

Supporting Information for

Assessment of activity and resistance mechanisms to cefepime in combination with the novel β -lactamase inhibitors zidebactam, taniborbactam, and enmetazobactam against a multicenter collection of carbapenemase-producing Enterobacterales

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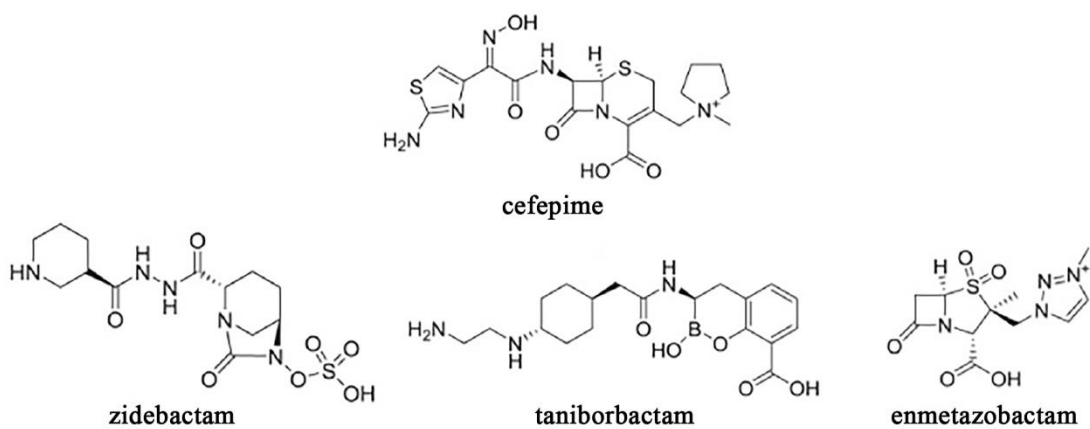


Figure S1. Structure of cephalosporin cefepime and β -lactamase inhibitors zidebactam (diazabicyclooctane), taniborbactam (boronate) and enmetazobactam (penicillanic acid sulfone).

