

1 **Impact of anticancer chemotherapy on the extension of beta-lactamase spectrum: an example with KPC-type carbapenemase activity**
2 **towards ceftazidime-avibactam**

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4 *Authors:* Claire Amaris Hobson¹, Stéphane Bonacorsi^{1,2}, Didier Hocquet³, André Baruchel⁴, Mony Fahd⁴, Thomas Storme⁵, Raksamy Tang⁵,
5 Catherine Doit^{1,2}, Olivier Tenaillon¹, André Birgy^{1,2*}.

6 ¹Université de Paris, IAME, INSERM, F-75018 Paris, France

7 ²AP-HP, Hôpital Robert Debré, Service de Microbiologie, F-75019 Paris, France

8 ³Hygiène Hospitalière, UMR CNRS 6249, Université de Bourgogne Franche-Comté, Besançon, France

9 ⁴Service d'Immuno-Hématologie Pédiatrique, Hôpital Robert Debré, AP-HP, Paris, France

10 ⁵Pharmacie Hospitalière, Hôpital Robert Debré, AP-HP, Paris, France

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13 ***Corresponding author:** André Birgy (AB), PD, PhD, Hôpital Robert-Debré (APHP), 48 Boulevard Sérurier F75019 Paris, France

14 (andre.birgy@aphp.fr). Phone +33 140034060

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16 **Supplementary Table S1:**

17 Rates, average and standard deviation (sd) of resistant mutants of 2 strains of KPC-producing *Enterobacteriaceae* (RD26 and RD29) selected on
 18 4 different antibiotic media (Ceftazidime/avibactam (CZA), Rifampicin (RMP), Meropenem (Mero) and the association Ceftazidime/avibactam
 19 and Meropenem (CZA-Mero)) after a culture with or without chemotherapy.

Resistance mutational rates to different antibiotics after exposure to cytotoxic molecules or non exposure (control)																		
Isolate	Selective plate medium	Cytarabine		Mercaptopurine		Azacitidine		Daunorubicin		Dacarbazine		Cyclophosphamide		Mitoxantrone		Control		
		Resistant mutational rates	average +/- sd (p-value)	Resistant mutational rates	average +/- sd (p-value)	Resistant mutational rates	average +/- sd (p-value)	Resistant mutational rates	average +/- sd (p-value)	Resistant mutational rates	average +/- sd (p-value)	Resistant mutational rates	average +/- sd (p-value)	Resistant mutational rates	average +/- sd (p-value)	Resistant mutational rates	average +/- sd	
<i>E. coli</i> KPC-2 (RD29)	CZA 2mg/L	1,0E-06		<1E-10		<1E-10		1.5E-07		3.4E-06		<1E-10		4.5E-08		4.0E-10		
		1.7E-08	2.7E-07 +/-		7.7E-09 +/-	1.9E-08	4.7E-09 +/-		8.3E-08 +/-	1.3E-06	3.3E-06 +/-		2.6E-08 +/-	3.0E-08	9.3E-08 +/-	1.2E-09	9.4E-09 +/-	
		3.7E-08	(0.15)	3.1E-08	1.5E-08	1.0E-10	(0.94)	1.0E-10	9.2E-09	9.7E-08	6.1E-06	2.1E-06	1.6E-08	4.0E-08	8.3E-08	8.2E-08	1.6E-09	1.6E-08
		1.4E-08		1.0E-10		1.0E-10		1.8E-07		2.4E-06		8.6E-08		2.1E-07		5.5E-09		
	RMP 50mg/L	1.5E-08		4.5E-08		3.7E-06		3.0E-07		1.8E-05		3.9E-08		6.5E-08		1.6E-08		
		4.1E-08	2.0E-08 +/-		4.2E-08 +/-	1.9E-06	2.3E-06 +/-		5.8E-06 +/-	1.7E-05	1.6E-04 +/-		3.8E-08 +/-	1.0E-07	5.6E-08 +/-	6.0E-08	3.2E-08 +/-	
6.2E-09		1.5E-08	6.1E-08	1.6E-08	1.4E-06	1.0E-06	4.0E-06	6.9E-06	5.9E-04	2.9E-04	2.6E-08	2.5E-08	5.0E-08	3.8E-08	5.5E-08	2.3E-08		
		1.9E-08		2.3E-08		2.4E-06		1.6E-05		6.7E-06		7.1E-08		8.1E-09		1.8E-08		
<i>E. cloacae</i> KPC-3 (RD26)	CZA 16mg/L	3.2E-09		2.1E-08		3.7E-07		1.3E-08		1.2E-07		1.1E-08		3.6E-08		6.1E-09		
		7.2E-09	6.2E-09 +/-		2.4E-08 +/-	3.8E-07	7.2E-07 +/-		1.7E-07 +/-	7.0E-08	9.6E-08 +/-		7.6E-09 +/-	7.1E-08	3.6E-08 +/-	5.9E-10	4.1E-09 +/-	
		9.8E-09	2.9E-09	3.7E-08	1.1E-08	1.1E-06	4.0E-07	2.8E-08	1.8E-07	3.0E-08	5.9E-08	3.9E-09	2.9E-09	1.7E-08	2.5E-08	2.6E-10	5.2E-09	
			4.6E-09		2.8E-08		1.0E-06		4.0E-07		1.7E-07		8.2E-09		1.9E-08		1.1E-09	
	RMP 50mg/L	4.4E-08		2.5E-07		1.6E-06		1.3E-07		2.5E-06		2.5E-07		1.1E-07		7.6E-08		
		6.9E-08	4.2E-08 +/-		9.5E-08 +/-	4.0E-06	3.0E-06 +/-		1.3E-06 +/-	3.6E-07	8.5E-07 +/-		1.4E-07 +/-	1.5E-07	1.2E-07 +/-	1.1E-07	7.3E-08 +/-	
		2.5E-08	1.9E-08	3.7E-08	1.0E-07	2.4E-06	1.2E-06	2.5E-07	1.5E-06	1.8E-07	1.1E-06	4.7E-08	9.8E-08	1.7E-07	4.8E-08	6.3E-08	2.1E-08	
			3.2E-08		5.0E-08		4.1E-06		1.4E-06		3.1E-07		2.0E-07		5.8E-08		4.8E-08	
	MEM 20mg/L	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
	CZA-MEM	<1E-10	0	<1E-10	0	<1E-10	0	<1E-10	0	<1E-10	0	<1E-10	0	<1E-10	0	<1E-10	0	
<1E-10		0	<1E-10	0	<1E-10	0	<1E-10	0	<1E-10	0	<1E-10	0	<1E-10	0	<1E-10	0		

