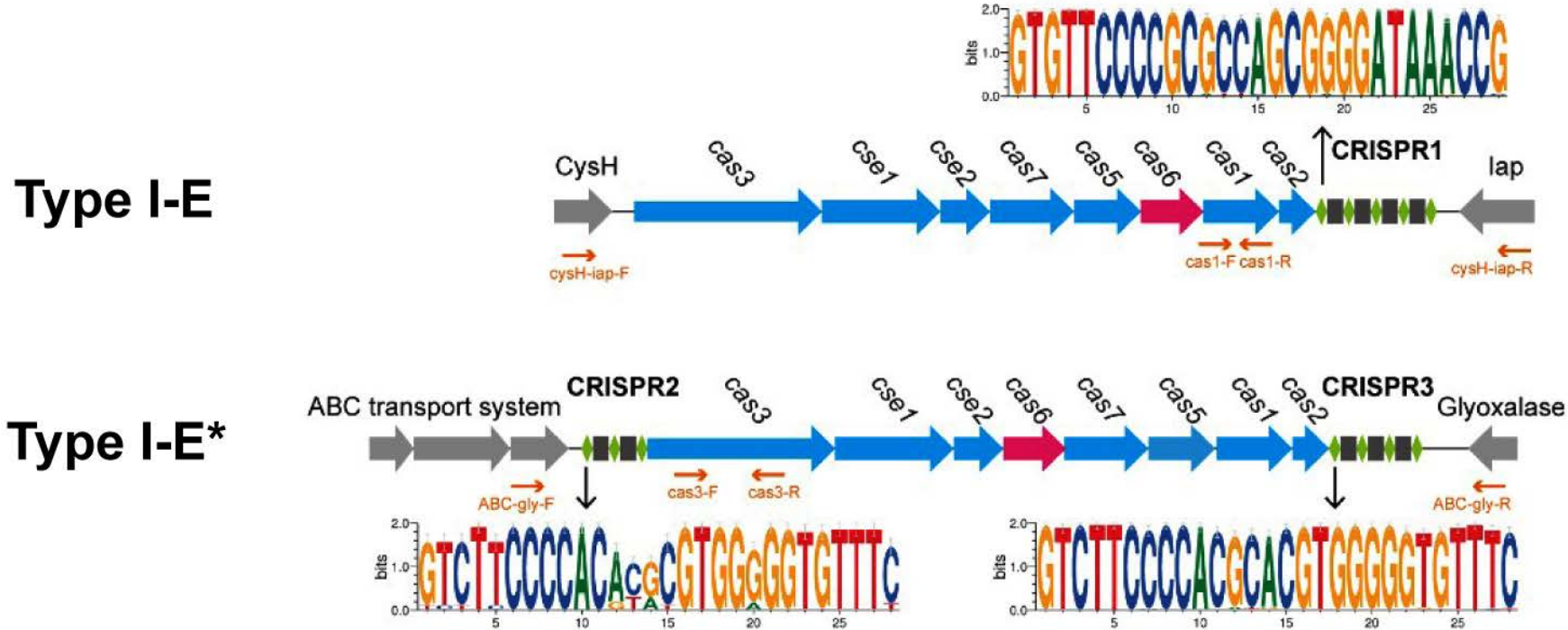
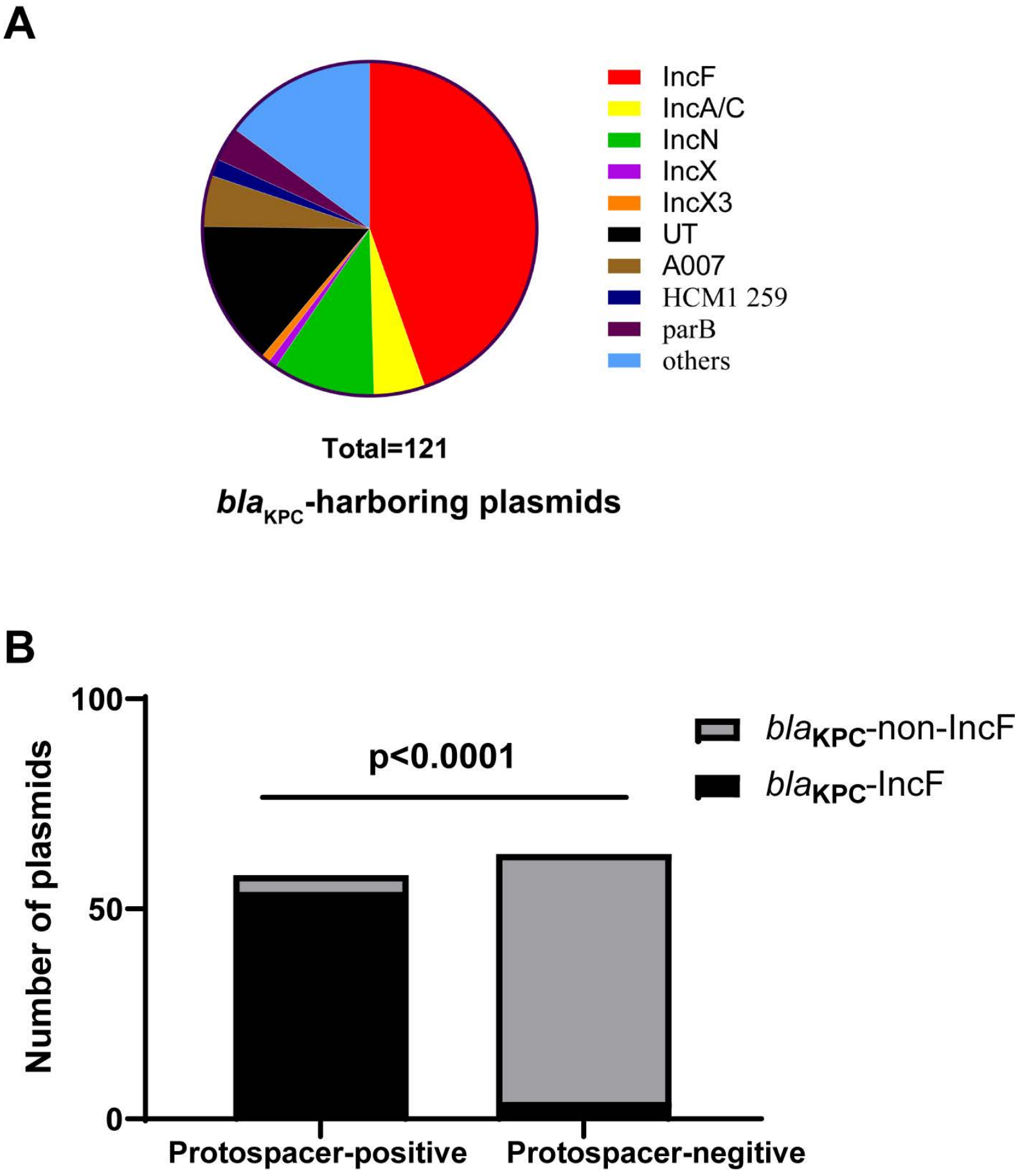
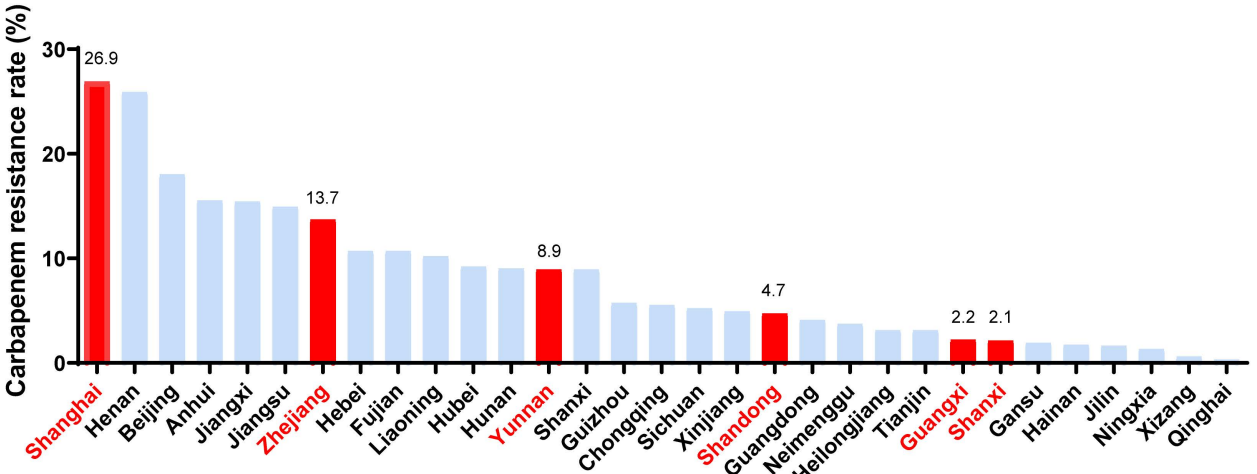
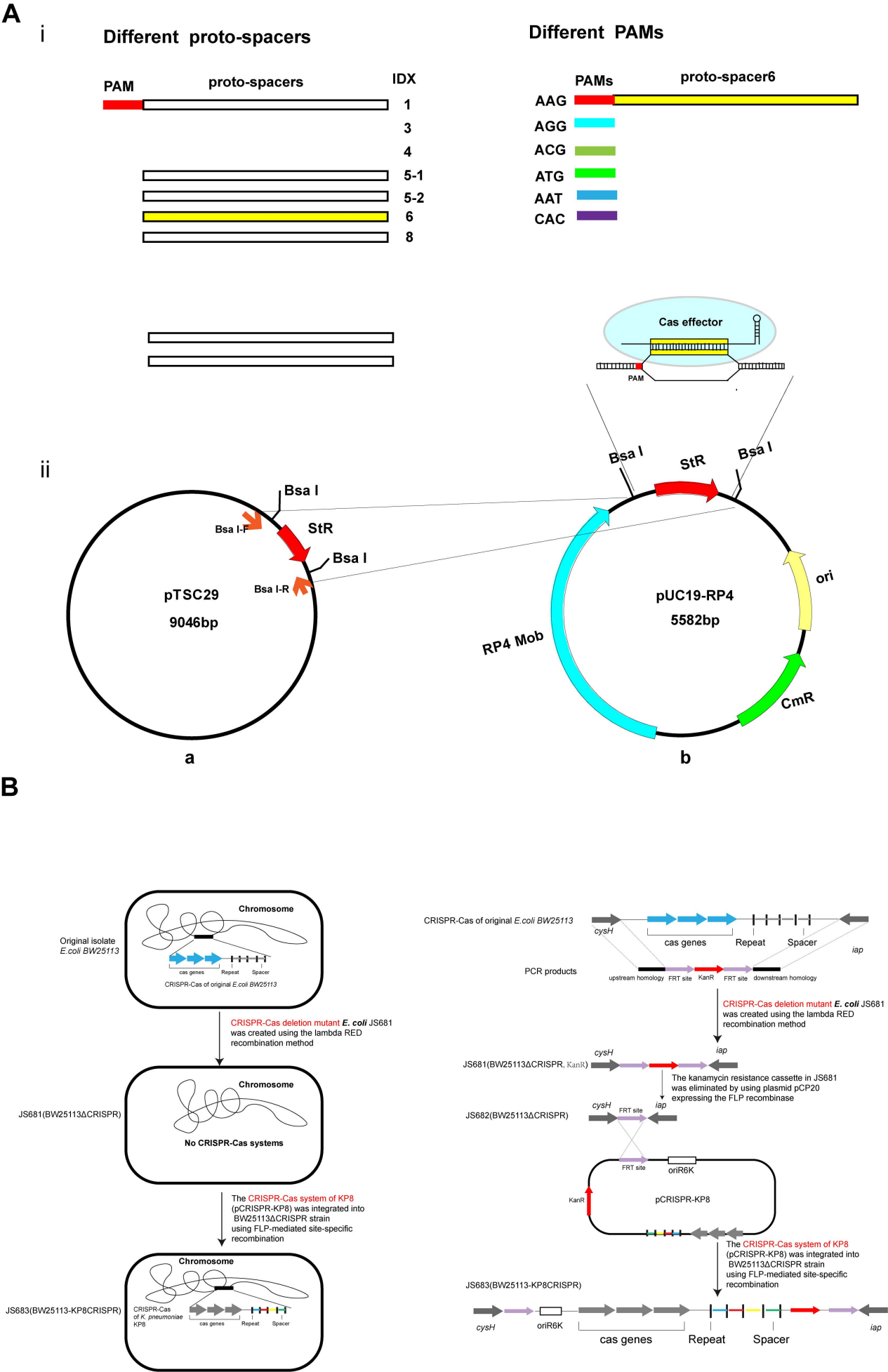