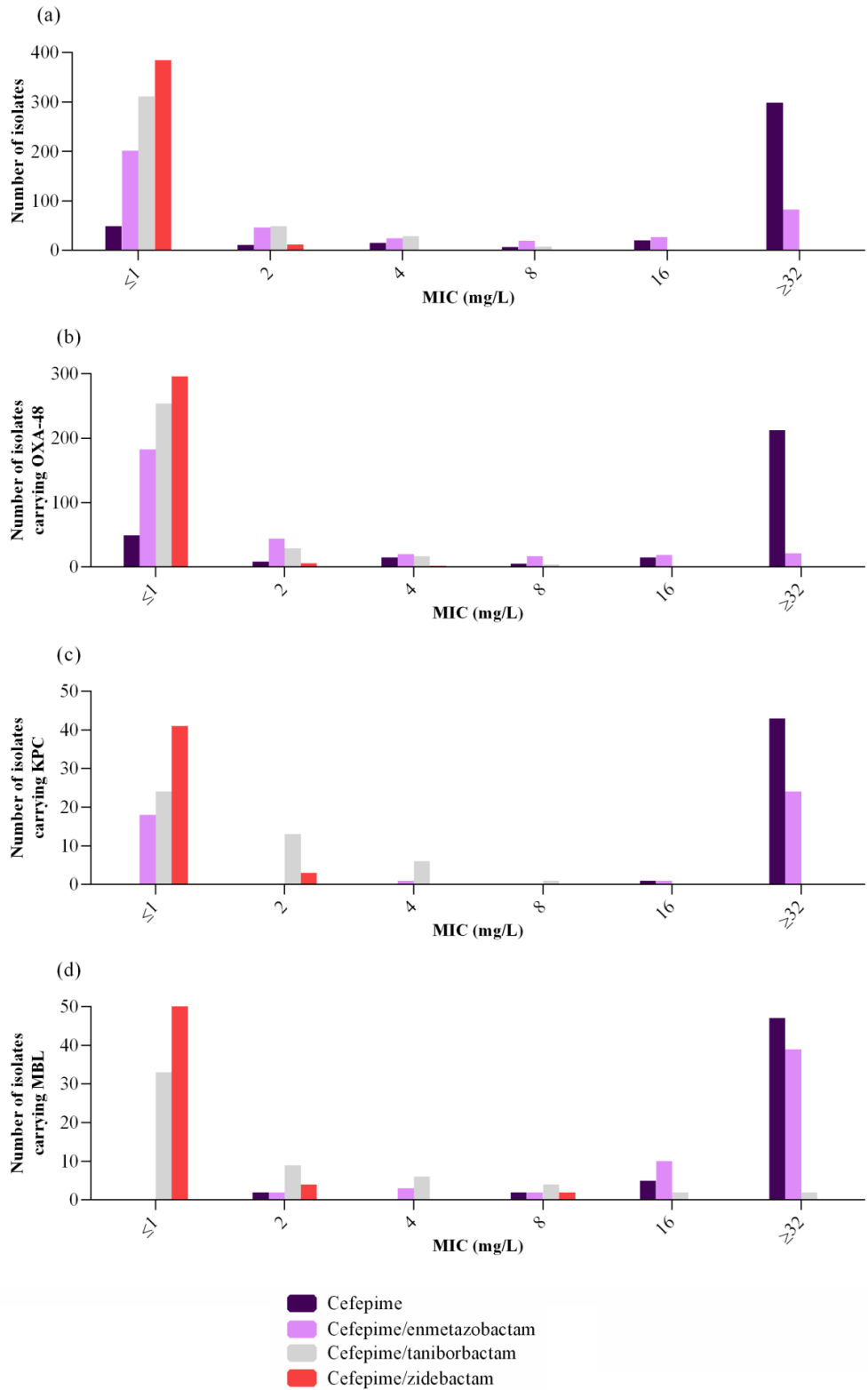


Figure S2. Distribution of MIC values for cefepime and cefepime/BLI combinations: cefepime/enmetazobactam, cefepime/taniborbactam and cefepime/zidebactam among a) the whole set of isolates of Enterobacterales (n=400), b) OXA-48-producing isolates (n=304), c) KPC-producing isolates (n=44) and d) MBL-producing isolates (n=56).

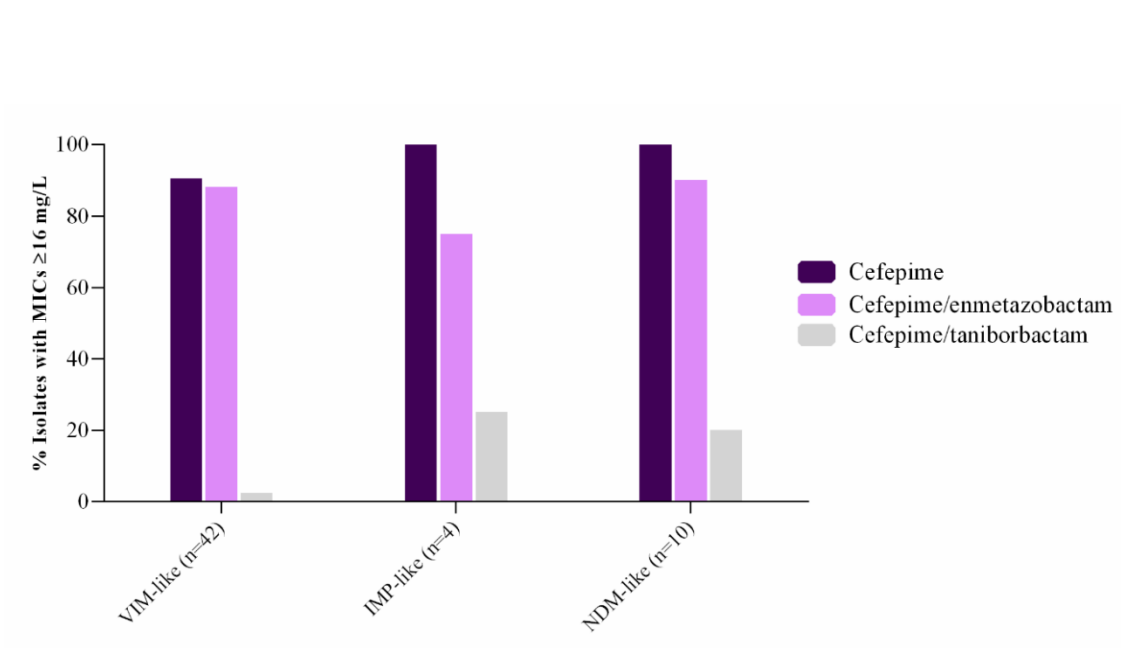


Figure S3. Rates of VIM-, IMP- and NDM-producing (MBLs) Enterobacteriales showing MIC ≥ 16 mg/L for cefepime and cefepime/BLIs (none of the strains showed MIC ≥ 16 mg/L for cefepime/zidebactam).

