrolysis assays				PCR		LFIA ^a	
		AMX	ATM	CRO	CAZ	CAZ/	CFX	CTX	FEP	IMP	MEM	ERT	Carba	RAPIDEC®	β-	MBT STAR-	In house	Xpert	NG-Test	Coris Resist-4
						AVI							NP test	CARBA NP	CARBA™	Carba IVD	<i>bla</i> _{KPC} PCR	Carba-R	CARBA 5	O.K.N.V. K-Set
	KPC-2	>256	32	16	4	0.38	4	8	2	8	3	1	+	+	+	+	+	+	+	+
H272Y	KPC-3	>256	>256	48	>256	0.75	12	>32	6	8	3	1	+	+	+	+	+	+	+	+
P104R	KPC-5	>256	>256	>256	>256	0.5	>256	8	2	1	0.25	0.19	+	+	+	+	+	+	+	+
V240G	KPC-6	>256	>256	48	32	0.75	16	>32	4	4	3	1	+	+	+	+	+	+	+	+
L168M	KPC-12	>256	12	6	4	0.38	3	4	1	0.75	0.25	0.125	+	+	+	+	+	+	+	+
D179Y	KPC-33	12	0.75	1	>256	8	6	1.5	1	0.25	0.032	0.008	-	-	-	-	+	+	-	-
H272Y, D179Y	KPC-31	6	0.75	2	>256	12	4	1.5	1	0.25	0.032	0.008	-	-	-	-	+	+	-	-
D92G		>256	64	12	4	0.25	4	4	2	1.5	0.5	0.38	+	+	+	+	+	+	+	+
W105A		>256	32	1	1	0.25	3	1.5	0.75	0.75	0.125	0.094	+	+	+	+	+	+	+	+
W105G		>256	24	1	1	0.25	2	0.75	0.25	1	0.19	0.125	+	+	+	+	+	+	+	+
A120L		>256	32	16	3	0.38	3	6	2	6	1.5	1.5	+	+	+	+	+	+	+	+
G147K		>256	64	32	6	0.38	6	24	6	6	1	0.75	+	+	+	+	+	+	+	+
C238S		>256	2	0.25	2	0.38	1	0.25	0.19	0.25	0.047	0.008	-	-	-	-	+	+	+	+
V240A		>256	>256	64	12	0.38	12	>32	4	8	2	1.5	+	+	+	+	+	+	+	+
<i>E. coli</i> TOP10		6	0.047	0.032	0.012	0.012	0.38	0.064	0.064	0.25	0.032	0.004	-	-	-	-	-	-	-	-

AMX: Amoxicillin, ATM: Aztreonam, CRO: Ceftriaxone, CAZ: Ceftazidime, AVI: Avibactam, CFX : Cefixime, CTX : Cefotaxime, FEP : Cefepime, IMP : Imipenem, MEM : Meropenem and ETP : Ertapenem
 Dark grey, light grey and white boxes represent MICs in the resistance, intermediate and susceptible range, respectively. Breakpoints were those of the European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters Version 9.0, valid from 2019-01-01 (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_9.0_Breakpoint_Tables.pdf; last accessed September 2019). Grey boxes represent negative test results.

^a LFIA: Lateral flow immunoassay

Supplementary data

A



B

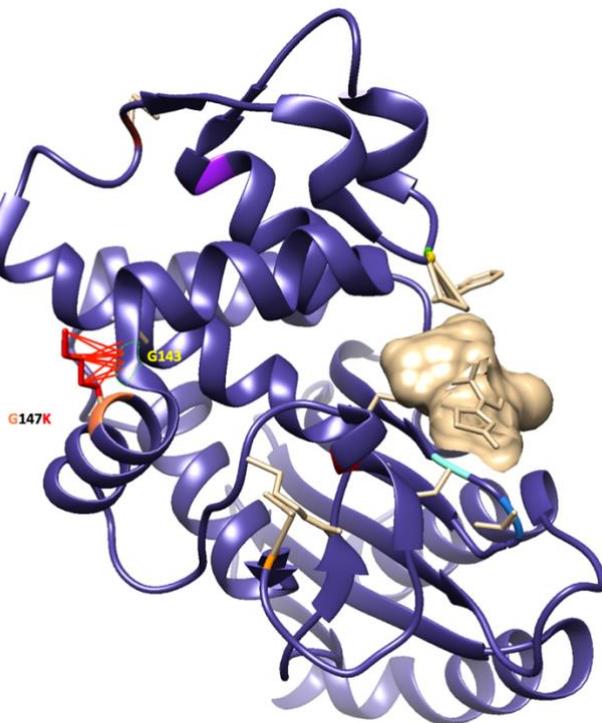


Figure S1. A Positions of the substituted residues included in this study on the Crystal structure of KPC-2 (PDB code 5UJ3) complex with cefotaxime (green), **B**. Representation of the steric clashes (red lines) between the side chains of K147 and G143.