Type I-E
CysH
cas3
cse1
cse2
cas7
cas5
cas6
cas1
cas2
CRISPR1
Iap
cysH-iap-F
cas1-F cas1-R
cysH-iap-R
Type I-E*
ABC transport system
CRISPR2
cas3
cse1
cse2
cas6
cas7
cas5
cas1
cas2
CRISPR3
Glyoxalase
ABC-gly-F
cas3-F
cas3-R
ABC-gly-R
bits


A
IncF
IncA/C
IncN
IncX
IncX3
UT
A007
HCM1 259
parB
others
Total=121
bla KPC-harboring plasmids
B
100
50
0
Number of plasmids
p<0.0001
bla KPC-non-IncF
bla KPC-IncF
Protospacer-positive
Protospacer-negitive


Carbapenem resistance rate (%)
30
20
10
0
26.9
13.7
8.9
4.7
2.2
2.1
Shanghai
Henan
Beijing
Anhui
Jiangxi
Jiangsu
Zhejiang
Hebei
Fujian
Liaoning
Hubei
Hunan
Yunnan
Shanxi
Guizhou
Chongqing
Sichuan
Xinjiang
Shandong
Guangdong
Neimenggu
Heilongjiang
Tianjin
Guangxi
Shanxi
Gansu
Hainan
Jilin
Ningxia
Xizang
Qinghai


A
i
Different proto-spacers
PAM
proto-spacers
IDX
1
3
4
5-1
5-2
6
8
Different PAMs
PAMs
proto-spacer6
AAG
AGG
ACG
ATG
AAT
CAC
Cas effector
PAM
ii
Bsa I
Bsa I-F
StR
Bsa I
Bsa I-R
pTSC29
9046bp
a
Bsa I
StR
Bsa I
ori
pUC19-RP4
5582bp
RP4 Mob
CmR
b
B
Original isolate
E.coli BW25113
Chromosome
cas genes
Repeat
Spacer
CRISPR-Cas of original E.coli BW25113
CRISPR-Cas deletion mutant E. coli JS681
was created using the lambda RED
recombination method
JS681(BW25113ΔCRISPR)
Chromosome
No CRISPR-Cas systems
The CRISPR-Cas system of KP8
(pCRISPR-KP8) was integrated into
BW25113ΔCRISPR strain
using FLP-mediated site-specific
recombination
JS683(BW25113-KP8CRISPR)
Chromosome
CRISPR-Cas
of K. pneumoniae
KP8
cas genes
Repeat
Spacer
CRISPR-Cas of original E.coli BW25113
cysH
cas genes
Repeat
Spacer
iap
PCR products
upstream homology
FRT site
KanR
FRT site
downstream homology
CRISPR-Cas deletion mutant E. coli JS681
was created using the lambda RED
recombination method
JS681(BW25113ΔCRISPR, KanR)
cysH
iap
The kanamycin resistance cassette in JS681
was eliminated by using plasmid pCP20
expressing the FLP recombinase
JS682(BW25113ΔCRISPR)
cysH
FRT site
iap
FRT site
oriR6K
KanR
pCRISPR-KP8
The CRISPR-Cas system of KP8
(pCRISPR-KP8) was integrated into
BW25113ΔCRISPR strain
using FLP-mediated site-specific
recombination
JS683(BW25113-KP8CRISPR)
cysH
oriR6K
cas genes
Repeat
Spacer
iap

Supplementary data 1a. 203 completely sequenced *K. pneumoniae* strains used in this study.