AN2350	<i>K. pneumoniae</i>	15	Urine	Hospital Universitario Lucus Augusti (Lugo)	OXA-48	≤0.5	≤0.5	≤0.5	≤0.5	≥128	128	0.25	0.5	S	S	S	S	R	S	S	SHV-28				
AN2351	<i>C. koseri</i>	0	Anal-rectal	Hospital Universitario Lucus Augusti (Lugo)	OXA-48	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≥256	0.5	0.5	S	S	S	S	R	S	S	TEM-1				
AN2352	<i>K. pneumoniae</i>	147	Sputum	Hospital Universitario Lucus Augusti (Lugo)	OXA-48	≥128	32	4	≤0.5	≤0.5	≥256	16	2	R	R	I	S	R	R	I	SHV-11	(2x)CTX-M-15			
AN2353	<i>C. koseri</i>	0	Anal-rectal	Hospital Universitario Lucus Augusti (Lugo)	OXA-48	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≥256	0.25	0.5	S	S	S	S	R	S	S					
AN2355	<i>K. pneumoniae</i>	307	Anal-rectal	Hospital Universitario Lucus Augusti (Lugo)	OXA-48	≥128	≤0.5	1	≤0.5	≤0.5	≥256	0.25	0.5	R	S	S	S	R	S	S	SHV-28	(5x)CTX-M-15	OXA-1	TEM-1	
AN2356	<i>K. pneumoniae</i>	15	Urine	Hospital Universitario Lucus Augusti (Lugo)	OXA-48	2	2	≤0.5	≤0.5	≤0.5	≥256	0.5	0.5	S	S	S	S	R	S	S	SHV-28				
AN2357	<i>K. pneumoniae</i>	15	Urine	Hospital Universitario Lucus Augusti (Lugo)	OXA-48	≥128	16	2	≤0.5	≤0.5	≥256	32	16	R	R	S	S	R	R	R	SHV-28	OXA-1	CTX-M-15		
AN2358	<i>K. pneumoniae</i>	15	Urine	Hospital Universitario Lucus Augusti (Lugo)	OXA-48	≥128	1	2	≤0.5	≤0.5	≥256	1	0.5	R	S	S	S	R	S	S	SHV-28	OXA-1	CTX-M-15	TEM-1	
AN2360	<i>E. cloacae</i> complex	93	Abscess	Hospital Arquitecto Marcide (Ferrol)	VIM-1	≥128	64	1	≤0.5	≤0.5	128	0.5	2	R	R	S	S	R	S	I	ACT-17	(2x)ACC-1	OXA-1		
AN2361	<i>K. pneumoniae</i>	15	Urine	Hospital Arquitecto Marcide (Ferrol)	OXA-48	≥128	16	1	≤0.5	≤0.5	128	1	1	R	R	S	S	R	S	S	SHV-28	OXA-1	CTX-M-15	TEM-1	
AN2362	<i>K. pneumoniae</i>	11	Anal-rectal	Hospital Arquitecto Marcide (Ferrol)	OXA-48	1	≤0.5	≤0.5	≤0.5	≤0.5	≥256	2	0.5	S	S	S	S	R	I	S	SHV-11	OXA-1			
AN2363	<i>K. oxytoca</i>	328	Anal-rectal	Hospital Arquitecto Marcide (Ferrol)	OXA-48	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≥256	0.25	0.5	S	S	S	S	R	S	S	OXY-2-7				
AN2364	<i>E. cloacae</i> complex	168	Urine	Hospital Arquitecto Marcide (Ferrol)	OXA-48	2	1	≤0.5	≤0.5	≤0.5	≥256	1	≤0.25	S	S	S	S	R	S	S	ACT-23	DHA-1			

Table S2. Bacterial species, sequence type, susceptibilities to cefepime and cefepime/enmetazobactam of KPC-producing Enterobacterales.