*Resistant rates were not evaluable since confluent growths were observed at this concentration

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24 **Supplementary Table S2:**

25 Fold increase of resistant mutants' rates, average and standard deviation (sd) of 2 strains of KPC-producing *Enterobacteriaceae* (RD26 and
 26 RD29) selected on 4 different antibiotic media (Ceftazidime/avibactam (CZA), Rifampicin (RMP), Meropenem (Mero) and the association
 27 Ceftazidime/avibactam and Meropenem (CZA-Mero)) after a culture with or without chemotherapy, compared to the control group.

Fold increased ¹ of resistant mutant rates to different antibiotics after exposure to different cytotoxics															
Isolates	Selective Plate medium	Cytarabine		Mercaptopurine		Azacitidine		Daunorubicin		Dacarbazine		Cyclophosphamide		Mitoxantrone	
		fold increase	average +/- sd	fold increase	average +/- sd	fold increase	average +/- sd	fold increase	average +/- sd	fold increase	average +/- sd	fold increase	average +/- sd	fold increase	average +/- sd
<i>E. coli</i> KPC-2 (RD29)	CZA 2mg/L	2490.0		0.0		0.0		373.5		8479.5		0.0		113.2	
		14.5	632.4 +/-	18.7	4.7 +/- 9.3	15.4	3.8 +/- 7.7	0.0	101.7 +/- 181.8	1051.3	3416.2 +/-	9.6	6.4 +/- 7.7	25.2	57.0 +/- 38.9
		22.6	1238.4	0.0		0.0		0.0		3700.7	3660.9	0.1		50.8	
	RMP 50mg/L	2.6		0.0		0.0		33.3		433.3		15.7		38.8	
		41.5		2.8		230.1		18.7		1103.7		2.5		4.0	
		0.7	10.8 +/- 20.4	1.1	2.1 +/- 1.1	31.0	105.2 +/- 97.3	73.2	309.1 +/- 389.8	288.3	3134.2 +/-	0.5	2.1 +/- 1.5	1.7	1.8 +/- 1.6
		0.1		3.4		25.0		274.5		10775.0	5107.0	1.2		0.9	
		1.0		1.3		134.7		870.0		370.0		3.9		0.4	
<i>E. cloacae</i> KPC-3 (RD26)	CZA 16mg/L	0.5		3.5		60.5		2.1		19.4		1.8		6.0	
		12.2	13.6 +/- 16.6	142.0	43.0 +/- 66.9	650.0	1492.5 +/-	107.6	125.2 +/- 170.9	118.8	102.2 +/- 58.0	14.8	6.2 +/- 6.5	119.8	52.1 +/- 51.7
		37.5		0.8		4329.3	1925.6	19.1		115.8		0.6		64.7	
	MEM 20mg/L	4.3		25.8		930.0		372.0		155.0		7.6		18.0	
		*		*		*		*		*		*		*	
		*		*		*		*		*		*		*	
		0.6		3.3		21.2		1.7		33.6		3.3		1.4	
		0.6	0.6 +/- 0.1	0.6	1.4 +/- 1.3	37.4	45.4 +/- 27.1	4.0	20.4 +/- 21.5	3.3	11.6 +/- 14.7	0.7	2.3 +/- 1.7	1.4	1.7 +/- 0.6
RMP 50mg/L	0.4		0.7		38.7		46.9		2.9		1.0		2.6		
	0.7		1.0		84.2		28.9		6.4		4.2		1.2		