* The '-'symbol indicates the absence of the CRISPR-Cas locus

On the basis of different layouts, the Type I-E CRISPR were furtherly classified and designated as Type I-E and Type I-E*

#The '-'symbol indicates the absence of the plasmid carrying bla_{KPC}

**Several strains have the same layout of the CRISPR array

203 completely sequenced K. pneumoniae strains used in this study											No. of spacer matches in bla_{KPC}-plasmid														
Number	CRISPR-Cas*	CRISPR array	Strain	Clonal complex	MLST	Collection location and date	GenBank accession no. of the chromosome	$bla_{KPC\#}$	Typing	No. of distinct spacer matches	Total	Spacer1	Spacer2	Spacer3	Spacer4	Spacer5	Spacer6	Spacer7	Spacer8	Spacer9	Spacer10	Spacer11	Spacer12	Spacer13	Spacer14
1	I-E	HZW25	HZW25	Non-CC258	34	USA(2016)	CP025211.1	-																	
2	I-E	KPNIH27	KPNIH27	Non-CC258	34	USA (2012)	CP007731.1	CP007732.1	IncA/C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	I-E	CAV1016**	AR_0126	Non-CC258	45	USA(2016)	CP021740.1	CP021743.1	HCM1 259	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	I-E	CAV1016	CAV1016	Non-CC258	45	USA (2007)	CP017934.1	CP017937.1	HCM1_259	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	I-E	QS17-0161	QS17-0161	Non-CC258	45	USA(2016)	CP024458.1	-																	
6	I-E	Kp52.145	Kp52.145	Non-CC258	66	France (2015)	FO834906.1	-																	
7	I-E	SB3432	SB3432	Non-CC258	67	USA(2016)	FO203501.1	-																	
8	I-E	002SK2	002SK2	Non-CC258	147	USA(2016)	CP025515.1	-																	
9	I-E	825795-1**	825795-1	Non-CC258	147	USA(2016)	CP017985.1	-																	
10	I-E	825795-1	Kp_Goe_822579	Non-CC258	147	USA(2016)	CP018140.1	-																	
11	I-E	825795-1	Kp_Goe_149473	Non-CC258	147	USA(2016)	CP018686.1	-																	
12	I-E	825795-1	Kp_Goe_149832	Non-CC258	147	USA(2016)	CP018695.1	-																	
13	I-E	825795-1	Kp_Goe_827024	Non-CC258	147	USA(2016)	CP018701.1	-																	
14	I-E	825795-1	Kp_Goe_827026	Non-CC258	147	USA(2016)	CP018707.1	-																	
15	I-E	825795-1	Kp_Goe_152021	Non-CC258	147	USA(2016)	CP018713.1	-																	
16	I-E	825795-1	KP_Goe_828304	Non-CC258	147	USA(2016)	CP018719.1	-																	
17	I-E	MS6671**	FDAARGOS_44	Non-CC258	147	USA(2016)	CP023925.1	CP023928.1	IncFIIK	8	8	1	1	0	0	0	1	1	0	1	1	1	0	1	0
18	I-E	MS6671	MS6671	Non-CC258	147	Australia(2015)	LN824133.1	-																	
19	I-E	MS6671	KP5	Non-CC258	147	Singapore(2015)	CP012426.1	-																	
20	I-E	MS6671	TGH13	Non-CC258	147	Belgium(2015)	CP012745.1	-																	
21	I-E	MS6671	AATZP	Non-CC258	147	USA(2016)	CP014755.1	-																	
22	I-E	MS6671	SKGH01	Non-CC258	147	USA(2016)	CP015500.1	-																	
23	I-E	MS6671	AR_0138	Non-CC258	147	USA(2016)	CP021757.1	-																	
24	I-E	MS6671	AR_0145	Non-CC258	147	USA(2016)	CP021939.1	-																	
25	I-E	MS6671	AR_0152	Non-CC258	147	USA(2016)	CP021944.1	-																	
26	I-E	MS6671	FDAARGOS_43	Non-CC258	147	USA(2016)	CP023913.1	-																	
27	I-E	MS6671	DA48896	Non-CC258	147	USA(2016)	CP024429.1	-																	
28	I-E	MS6671	CRKP-2297	Non-CC258	147	USA(2016)	CP024834.1	-																	
29	I-E	MS6671	CRKP-1215	Non-CC258	147	USA(2016)	CP024838.1	-																	
30	I-E	AR_0158**	AR_0158	Non-CC258	163	USA(2016)	CP021696.1	-																	
31	I-E	MS6671	Kpn223	Non-CC258	273	USA(2016)	CP015025.1	-																	
32	I-E	WCHKP34	WCHKP34	Non-CC258	273	-	CP025963.1	-																	
33	I-E	TGH8**	TGH8	Non-CC258	383	USA(2016)	CP012743.1	-																	
34	I-E	TGH8	TGH10	Non-CC258	383	USA(2016)	CP012744.1	-																	
35	I-E	KPNIH31**	KPNIH31	Non-CC258	392	USA (2013)	CP009876.1	in chromosome																	
36	I-E	KPNIH31	CN1	Non-CC258	392	USA(2016)	CP015382.1	-																	
37	I-E	GN-2	GN-2	Non-CC258	485	USA(2016)	CP019160.1	-																	
38	I-E	YH43	YH43	Non-CC258	906	Japan	CP020847.1	-																	
39	I-E	CAV1344**	CAV1193	Non-CC258	941	USA (2010)	CP013322.1	CP013325.1	HCM1 259	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	I-E	CAV1344	CAV1344	Non-CC258	941	USA (2010)	CP011624.1	CP011622.1	IncA/C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	I-E*	FDAARGOS_15	FDAARGOS_15	Non-CC258	13	USA(2016)	CP014123.1	-																	
42	I-E*	PittNDM01**	PittNDM01	Non-CC258	14	USA(2016)	CP006798.1	-																	
43	I-E*	PittNDM01	KP617	Non-CC258	14	USA(2016)	CP012753.1	-																	
44	I-E*	PittNDM01	U25	Non-CC258	14	USA(2016)	CP012043.1	-																	
45	I-E*	PittNDM01	K.pneumoniae 11	Non-CC258	14	USA(2016)	CP016923.1	-																	
46	I-E*	PittNDM01	AR_0068	Non-CC258	14	USA(2016)	CP020067.1	-																	
47	I-E*	PittNDM01	KPN528	Non-CC258	14	USA(2016)	CP020853.1	-																	
48	I-E*	PittNDM01	AR_0143	Non-CC258	14	USA(2016)	CP021708.1	-																	
49	I-E*	PittNDM01	AR_0066	Non-CC258	14	USA(2016)	CP026751.1	-																	
50	I-E*	NUHL24835**	NUHL24835	Non-CC258	14	USA(2016)	CP014004.1	-																	