Genome no.	Species	ST	MIC (mg/L)		Carbapenemase	Other β -lactamases		Porins	PBP _s	Transcription regulators AcrAB-ToIC
			cefepime	cefepime/enmetazob.		ESBL β -lactamases	No ESBL β -lactamases			
AI2588	<i>K. pneumoniae</i>	512	32	$\leq 0,5$	KPC-3	TEM-1, SHV-11	OmpK35 NF (E42fs)	-	-	
AI2627	<i>K. pneumoniae</i>	512	≥ 128	≥ 128	KPC-3	TEM-1, SHV-11	OmpK35 NF (E42fs) OmpK36 (G134_D135insGD)	-	-	
AI2628	<i>K. pneumoniae</i>	512	≥ 128	≥ 128	KPC-3	TEM-1, SHV-11	OmpK35 NF (E42fs) OmpK36 (G134_D135insGD)	-	-	
AI2629	<i>K. pneumoniae</i>	512	≥ 128	≥ 128	KPC-3	TEM-1, SHV-11	OmpK35 NF (E42fs) OmpK36 (G134_D135insGD)	-	-	
AI2631	<i>K. pneumoniae</i>	512	≥ 128	≥ 128	KPC-3	TEM-1, SHV-11	OmpK35 NF (E42fs) OmpK36 (G134_D135insGD)	-	-	
AI2632	<i>K. pneumoniae</i>	512	≥ 128	≥ 128	KPC-3	TEM-1, SHV-11	OmpK35 NF (E42fs) OmpK36 (G134_D135insGD)	-	-	
AI2805	<i>K. pneumoniae</i>	512	≥ 128	≥ 128	KPC-3	TEM-1, (2x)SHV-11	OmpK35 NF (E42fs) OmpK36 (G134_D135insGD)	-	-	
AI2806	<i>K. pneumoniae</i>	512	≥ 128	≥ 128	KPC-3	TEM-1, (2x)SHV-11	OmpK35 NF (E42fs) OmpK36 (G134_D135insGD)	-	-	
AI2807	<i>K. pneumoniae</i>	512	≥ 128	≥ 128	KPC-3	TEM-1, SHV-11	OmpK35 NF (E42fs) OmpK36 (G134_D135insGD)	-	-	
AI2808	<i>K. pneumoniae</i>	512	≥ 128	≥ 128	KPC-3	TEM-1, (2x)SHV-11	OmpK35 NF (E42fs) OmpK36 (G134_D135insGD)	-	-	
AI2809	<i>K. pneumoniae</i>	512	≥ 128	≥ 128	KPC-3	TEM-1, (2x)SHV-11	OmpK35 NF (E42fs) OmpK36 (G134_D135insGD)	-	-	
AI2810	<i>K. pneumoniae</i>	512	≥ 128	≥ 128	KPC-3	TEM-1, (2x)SHV-11	OmpK35 NF (E42fs) OmpK36 (G134_D135insGD)	-	-	
AI2811	<i>K. pneumoniae</i>	512	≥ 128	≥ 128	KPC-3	TEM-1, (2x)SHV-11	OmpK35 NF (E42fs) OmpK36 (G134_D135insGD)	-	-	
AI2812	<i>K. pneumoniae</i>	512	≥ 128	≥ 128	KPC-3	TEM-1, (2x)SHV-11	OmpK35 NF (E42fs) OmpK36 (G134_D135insGD)	-	-	
AI2813	<i>K. pneumoniae</i>	512	≥ 128	≥ 128	KPC-3	TEM-1, (2x)SHV-11	OmpK35 NF (E42fs) OmpK36 (G134_D135insGD)	-	-	
AI2814	<i>K. pneumoniae</i>	512	≥ 128	≥ 128	KPC-3	SHV-11	OmpK35 NF (E42fs) OmpK36 (G134_D135insGD)	-	-	
AI2815	<i>K. pneumoniae</i>	512	≥ 128	≥ 128	KPC-3	SHV-11	OmpK35 NF (E42fs) OmpK36 (G134_D135insGD)	-	-	
AI2816	<i>K. pneumoniae</i>	512	≥ 128	≥ 128	KPC-3	TEM-1, (2x)SHV-11	OmpK35 NF (E42fs) OmpK36 (G134_D135insGD)	-	-	
AI2817	<i>K. pneumoniae</i>	512	≥ 128	≥ 128	KPC-3	TEM-1, SHV-11	OmpK35 NF (E42fs) OmpK36 (G134_D135insGD)	-	-	
AI2835	<i>K. pneumoniae</i>	512	≥ 128	≥ 128	KPC-3	TEM-1, SHV-11	OmpK35 NF (E42fs) OmpK36 (G134_D135insGD)	-	-	
AI2836	<i>K. pneumoniae</i>	512	≥ 128	≥ 128	KPC-3	TEM-1, SHV-11	OmpK35 NF (E42fs) OmpK36 (G134_D135insGD)	-	-	
AI2837	<i>K. pneumoniae</i>	512	≥ 128	≥ 128	KPC-3	TEM-1, SHV-11	OmpK35 NF (E42fs) OmpK36 (G134_D135insGD)	-	-	
AI2940	<i>K. pneumoniae</i>	258	≥ 128	≥ 128	KPC-3	TEM-1, SHV-11	OmpK35 NF (E42fs)	-	-	
AI2643	<i>K. pneumoniae</i>	307	16	$\leq 0,5$	KPC-3	SHV-28	OXA-1	-	-	
AI2644	<i>K. pneumoniae</i>	307	≥ 128	$\leq 0,5$	KPC-3	SHV-28, CTX-M-15	OXA-1, (2x)TEM-1	-	-	
AI2645	<i>K. pneumoniae</i>	307	64	$\leq 0,5$	KPC-3	SHV-28, CTX-M-15	OXA-1, TEM-1	-	-	