¹Four experiments were performed for each antibiotics

*Resistant rates were not evaluable since confluent growths were observed at this concentration

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33 **Supplementary Table S3:**

34 Rates, average and standard deviation (sd) of resistant mutants of 5 strains of KPC-producing *Enterobacteriaceae* selected on 2 different
 35 antibiotic media (Ceftazidime/avibactam (CZA), Rifampicin (RMP), after a culture with or without chemotherapy.

	Mutation rate on RMP medium				Mutation rate on CZA medium			
	with chemotherapy	average +/- sd (p-value)	without chemotherapy	average +/- sd (p-value)	with chemotherapy	average +/- sd (p-value)	without chemotherapy	average +/- sd (p-value)
RD27	3.66E-07		2.62E-08		6.21E-08		3.28E-10	
	4.56E-07	4.97E-07 +/-	1.32E-08	2.32E-08 +/-	1.32E-07	1.74E-07 +/-	2.63E-10	3.61E-10 +/-
	5.05E-07	1.47E-07	5.26E-08	1.79E-08	1.29E-07	1.48E-07	4.39E-10	7.39E-11
	7.43E-07	(<0.01)	1.72E-08		4.34E-07	(<0.01)	4.31E-10	
	4.15E-07		6.90E-09		1.12E-07		3.45E-10	
DD34	4.85E-07		1.36E-08		1.29E-07		2.71E-10	
	3.51E-07	3.98E-07 +/-	1.01E-08	1.14E-08 +/-	1.70E-07	2.15E-07 +/-	2.53E-10	2.96E-09 +/-
	4.31E-07	6.87E-08	4.74E-09	4.50E-09	5.38E-07	1.96E-07	7.11E-09	3.63E-09
	4.11E-07	(<0.01)	1.69E-08		2.24E-07	(<0.01)	6.76E-09	
	3.10E-07		1.19E-08		1.63E-08		3.97E-10	
DD36	1.13E-05		7.96E-07		6.71E-06		3.12E-07	
	7.16E-06	1.99E-05 +/-	5.98E-07	6.11E-07 +/-	5.39E-06	4.68E-06 +/-	1.86E-07	1.99E-07 +/-
	6.79E-05	2.70E-05	5.70E-07	1.07E-07	3.49E-06	1.76E-06	9.00E-08	1.04E-07
	3.95E-06	(<0.01)	5.27E-07		2.30E-06	(<0.01)	3.01E-07	
	8.98E-06		5.62E-07		5.52E-06		1.07E-07	
DD37	4.69E-07		1.63E-08		8.97E-08		2.72E-10	
	1.37E-06	1.29E-06 +/-	4.03E-09	8.43E-09 +/-	3.17E-08	5.47E-08 +/-	4.03E-10	3.51E-10 +/-
	3.40E-06	1.24E-06	1.20E-08	5.50E-09	1.04E-07	3.89E-08	3.98E-10	5.87E-11
	4.30E-07	(<0.01)	3.75E-09		2.65E-08	(<0.01)	3.75E-10	
	7.61E-07		6.13E-09		2.17E-08		3.07E-10	
DD38	4.54E-07		8.38E-09		3.21E-08		2.79E-10	
	7.64E-07	8.04E-07 +/-	1.60E-08	1.77E-08 +/-	1.39E-08	1.81E-08 +/-	2.67E-09	7.55E-10 +/-
	1.57E-06	4.44E-07	3.36E-08	1.05E-08	1.70E-08	8.53E-09	2.58E-10	1.07E-09
	6.76E-07	(<0.01)	8.72E-09		1.80E-08	(<0.01)	2.91E-10	
	5.57E-07		2.17E-08		9.43E-09		2.71E-10	

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38 **Supplementary Table S4:**

39 Genome sequencing and analysis: identification of the beta-lactamases genes, sequence types and quality of the sequencing data illustrated by
 40 the mean N50 (bp), mean coverage and number of contigs.