51	I-E*	NUHL24835	23	Non-CC258	14	USA(2016)	CP016926.1	-																	
52	I-E*	19051	19051	Non-CC258	15	USA(2016)	CP022023.1	-																	
53	I-E*	PMK1**	BR#	Non-CC258	15	USA(2016)	CP015990.1	CP015991.1	repN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
54	I-E*	PMK1	PMK1	Non-CC258	15	USA(2016)	CP008929.1	-																	
55	I-E*	PMK1	KP36	Non-CC258	15	USA(2016)	CP017385.1	-																	
56	I-E*	PMK1	DHQP1605752_	Non-CC258	15	USA(2016)	CP022127.1	-																	
57	I-E*	RJF999	RJF999	Non-CC258	23	USA(2016)	CP014010.1	-																	
58	I-E*	RJA166	RJA166	Non-CC258	23	USA(2016)	CP019047.1	-																	
59	I-E*	1084**	1084	Non-CC258	23	USA(2016)	CP003785.1	-																	
60	I-E*	1084	ED2	Non-CC258	23	USA(2016)	CP016813.1	-																	
61	I-E*	1084	SGH10	Non-CC258	23	USA(2016)	CP025080.1	-																	
62	I-E*	P1428	P1428	Non-CC258	23	USA(2016)	CP017994.1	-																	
63	I-E*	KPHS1249	KPHS1249	Non-CC258	23	USA(2016)	CP027189.1	-																	
64	I-E*	ED23	ED23	Non-CC258	23	USA(2016)	CP016814.1	-																	
65	I-E*	NTUH-K2044	NTUH-K2044	Non-CC258	23	USA(2016)	AP006725.1	-																	
66	I-E*	J1	J1	Non-CC258	111	USA(2016)	CP013711.1	-																	
67	I-E*	342	342	Non-CC258	146	USA(2016)	CP000964.1	-																	
68	I-E*	AR_0117	AR_0117	Non-CC258	234	USA(2016)	CP020061.1	CP020066.1	IncN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
69	I-E*	RJF293	RJF293	Non-CC258	374	USA(2016)	CP014008.1	-																	
70	I-E*	ATCC 43816	ATCC 43816	Non-CC258	493	USA(2016)	CP009208.1	-																	
71	I-E*	ATCC 35657	ATCC 35657	Non-CC258	505	USA(2016)	CP015134.1	-																	
72		-	Kp_Goe_39795	Non-CC258	15	Germany（2016	CP018458.1	-																	
73		-	QS17-0029	Non-CC258	16	Thailand(2017)	CP024038.1	-																	
74		-	UCLAOXA232K	Non-CC258	16	USA(2015)	CP012560.1	-																	
75		-	UCLAOXA232K	Non-CC258	16	USA(2015)	CP012568.1	-																	
76		-	FDAARGOS_44	Non-CC258	16	USA（2017）	CP023919.1	-																	
77		-	XH209	Non-CC258	17	China (before 201	CP009461.1	-																	
78		-	Kp_Goe_154414	Non-CC258	23	Germany（2016	CP018337.1	-																	
79		-	KP-1	Non-CC258	29	Australia	CP012883.1	-																	
80		-	INF322	Non-CC258	29	Australia(2017)	CP024482.1	-																	
81		-	INF249	Non-CC258	29	Australia(2017)	CP024489.1	-																	
82		-	KpvK54	Non-CC258	29	UK(2017)	CP023134.2	-																	
83		-	DHQP1002001	Non-CC258	34	USA(2016)	CP016811.1	-																	
84		-	KPNIH39	Non-CC258	37	USA(2016)	CP014762.1	CP014765.1	IncFIIK	10	12	1	3	0	0	1	1	1	0	1	1	1	0	1	1
85		-	KSB1_7J	Non-CC258	37	Australia	CP024548.1	-																	
86		-	459	Non-CC258	37	China（2016）	CP018306.1	-																	
87		-	KPN1482	Non-CC258	37	USA（2017）	CP020841.1	-																	
88		-	AR_0139	Non-CC258	37	USA（2017）	CP021960.1	-																	
89		-	INF042	Non-CC258	37	Australia(2017)	CP024542.1	-																	
90		-	INF059	Non-CC258	37	Australia(2017)	CP024545.1	-																	
91		-	MGH 78578	Non-CC258	38	USA (1994)	CP000647.1	-																	
92		-	1158	Non-CC258	65	China (2002)	CP006722.1	-																	
93		-	NUHL30457	Non-CC258	86	China(2018)	CP026586.1	CP026589.1	IncFII	2	2	0	0	1	0	0	0	0	1	0	0	0	0	0	0
94		-	CG43	Non-CC258	86	China	CP006648.1	-																	
95		-	KP5-1	Non-CC258	86	USA (2005)	CP008700.1	-																	
96		-	HK787	Non-CC258	86	China	CP006738.1	-																	
97		-	kp757	Non-CC258	86	USA(2016)	CP015120.1	-																	
98		-	Kp_Goe_33208	Non-CC258	101	Germany(2016)	CP018447.1	-																	
99		-	Kp_Goe_71070	Non-CC258	101	Germany(2016)	CP018450.1	-																	
100		-	Kp_Goe_121641	Non-CC258	101	Germany（2016	CP018735.1	-																	
101		-	S11101:960186733	Non-CC258	101	South Africa(2016	CP023487.1	-																	
102		-	FDAARGOS_44	Non-CC258	152	USA（2017）	CP023949.1	CP023952.1	IncA/C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
103		-	KPNIH48	Non-CC258	252	USA（2018）	CP026392.1	CP026395.1,C	UT/IncFIIS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
104		-	KPNIH50	Non-CC258	252	USA（2018）	CP026177.1	CP026175.1	A058	12	15	2	3	1	1	0	1	1	0	1	1	1	1	1	1
105		-	KpN01	Non-CC258	278	Canada (2013)	CP012987.1	-																	
106		-	KpN06	Non-CC258	278	Canada (2013)	CP012992.1	-																	
107		-	KP1766	Non-CC258	307	USA（2017）	CP025146.1	CP025148.1,C	pilL/pilL	11	15	2	4	1	0	1	1	1	0	1	1	0	1	1	1
108		-	NR5632	Non-CC258	307	USA（2017）	CP025143.1	CP025144.1	IncA/C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