AI2646	<i>K. pneumoniae</i>	307	64	≤0,5	KPC-3	CTX-M-15, SHV-28	OXA-1, (2x)TEM-1	-	-	-
AI2647	<i>K. pneumoniae</i>	307	64	≤0,5	KPC-3	CTX-M-15, SHV-28	OXA-1, (2x)TEM-1	-	-	-
AI2648	<i>K. pneumoniae</i>	307	≥128	≤0,5	KPC-3	CTX-M-15, SHV-28	OXA-1, (2x)TEM-1	-	-	-
AI2649	<i>K. pneumoniae</i>	307	≥128	1	KPC-3	(2x)CTX- M-15, SHV-28	OXA-1, TEM-1	-	-	-
AI2650	<i>K. pneumoniae</i>	307	64	≤0,5	KPC-3	CTX-M-15, SHV-28	OXA-1, (2x)TEM-1	-	-	-
AI2651	<i>K. pneumoniae</i>	459	32	≤0,5	KPC-3		SHV-26, (2x)TEM-30	-	PBP-3 (P372R)	AcrR NF (Y194*)
AI2652	<i>K. pneumoniae</i>	307	32	≤0,5	KPC-3	CTX-M-15, SHV-28	OXA-1, (2x)TEM-1	-	-	-
AI2653	<i>K. pneumoniae</i>	307	32	≤0,5	KPC-3	CTX-M-15, SHV-28	OXA-1, (2x)TEM-1	-	-	-
AI2654	<i>K. pneumoniae</i>	307	32	≤0,5	KPC-3	CTX-M-15, SHV-28	OXA-1, TEM-1	-	-	-
AI2655	<i>K. pneumoniae</i>	307	32	≤0,5	KPC-3	CTX-M-15, SHV-28	OXA-1, (2x)TEM-1	-	-	-
AI2883	<i>K. pneumoniae</i>	307	64	1	KPC-3	SHV-28	OXA-1, TEM-1	-	-	-
AI2888	<i>K. pneumoniae</i>	15	64	≤0,5	OXA- 48+KPC	CTX-M-15, SHV-28		-	-	-
AI2889	<i>K. pneumoniae</i>	307	32	≤0,5	KPC-3	CTX-M-15, SHV-28	OXA-1, (2x)TEM-1	-	-	-
AI2901	<i>K. pneumoniae</i>	307	32	≤0,5	KPC-3	SHV-28	(2x)TEM-1	-	-	-
AI2602	<i>C. freundii</i>	112	≥128	≥128	KPC-2		CMY-like, OXA-1	OmpK35 NF (Q174*)	PBP3 (A310V)	-
AI2614	<i>K. oxytoca</i>	324	32	4	KPC-2	CTX-M-9, OXY-1-like	SHV-12	-	-	-
AI2826	<i>C. freundii</i>	22	≥128	64	KPC-2		CMY-48	OmpK35 NF (A49fs)	-	-

(2x): indicates that the strain has 2 copies of the gene coding for the specified β-lactamase. NF: indicates non-functional; asterisk (*): indicates premature stop codon; fs: indicates frameshifts

Table S3. Bacterial species, sequence type, susceptibilities to cefepime and cefepime/enmetazobactam of MBL-producing Enterobacterales showing low MIC values for cefepime/enmetazobactam (MIC <16mg/L).