Isolates	Genome_size	Number of contigs	n50	Coverage	Species	m1st	beta-lactamases genes
RD26	5020989	75	302644	101	<i>E. cloacae</i>	-	blaKPC-3,blaOXA-9,blaTEM-1A
RD29	4980967	90	309405	84	<i>Escherichia coli</i>	ST-131	blaKPC-2,blaTEM-1B
RD27	5542544	88	248804	80	<i>Klebsiella pneumoniae</i>	ST-512	blaKPC-3,blaOXA-9,blaSHV-11,blaTEM-1A
DD34	5619986	120	233237	72	<i>Klebsiella pneumoniae</i>	ST-258	blaKPC-2,blaOXA-9,blaSHV-12,blaTEM-1A
DD36	5747097	153	186314	75	<i>Klebsiella pneumoniae</i>	ST-11	blaKPC-2,blaSHV-11,blaTEM-1B
DD37	5710690	148	342058	75	<i>Klebsiella pneumoniae</i>	ST-101	blaKPC-2,blaOXA-1,blaOXA-9,blaSHV-1,blaTEM-1A
DD38	5579702	96	236649	106	<i>Klebsiella pneumoniae</i>	ST-307	blaKPC-3,blaOXA-9,blaSHV-28,blaTEM-1A

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44 **Supplementary Table S5:**

45 Relative quantification of *bla*_{KPC} expression using transcription analysis by RT-qPCR of recombinant strains (*E. coli* Top10 with pBR322 harboring
46 either wild-type allele (KPC-3) or alleles conferring ceftazidime-avibactam resistance. *dinB* a chromosomal housekeeping gene and *tetA* a
47 pBR322 plasmid gene (coding for tetracyclin resistance) were used for normalization.

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Isolates	Replicates	Ct* <i>blaKPC</i>	mean Ct* KPC	Ct* <i>dinB</i>	mean Ct* <i>dinB</i>	Ct* <i>tetA</i>	mean Ct* <i>tetA</i>	Ct* <i>blaKPC</i> - Ct* chromosomal internal control	Ct* <i>blaKPC</i> - Ct* plasmidic internal control	delta delta Ct* chromosomal control	delta delta Ct* plasmidic control	Fold change compared to RD26 (chromosomal control)	Fold change compared to RD26 (plasmidic control)
Top10-pBR322- RD26 (KPC-3)	1	16.74		29.9		19.8							
	2	16.84	16.77	29.47	29.4	19.82	19.8	-12.6	-3.04	-	-	-	-
	3	16.73		28.71		19.81							
Top10-pBR322- RD26-7	1	16.5		29.18		19.94							
	2	16.72	16.61	29.2	29.2	19.91	19.9	-12.6	-3.3	0.0	-0.3	1.0	1.2
	3	16.6		29.27		19.85							
Top10-pBR322- RD26-1	1	17		29.8		20.48							
	2	17.01	17,0	29.72	29.7	20.43	20.4	-12.7	-3.4	-0.1	-0.4	1.1	1.3
	3	16.95		29.61		20.33							
Top10-pBR322- RD26-3	1	16.68		29.62		20.11							
	2	16.77	16.76	29.82	29.7	20.04	20.0	-13.0	-3.3	-0.4	-0.2	1.3	1.2
	3	16.81		29.68		19.93							
Top10-pBR322- RD26-6	1	16.78		29.49		20.19							
	2	16.89	16.8	29.62	29.6	20.07	20.1	-12.7	-3.3	-0.1	-0.2	1.1	1.2
	3	16.85		29.55		20.07							
Top10-pBR322- RD26-8	1	16.59		29.58		19.87							
	2	16.6	16.6	29.67	29.6	19.98	19.9	-13.0	-3.3	-0.4	-0.3	1.3	1.2
	3	16.55		29.59		19.79							
Top10-pBR322- RD26-5	1	15.8		28.12		18.24							
	2	15.86	15.8	28.17	28.1	18.26	18.3	-12.2	-2.4	0.4	0.6	0.8	0.7
	3	15.88		27.94		18.31							
Top10-pBR322- RD26-9	1	16.7		29.91		20							
	2	16.72	16.7	29.68	29.7	19.98	20.0	-13.0	-3.3	-0.4	-0.2	1.3	1.2
	3	16.74		29.53		20.03							
negative control	1	39.57	-	38.62	-	32.87	-	-	-	-	-	-	-

* Ct Cycle threshold

dinB chromosomal gene used as control

tetA plasmid gene used as control