109		-	KP1768	Non-CC258	307	USA（2017）	CP025140.1	CP025141.1	IncA/C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
110		-	KSB1_7E	Non-CC258	323	Australia(2017)	CP024496.1	-																	
111		-	KSB1_4E	Non-CC258	323	Australia(2017)	CP024499.1	-																	
112		-	KSB2_1B	Non-CC258	323	Australia(2017)	CP024504.1	-																	
113		-	KSB1_10J	Non-CC258	323	Australia(2017)	CP024515.1	-																	
114		-	KCTC 2242	Non-CC258	375	Korea	CP002910.1	-																	
115		-	blaNDM-1	Non-CC258	395	USA (2011-2013)	CP009114.1	-																	
116		-	Kp_Goe_62629	Non-CC258	395	Germany(2016)	CP018364.1	-																	
117		-	Kp13	Non-CC258	442	Brazil(2009)	CP003999.1	CP003997.1	parB	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
118		-	234-12	Non-CC258	514	Germany (2011)	CP011313.1	-																	
119		-	H11	Non-CC258	659	China（2016）	CP018056.1	-																	
120		-	AR_0107	Non-CC258	1161	USA（2017）	CP021955.1	-																	
121		-	KPNIH29	Non-CC258	1518	USA (2013)	CP009863.1	CP009864.1	korA, repN, tra	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
122		-	W14	Non-CC258	1536	China(2016)	CP015753.1	-																	
123		-	TH1	Non-CC258	1536	China(2016)	CP016159.1	-																	
124		-	1756	Non-CC258	2549	Taiwan(2017)	CP019219.1	-																	
125		-	HKUOPLC	Non-CC258	ND	China (2013)	CP012300.1	-																	
126		-	ATCC BAA-214	CC258	11	USA (2010)	CP006659.2	-																	
127		-	SWU01	CC258	11	China(2016)	CP018454.1	CP018455.1	IncFII	10	14	2	3	2	1	0	1	1	1	1	1	0	0	1	0
128		-	WCHKP8F4	CC258	11	China(2018)	CP027068.2	CP027067.1	IncFII	7	11	2	3	2	1	0	1	1	0	1	0	0	0	0	0
129		-	WCHKP649	CC258	11	China(2018)	CP026585.2	CP026584.1	IncFII	10	14	2	3	2	1	0	1	1	1	1	1	0	0	1	0
130		-	GD4	CC258	11	Hong Kong(2017)	CP025951.1	CP025952.1	IncFII	11	15	2	3	2	1	1	1	1	1	1	1	0	0	1	0
131		-	K. pneumoniae 1	CC258	11	China (2009)	CP025466.1	CP025468.1	IncFIIK	10	13	2	3	1	1	0	1	1	0	1	1	1	0	1	0
132		-	K. pneumoniae F	CC258	11	China (2016)	CP025461.1	CP025463.1	IncFII	8	12	2	3	2	1	0	1	1	1	1	0	0	0	0	0
133		-	FDAARGOS_44	CC258	11	USA（2017）	CP023941.1	CP023942.1	IncFII	10	15	2	3	2	1	0	1	1	2	1	1	0	0	1	0
134		-	FDAARGOS_44	CC258	11	USA（2017）	CP023933.1	CP023938.1	A054	7	11	2	3	2	1	0	1	1	0	1	0	0	0	0	0
135		-	CAV1392	CC258	11	UK(2011)	CP011578.1	CP011575.1	trbA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
136		-	JM45	CC258	11	China (2010)	CP006656.1	CP006657.1	IncFIIK	11	15	2	4	1	0	1	1	1	0	1	1	0	1	1	1
137		-	HS11286	CC258	11	China (2011)	CP003200.1	CP003224.1	IncFIIK	11	15	2	4	1	1	0	1	1	0	1	1	1	1	1	0
138		-	KP38731	CC258	11	USA(2016)	CP014294.1	-																	
139		-	Kp_Goe_822917	CC258	11	Germany(2016)	CP018438.1	-																	
140		-	Kp_Goe_821588	CC258	11	Germany（2016	CP018692.1	-																	
141		-	AR_0049	CC258	11	USA（2016）	CP018816.1	-																	
142		-	K66-45	CC258	11	Norway(2017)	CP020901.1	-																	
143		-	AR_0146	CC258	11	USA（2017）	CP021685.1	-																	
144		-	AR_0148	CC258	11	USA（2017）	CP021950.1	-																	
145		-	911021	CC258	11	China(2017)	CP022882.1	-																	
146		-	721005	CC258	11	China(2017)	CP022997.1	-																	
147		-	500_1420	CC258	258	USA(2004-2012)	CP011980.1	CP011981.1	IncFIIK	10	13	2	3	1	0	0	1	1	0	1	1	0	1	1	1
148		-	AR_0361	CC258	258	USA（2018）	CP027160.1	CP027158.1	IncFIIK	8	8	1	1	0	0	0	1	1	0	1	1	1	0	1	0
149		-	NU-CRE047	CC258	258	USA（2017）	CP025037.1	CP025039.1	IncFIA	4	4	0	0	1	0	0	0	0	0	0	1	1	0	1	0
150		-	AUSMDU00008	CC258	258	Australia(2017)	CP025008.1	CP025010.1	IncFIIK	8	8	1	1	0	0	0	1	1	0	1	1	1	0	1	0
								,CP025009.1	IncFIIK	10	14	2	4	1	0	1	1	1	0	1	1	0	0	1	1
151		-	AUSMDU00003	CC258	258	Australia(2017)	CP025005.1	CP025006.1	IncFIIK	10	14	2	4	1	0	1	1	1	0	1	1	0	0	1	1
152		-	AUSMDU00008	CC258	258	Australia (2012)	CP022691.1	CP022693.1	IncFIIK	10	14	2	4	1	0	1	1	1	0	1	1	0	0	1	1
153		-	BIC-1	CC258	258	France (2009)	CP022573.1	CP022574.1	IncFIIK	10	14	2	4	1	0	1	1	1	0	1	1	0	0	1	1
154		-	AR_0125	CC258	258	USA（2017）	CP021859.1	CP021861.1	UT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
								,CP021860.1	A007	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
155		-	AR_0120	CC258	258	USA（2017）	CP021833.1	CP021835.1	IncA/C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
156		-	AR_0113	CC258	258	USA（2017）	CP021751.1	CP021756.1	A007	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
								,CP021754.1	UT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
157		-	AR_0129	CC258	258	USA（2017）	CP021718.1	CP021716.1	HCM1_259	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
158		-	AR_0112	CC258	258	USA（2017）	CP021549.1	CP021546.1	UT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
								,CP021548.1	A007	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
159		-	AR_0098	CC258	258	USA（2017）	CP020108.1	CP020110.1	repN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
160		-	AR_0115	CC258	258	USA（2017）	CP020071.1	CP020075.1	traJ	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
161		-	MNCRE53	CC258	258	USA (2012)	CP018437.1	CP018436.1	HCM1_259	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
162		-	MNCRE78	CC258	258	USA (2013)	CP018428.1	CP018432.1	HCM1_259	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