Genome no.	Species	MLST	MIC (mg/L)		Carbapenemase	Other β -lactamases		Porins	PBPs	Transcription regulators AcrAB-TolC
			Cefepime	Cefepime-enmetaz.		ESBL β -lactamases	Non-ESBL β -lactamases			
AI2830	<i>K. pneumoniae</i>	147	16	4	NDM-1	CTX-M-15	SHV-11	-	-	-
AI2885	<i>K. pneumoniae</i>	307	32	4	VIM-1	CTX-M-15, SHV-28	TEM-1	-	-	RamR (I88N, A110E)

Table S4. Bacterial species, sequence type, susceptibilities to cefepime and cefepime/enmetazobactam of OXA-48-producing Enterobacterales showing high MIC values for cefepime/enmetazobactam (MIC \geq 16mg/L).

Genome no.	Species	ST	MIC (mg/L)		Carbapenemase	Other β -lactamases		Porins	PBPs	Transcription regulators AcrAB-TolC
			cefepime	cefepime/ enmetazob.		ESBL β - lactamases	No ESBL β - lactamases			
AH0326	<i>K. pneumoniae</i>	392	\geq 128	32	OXA-48	(2x)CTX-M-15	TEM-1, SHV-11	-	-	-
AI2585	<i>K. pneumoniae</i>	405	\geq 128	32	OXA-48	SHV-76, CTX-M-15	OXA-1, TEM-1	-	-	-
AI2600	<i>K. pneumoniae</i>	405	\geq 128	64	OXA-48	SHV-76, (4x)CTX-M-15	OXA-1, TEM-1	OmpK36 (L9M)	-	-
AI2624	<i>E. cloacae</i> complex	114	32	32	OXA-48		ACT-like	-	-	-
AI2638	<i>K. pneumoniae</i>	307	64	32	OXA-48	SHV-28, CTX-M-15	TEM-1	OmpK35 NF (Y117*)	-	RamR (P159A)
AI2662	<i>E. cloacae</i> complex	171	\geq 128	32	OXA-48	CTX-M-9, (2x)CTX-M-15	(2x)OXA-1, ACT-25	-	-	-
AI2668	<i>C. freundii</i>	18	32	16	OXA-48	CTX-M-9, SHV-12	CMY-117, TEM-1	-	-	-
AI2739	<i>K. pneumoniae</i>	11	\geq 128	32	OXA-48	(2x)CTX-M-15	OXA-1, SHV-11, TEM-1	-	-	RamR NF (D75fs)
AI2756	<i>K. pneumoniae</i>	11	\geq 128	64	OXA-48	(3x)CTX-M-15	SHV-11	-	-	RamR NF (D75fs)
AI2761	<i>K. pneumoniae</i>	5000	\geq 128	16	OXA-48	(2x)CTX-M-15	OXA-1, TEM-1, SHV-11	-	-	RamR NF (D75fs)
AI2765	<i>K. pneumoniae</i>	147	\geq 128	64	OXA-48	(2x)CTX-M-15	SHV-11	OmpK36 (G134_D135insGD)	-	-
AI2773	<i>K. pneumoniae</i>	307	\geq 128	16	OXA-48	SHV-28, CTX-M-15	DHA-like, TEM-1	-	-	-
AI2829	<i>K. pneumoniae</i>	15	\geq 128	16	OXA-48	SHV-28, CTX-M-15	OXA-1	-	-	-
AI2838	<i>K. pneumoniae</i>	307	\geq 128	\geq 128	OXA-48	SHV-28, CTX-M-15		-	-	-
AI2847	<i>K. pneumoniae</i>	307	\geq 128	\geq 128	OXA-48	SHV-28, CTX-M-15	TEM-1	-	-	-
AI2849	<i>K. pneumoniae</i>	147	\geq 128	16	OXA-48	(2x)CTX-M-15	SHV-11	OmpK36 (G134_D135insGD)	-	-
AI2855	<i>K. pneumoniae</i>	147	\geq 128	\geq 128	OXA-48	(2x)CTX-M-15	SHV-11	-	-	-
AI2856	<i>K. pneumoniae</i>	147	\geq 128	16	OXA-48	(2x)CTX-M-15	SHV-11	-	-	-
AI2862	<i>K. pneumoniae</i>	405	\geq 128	32	OXA-48	(4x)CTX-M-15	SHV-76, (2x)OXA-1, TEM-1	-	-	-
AI2871	<i>K. pneumoniae</i>	11	\geq 128	16	OXA-48	(4x)CTX-M-15	OXA-1, SHV-11	-	PBP-2 (M522V)	RamR NF (D75fs)
AI2873	<i>K. pneumoniae</i>	11	\geq 128	32	OXA-48	(3x)CTX-M-15	OXA-1, SHV-11	-	PBP-2 (M522V)	RamR NF (D75fs)
AI2876	<i>K. pneumoniae</i>	11	\geq 128	32	OXA-48	(3x)CTX-M-15	SHV-11	OmpK35 NF (L103fs)	-	RamR NF (D75fs)
AI2877	<i>K. pneumoniae</i>	11	\geq 128	16	OXA-48	(3x)CTX-M-15	OXA-1, SHV-11	OmpK36 (AL233-234GT)	PBP-2 (M522V)	RamR NF (D75fs)
AI2913	<i>K. aerogenes</i>	57	16	16	OXA-48		(4x)OXA-1, EC-like, TEM-1	OmpK36 (M70T, T45S)	-	-
AI2914	<i>K. pneumoniae</i>	11	\geq 128	16	OXA-48	(2x)CTX-M-15	SHV-11	OmpK36 (L9Q, W94_T95del/insA)	-	RamR NF (D75fs)