163		-	MNCRE69	CC258	258	USA (before 2016	CP018427.1	CP018426.1	HCM1_259	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
164		-	CR14	CC258	258	USA(2016)	CP015392.1	CP015395.1	IncA/C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
165		-	KPNIH36	CC258	258	USA(2016)	CP014647.1	CP014650.1	IncFIIK	8	8	1	1	0	0	0	1	1	0	1	1	1	0	1	0
166		-	UHKPC33	CC258	258	USA(2004-2012)	CP011989.1	CP011990.1	IncFIIK	11	15	2	4	1	0	1	1	1	0	1	1	0	1	1	1
167		-	UHKPC07	CC258	258	USA(2004-2012)	CP011985.1	CP011986.1	IncFIIK	8	8	1	1	0	0	0	1	1	0	1	1	1	0	1	0
168		-	DMC1097	CC258	258	USA(2004-2012)	CP011976.1	CP011978.1	UT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
169		-	CAV1596	CC258	258	USA (2012)	CP011647.1	CP011646.1	trbA	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
								,CP011645.1	repN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
170		-	32192	CC258	258	USA (2010)	CP010361.1	CP010575.1	repN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
171		-	34618	CC258	258	USA (2011)	CP010392.1	CP010396.1	repN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
172		-	KPNIH30	CC258	258	USA (2013)	CP009872.1	CP009875.1	IncFIIK	8	8	1	1	0	0	0	1	1	0	1	1	1	0	1	0
173		-	KPNIH32	CC258	258	USA (2013)	CP009775.1	CP009776.1	IncFIA	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
174		-	KPNIH33	CC258	258	USA (2013)	CP009771.1	CP009773.1	IncFIA	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
175		-	KPR0928	CC258	258	USA (2012)	CP008831.1	CP008833.1	IncFIIK	10	15	1	4	0	0	3	1	1	0	1	1	1	1	1	0
176		-	KPNIH1	CC258	258	USA (2011)	CP008827.1	CP008830.1	IncFIIK	8	8	1	1	0	0	0	1	1	0	1	1	1	0	1	0
177		-	KPNIH24	CC258	258	USA (2012)	CP008797.1	CP008798.1	repN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
178		-	KPNIH10	CC258	258	USA (2011)	CP007727.1	CP007730.1	IncFIIK	10	15	1	4	0	0	3	1	1	0	1	1	1	1	1	0
179		-	30660/NJST258_	CC258	258	USA (2010)	CP006923.1	CP006926.1	IncFIA	6	7	1	2	0	0	0	0	0	0	0	1	0	1	1	1
180		-	30684/NJST258_	CC258	258	USA (2010)	CP006918.1	CP006919.1	A011	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
181		-	CAV1453	CC258	258	UK(2017)	CP018356.1	-																	
182		-	BK13043	CC258	258	USA（2017）	CP020837.1	-																	
183		-	AR_0047	CC258	258	USA（2017）	CP021539.1	-																	
184		-	FDAARGOS_44	CC258	340	Canada (2013)	CP023502.1	-																	
185		-	CAV1042	CC258	340	USA (2008)	CP018671.1	in chromosome																	
186		-	CAV1217	CC258	340	USA (2010)	CP018676.1	in chromosome																	
187		-	NY9	CC258	340	USA(2016)	CP015385.1	CP015387.1	IncFIA	4	4	0	0	1	0	0	0	0	0	0	1	1	0	1	0
188		-	INF274	CC258	340	Australia	CP024570.1	-																	
189		-	INF278	CC258	340	Australia	CP024563.1	-																	
190		-	INF164	CC258	340	Australia	CP024556.1	-																	
191		-	INF163	CC258	340	Australia	CP024549.1	-																	
192		-	CAV1417	CC258	340	UK(2016)	CP018352.1	-																	
193		-	FDAARGOS_43	CC258	340	USA(2017)	CP023907.1	-																	
194		-	FDAARGOS_44	CC258	340	USA(2017)	CP023946.1	-																	
195		-	KSB1_5D	CC258	340	Australia(2017)	CP024191.1	-																	
196		-	INF158	CC258	340	Australia(2017)	CP024521.1	-																	
197		-	INF157	CC258	340	Australia(2017)	CP024528.1	-																	
198		-	KSB1_9D	CC258	340	Australia(2017)	CP024535.1	-																	
199		-	BR21	CC258	437	USA（2016）	CP018885.1	CP018887.1	korA, repN, tra	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
200		-	BR7	CC258	437	USA（2016）	CP018883.1	CP018884.1	korA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
201		-	KPN_KPC_HUG	CC258	512	Switzerland(2015)	CP019772.1	CP019774.1	IncFIIK	8	8	1	1	0	0	0	1	1	0	1	1	1	0	1	0
202		-	blood sample 2	CC258	512	Switzerland(2016)	CP015822.1	-																	
203		-	K. pneumoniae 6	CC258	1326	China (2017)	CP025456.1	CP025458.1	IncFII	7	11	2	3	2	1	0	1	1	0	1	0	0	0	0	0

Supplementary data 1b. 121 bla_{KPC}- positive plasmids in *K. pneumoniae* used in this study.

No.	Name[a]	GenBank accession no	Resistance gene	Typing	Number of the regions matching to the spacers of the *K. pneumoniae* CRISPR loci														
					Total	Spacer1	Spacer2	Spacer3	Spacer4	Spacer5	Spacer6	Spacer7	Spacer8	Spacer9	Spacer10	Spacer11	Spacer12	Spacer13	Spacer14
1	p187-2	CP025468.1	KPC	IncFIIK	13	2	3	1	1	0	1	1	0	1	1	1	0	1	0
2	p12139-KPC	MF168403.1	KPC	IncFII	23	2	6	2	2	4	1	1	1	1	1	0	1	1	0
3	p64917-KPC	MF168405.1	KPC	IncFII	21	2	6	2	1	4	1	1	1	1	1	0	0	1	0
4	pCT-KPC	KT185451.1	KPC	IncFII	19	2	5	2	1	3	1	1	0	1	1	0	1	1	0
5	p0716-KPC	KY270849.1	KPC	IncFIIY	21	2	5	1	1	4	1	1	1	1	1	1	1	1	0
6	p20049-KPC	MF168404.1	KPC	IncFII	17	2	5	0	1	3	1	1	0	1	1	0	1	1	0
7	pKpQIL-307_48	KY271403.1	KPC	IncFIIK	21	2	4	0	0	4	2	2	0	2	1	1	1	2	0
8	p12181-KPC	KY270850.1	KPC	IncFIIY	20	2	5	1	1	3	1	1	1	1	1	1	1	1	0
9	pKP1034	KP893385.1	KPC	IncFII	19	2	6	2	1	3	1	1	1	1	0	0	0	1	0
10	pBIC-1a	CP022574.1	KPC	IncFIIK	16	2	4	1	0	3	1	1	0	1	1	0	1	1	0
11	p44-2	CP025463	KPC	IncFII	19	2	6	2	1	3	1	1	1	1	0	0	0	1	0
12	p1068-KPC	MF168402.1	KPC	IncFII	19	2	5	2	1	3	1	1	0	1	1	0	1	1	0
13	p283747-KPC	MF168406.1	KPC	IncFII	17	1	5	2	1	3	1	1	1	1	0	0	0	1	0
14	pKPHS2	CP003224.1	KPC	IncFIIK	18	2	4	1	1	3	1	1	0	1	1	1	1	1	0
15	pHS091147	KX236178.1	KPC	IncFIIK	19	2	4	1	1	4	1	1	0	1	1	1	1	1	0
16	p628-KPC	KP987218.1	KPC	IncFIIK	17	2	4	1	0	3	1	1	0	1	1	1	1	1	0
17	pGR-1504	KF874496.1	KPC	IncFIIK	16	2	4	0	0	3	1	1	0	1	1	1	1	1	0
18	pGR-1870	KF874498.1	KPC	IncFIIK	18	2	4	1	0	4	1	1	0	1	1	1	1	1	0
19	pKPC_CAV1217	CP018675.1	KPC	IncFIIK	17	2	4	1	0	3	1	1	0	1	1	1	1	1	0
20	pIT-01C03	HG969995.1	KPC	IncFIIK	15	1	4	0	0	3	1	1	0	1	1	1	1	1	0
21	pIT-11C07	HG969998.1	KPC	IncFIIK	17	2	3	1	0	4	1	1	0	1	1	1	1	1	0
22	pKpQIL-10	KJ146687.1	KPC	IncFIIK	15	1	4	0	0	3	1	1	0	1	1	1	1	1	0
23	pKpQIL-234	KJ146689.1	KPC	IncFIIK	15	1	4	0	0	3	1	1	0	1	1	1	1	1	0
24	pAUSMDU8079-2	CP022693.1	KPC	IncFIIK	15	1	4	0	0	3	1	1	0	1	1	1	1	1	0
25	pG12-KPC-2	KU665642.1	KPC	IncFIIK	15	1	4	0	0	3	1	1	0	1	1	1	1	1	0
26	pIT-01C22	HG969997.1	KPC	IncFIIK	15	1	4	0	0	3	1	1	0	1	1	1	1	1	0
27	pKpQIL	GU595196.1	KPC	IncFIIK	14	1	4	0	0	2	1	1	0	1	1	1	1	1	0
28	p2	CP019774.1	KPC	IncFIIK	15	1	4	0	0	3	1	1	0	1	1	1	1	1	0
29	pGR-1780	KF874497.2	KPC	IncFIIK	15	1	4	0	0	3	1	1	0	1	1	1	1	1	0
30	pGR-3913	KF874499.1	KPC	IncFIIK	15	1	4	0	0	3	1	1	0	1	1	1	1	1	0
31	pIT-06C07	LT009688.1	KPC	IncFIIK	16	2	4	1	0	2	1	1	0	1	1	1	1	1	0
32	pKpQIL-D1	KY798505.1	KPC	IncFIIK	16	2	4	0	0	3	1	1	0	1	1	1	1	1	0
33	pKpQIL-IT	JN233705.2	KPC	IncFIIK	15	1	4	0	0	3	1	1	0	1	1	1	1	1	0
34	pKpQIL-UK	KY798507.1	KPC	IncFIIK	15	1	4	0	0	3	1	1	0	1	1	1	1	1	0
35	pUHKPC33	CP011991.1	KPC	IncFIIK	15	1	4	0	0	3	1	1	0	1	1	1	1	1	0
36	p69-2	CP025458	KPC	IncFII	14	2	3	2	1	2	1	1	0	1	0	0	0	1	0
37	p500_1420	CP011981.1	KPC	IncFIIK	15	2	4	0	0	3	1	1	0	1	1	0	1	1	0
38	pKp41	CP012000.1	KPC	IncFIIK	15	1	4	0	0	3	1	1	0	1	1	1	1	1	0
39	pVGH151	KJ721790.1	KPC	IncFIIK	15	1	4	0	0	3	1	1	0	1	1	1	1	1	0
40	pKpQIL-LS6	JX442975.1	KPC	IncFIB	13	1	4	0	0	4	1	1	0	1	0	0	0	1	0
41	pIT-FIPP-1	HG969999.1	KPC	IncFIB	13	2	4	0	0	3	1	1	0	1	0	0	0	1	0
42	pBK32179	JX430448.1	KPC	IncFIIK	13	2	2	1	0	2	1	1	0	1	1	0	1	0	1
43	pIT-12C47	HG969996.1	KPC	IncFIIK	8	1	2	0	0	2	0	0	0	0	1	1	1	0	0
44	PKPCAPSS	KP008371.1	KPC	IncFIIK	13	0	4	0	0	3	1	1	0	0	1	1	1	1	0
45	p13294-KPC	MF156708.1	KPC	IncFIIY	9	1	3	0	0	3	0	0	0	0	1	1	0	0	0
46	pUSKPC3	KJ721789.1	KPC	IncFIA	8	0	2	1	0	2	0	0	0	0	1	1	1	0	0
47	pNY9_2	CP015387.1	KPC	IncFIA	7	0	2	1	0	1	0	0	0	0	1	1	1	0	0
48	pBK30683	KF954760.1	KPC	IncFIA	7	0	2	1	0	1	0	0	0	0	1	1	1	0	0
49	pKP048	FJ628167.2	KPC	IncFIIK	5	0	1	0	0	1	0	0	0	0	1	1	1	0	0
50	pIT-12C73	LT009689.1	KPC	IncFIIK	3	0	0	0	0	0	0	0	0	0	1	1	1	0	0
51	pKPN101-IT	JX283456.1	KPC	IncFIIK	3	0	0	0	0	0	0	0	0	0	1	1	1	0	0
52	pKp28	CP011999.1	KPC	IncFIA	3	1	1	0	0	1	0	0	0	0	0	0	0	0	0
53	pMNCRE44_5	CP010881.1	KPC	IncFIA	3	0	1	1	0	1	0	0	0	0	0	0	0	0	0
54	pBK30661	KF954759.1	KPC	IncFIA	3	0	1	1	0	1	0	0	0	0	0	0	0	0	0

55	pKPC-LK30	KC405622.1	KPC	UT	17	2	5	1	1	3	1	1	1	1	0	0	0	1	0
56	pKPC_P16	KY689238.1	KPC	UT	15	1	5	1	1	2	1	1	1	1	0	0	0	1	0
57	pKP1766_p2	CP025148.1	KPC	UT	16	2	4	0	0	3	1	1	0	1	1	1	1	0	1
58	pBK32533	KP345882.1	KPC	IncA/C	3	0	1	1	0	1	0	0	0	0	0	0	0	0	0
59	pWSZBR	CP015991.1	KPC	repN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
60	pCRKP-59-KPC	KX928752.1	KPC	Inc FIA, FIB,FII	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
61	pSLMT	HQ589350.1	KPC	IncFIIK	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
62	pGN2-KPC	MF156710.1	KPC	IncFIIY	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
63	pGN28-KPC	MF156712.1	KPC	IncFIIY	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
64	pE20-KPC	MF156709.1	KPC	IncFIIY	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
65	pKP1194a	KX756453.1	KPC	A005	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
66	15S	FJ223606.1	KPC	A007	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
67	pKPC-SMH	KT148595.1	KPC	A007	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
68	IncX-3	KU934011.1	KPC	parB	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
69	pEC4365	KX783439.1	KPC	A007	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
70	pKP4365	KX783440.1	KPC	A007	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
71	pCr-145	KY659388.1	KPC	A007	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
72	pRYCKPC3.1	NC_019151.1	KPC	A007	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
73	pHS10842	KP125892.1	KPC	A010	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
74	pNE1280	JQ837276.1	KPC	A011	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
75	pKPC_CAV1042-44	CP018668.1	KPC	HCM1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
76	pD5170990	KX169264.1	KPC	HCM1 043	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
77	pHS062105	KF623109.1	KPC	HCM1 116	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
78	pHS102707	KF701335.1	KPC	HCM1 178ac	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
79	pKPC_CAV1193*	CP013325.1	KPC	HCM1 259	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
80	pMNCRE78_3	CP018432.1	KPC	HCM1 259	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
81	pKPC_CAV1344*	CP011622.1	KPC	IncA/C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
82	pKEC-dc3*	CP007732.1	KPC	IncA/C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
83	KP1768_p1	CP025141.1	KPC	IncA/C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
84	NR5632_p1	CP025144.1	KPC	IncA/C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85	KP1766_p1	CP025147.1	KPC	IncA/C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86	pCRKP-1-KPC	KX928750.1	KPC	IncN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
87	pCRKP-5-KPC	KX928751.1	KPC	IncN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
88	pJF-WMKPCN1	KX881941.1	KPC	IncN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
89	pKp145_11a	KX154765.1	KPC	IncN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
90	pKP148	KX062091.1	KPC	IncN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
91	pKp196	KX397572.1	KPC	IncN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
92	pKp314_11a	KX276209.1	KPC	IncN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
93	pKPC_FCF_3SP	NC_021660.2	KPC	IncN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
94	pKPC_FCF13_05	NC_021664.2	KPC	IncN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
95	12	FJ223605.1	KPC	IncN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
96	9	FJ223607.1	KPC	IncN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
97	pBK31551	JX193301.1	KPC	IncN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
98	pIncX-SHV	NC_019157.1	KPC	IncX	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
99	8-p45-IncX3	KT362706.1	KPC	IncX3		0	0	0	0	0	0	0	0	0	0	0	0	0	0
100	pKP1433	NC_021238.1	KPC	korA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
101	pYD626E	KJ933392.1	KPC	korA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
102	pKP13d	CP003997.1	KPC	parB	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
103	pKPC_Kp01	KX348144.1	KPC	parB	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
104	pCfr-30	KY659387.1	KPC	parB	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
105	pKPC-DK05	KR091915.1	KPC	repN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
106	pBK31567	JX193302.1	KPC	traJ	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
107	pUCLAKPC1	KY930324.1	KPC	trbA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
108	pKPC-NY79	NC_019161.1	KPC	UT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
109	pKpS90	NC_019384.1	KPC	UT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
110	pKPC-NY79	NC_019161.1	KPC	UT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
111	pDMC1097	CP011978.1	KPC	UT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

112	pFOS18	KJ653815.1	KPC	UT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
113	pKP64477d	MF150120.1	KPC	UT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
114	pKPC_CAV1042-89	CP018669.1	KPC	UT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
115	pKPC_Kp02	KX348145.1	KPC	UT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
116	pKPC_Kp46	KX348146.1	KPC	UT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
117	pKPC_UVA01*	CP009465.1	KPC	UT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
118	pMNCRE53_3	CP018436.1	KPC	UT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
119	pMNCRE69_3	CP018426.1	KPC	UT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
120	pUCLAKPC2	KY930325.1	KPC	UT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
121	pBK15692	KC845573.1	KPC	UT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Supplementary data 1c. 14 proto-spacers carried by *bla*$_{KPC}$- positive plasmids matched for the CRISPR system in *K. pneumoniae*.