AI2917	<i>K. pneumoniae</i>	392	≥128	≥128	OXA-48	CTX-M-15	OXA-1, TEM-1, SHV-11	-	-	-
AI2935	<i>C. freundii</i>	112	≥128	32	OXA-48	CTX-M-9-like	CMY-75	OmpK36 NF (D181_F187del/insGM)	PBP2 (A438S, F489L) PBP3 (A462S)	AcrR NF (S219*)
AI2950	<i>E. cloacae</i> complex	66	16	16	OXA-48	CTX-M-15	OXA-1, TEM-1, ACT-like-like	-	-	-
AI2953	<i>K. pneumoniae</i>	307	≥128	16	OXA-48	SHV-28, CTX-M-15	OXA-1, TEM-1	-	-	-
AI2954	<i>K. pneumoniae</i>	11	≥128	≥128	OXA-48	(2x)CTX-M-15	OXA-1, SHV-11	OmpK36 (Q313K)	-	RamR NF (D75fs)
AI2962	<i>K. pneumoniae</i>	307	≥128	16	OXA-48	SHV-28, CTX-M-15	OXA-1, TEM-1	OmpK35 (E269*) OmpK36 (W125*)	-	-
AI3039	<i>K. pneumoniae</i>	307	≥128	16	OXA-48	SHV-28, CTX-M-15	OXA-1, TEM-1	OmpK36 (G134_D135insGD)	-	-
AI3062	<i>K. pneumoniae</i>	5000	≥128	32	OXA-48	(2x)CTX-M-15	OXA-1, TEM-1, SHV-11	-	-	-
AN2342	<i>K. pneumoniae</i>	147	≥128	16	OXA-48	CTX-M-15	SHV-11	-	-	-
AN2352	<i>K. pneumoniae</i>	147	≥128	32	OXA-48	(2x)CTX-M-15	SHV-11	OmpK36 (G134_D135insGD)	-	-
AN2357	<i>K. pneumoniae</i>	15	≥128	16	OXA-48	SHV-28, CTX-M-15	OXA-1	-	-	-
AN2361	<i>K. pneumoniae</i>	15	≥128	16	OXA-48	CTX-M-15	OXA-1, SHV-28, TEM-1	-	-	-

(2x), (3x) and (4x): indicate that the strain has 2, 3 or 4 copies of the gene coding for the specified β -lactamase, respectively. NF: indicates non-functional; asterisk (*): indicates premature stop codon; fs: indicates frameshifts.