Name of spacer	Sequence (5' to 3')
Spacer1	CAGACAGACAGCAGGCAGCAAACAGGGAAGAC
Spacer2	GAGCAGGCACCCGCCGCAACGACGAAGAGCGC
Spacer3	GTGGTTTGTTACCGTGTTGTGTGGCAAAAAGC
Spacer4	GAACGGAGGAATATAAGAACAAAAGCCCGCAG
Spacer5	TTAATACCAGGGGGCAGGTTCAGCAGGTCCCC
Spacer6	CGATAACCGGGCGTTTCGACTGAACTCACCTC
Spacer7	CCGCCGTTTAATCGCGGTGATGATATCCGGCA
Spacer8	TCGTCTGAGTTCCGGCTTACGCCGTGCCGACA
Spacer9	CCCCGTCGTCATTCGCGCATTCTGCGCACAGA
Spacer10	TACTGCAGCAGGATGTCGTAGCCGATATAGTC
Spacer11	GAAATAACCGTCTTCATTTCCACCCTCCCTCA
Spacer12	TACTGAAACGGGTAATCAGCACAAATACCAAA
Spacer13	ATTTTCAAATACTTTTCAGACCGGGCAATGTG
Spacer14	CGTGATAGCGCGTTTAAGCATGTGCGCGGGGG

Supplementary data 1d. 459 clinical isolates in China.

459 clinical isolates			
ID	MLST	CRISPR-Cas	*bla* $_{KPC}$
1	218		-
2	690		-
3	65		-
4	23	I-E*	-
5	11		-
6	23	I-E*	-
7	86		-
8	65		-
9	1		-
10	17		-
11	17		-
12	23	I-E*	-
13	29		-
14	39	I-E	-
15	11		-
16	20		-
17	147		-
18	65		-
19	14	I-E*	-
20	793	I-E*	-
21	14	I-E*	-
22	14	I-E	-
23	686		-
24	1938	I-E	-
25	1933		-
26	660		-
27	1419		-
28	101		-
29	872	I-E	-
30	23	I-E*	-
31	37		-
32	29		-
33	1934	I-E*	-
34	23	I-E*	-
35	152	I-E*	-

232 non-CG258 isolates			
ID	MLST	CRISPR-Cas	*bla* $_{KPC}$
9	1		-
118	1		-
183	6		-
19	14	I-E*	-
21	14	I-E*	-
22	14	I-E	-
116	14		-
52	15	I-E*	-
68	15	I-E*	-
166	15	I-E*	-
170	15	I-E*	-
182	15	I-E*	-
348	15	I-E*	+
368	15	I-E*	+
369	15	I-E*	+
370	15	I-E*	+
374	15	I-E*	+
375	15	I-E*	+
378	15	I-E*	+
381	15	I-E*	+
382	15	I-E*	+
389	15	I-E*	+
392	15	I-E*	+
403	15		+
10	17		-
11	17		-
55	17		-
16	20		-
37	20		-
65	20		-
139	20		-
140	20		-
142	20		-
147	20		-
148	20		-

227 CG258 isolates			
ID	MLST	CRISPR-Cas	*bla* $_{KPC}$
5	11		-
15	11		-
66	11		-
67	11	I-E*	-
158	11	I-E*	-
220	11		+
221	11		+
223	11		+
224	11		+
225	11		+
226	11		+
227	11		+
228	11		+
229	11		+
230	11		+
231	11		+
232	11		+
233	11		+
234	11		+
235	11		+
236	11		+
237	11		+
238	11		+
239	11		+
240	11		+
241	11		+
242	11		+
243	11		+
244	11		+
245	11		+
246	11		+
247	11		+
248	11		+
249	11		+
250	11		+

36	23	I-E*	-
37	20		-
38	29		-
39	23	I-E*	-
40	1938	I-E	-
41	494	I-E*	-
42	23	I-E*	-
43	309		-
44	309		-
45	218		-
46	690	I-E	-
47	626	I-E	-
48	592	I-E	-
49	461		-
50	23	I-E*	-
51	37	I-E	-
52	15	I-E*	-
53	29		-
54	23	I-E*	-
55	17		-
56	1939		-
57	494	I-E*	-
58	494	I-E*	-
59	36		-
60	1940		-
61	35	I-E*	-
62	39	I-E	-
63	261		-
64	655		-
65	20		-
66	11		-
67	11	I-E*	-
68	15	I-E*	-
69	494	I-E*	-
70	101		-
71	494		-
72	23		-
73	86	I-E*	-

150	20		-
152	20		-
153	20		-
154	20		-
155	20		-
156	20		-
168	20		-
4	23	I-E*	-
6	23	I-E*	-
12	23	I-E*	-
30	23	I-E*	-
34	23	I-E*	-
36	23	I-E*	-
39	23	I-E*	-
42	23	I-E*	-
50	23	I-E*	-
54	23	I-E*	-
72	23		-
105	23	I-E*	-
109	23	I-E*	-
113	23	I-E*	-
185	23	I-E*	-
186	23	I-E*	-
191	23	I-E*	-
193	23	I-E*	-
404	23	I-E*	+
203	25		-
13	29		-
32	29		-
38	29		-
53	29		-
92	29		-
93	29		-
97	29		-
98	29		-
125	29		-
114	34	I-E	-
61	35	I-E*	-

251	11		+
252	11		+
253	11		+
254	11		+
255	11		+
256	11		+
257	11		+
258	11		+
259	11		+
260	11		+
261	11		+
262	11		+
263	11		+
264	11		+
265	11		+
266	11		+
267	11		+
268	11		+
269	11		+
270	11		+
271	11		+
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273	11		+
274	11		+
275	11		+
276	11		+
277	11		+
278	11		+
279	11		+
280	11		+
281	11		+
282	11		+
283	11		+
284	11		+
285	11		+
286	11		+
287	11		+
288	11		+

74	134		-
75	1935		-
76	36	I-E*	-
77	36		-
78	36		-
79	1936		-
80	101		-
81	ND		-
82	1937	I-E	-
83	1764		-
84	43		-
85	1770		-
86	39	I-E	-
87	188		-
88	35	I-E*	-
89	1031	I-E*	-
90	881		-
91	881		-
92	29		-
93	29		-
94	86		-
95	37		-
96	35	I-E*	-
97	29		-
98	29		-
99	1914		-
100	494	I-E*	-
101	152		-
102	1419		-
103	455		-
104	1419		-
105	23	I-E*	-
106	1326		-
107	896		-
108	584		-
109	23	I-E*	-
110	48		-
111	133		-

88	35	I-E*	-
96	35	I-E*	-
169	35	I-E*	-
190	35	I-E*	-
59	36		-
76	36	I-E*	-
77	36		-
78	36		-
31	37		-
51	37	I-E	-
95	37		-
119	37		-
14	39	I-E	-
62	39	I-E	-
86	39	I-E	-
84	43		-
110	48		-
112	48		-
133	54		-
135	54		-
136	54		-
137	54		-
138	54		-
144	54		-
145	54		-
146	54		-
149	54		-
151	54		-
157	64		-
3	65		-
8	65		-
18	65		-
174	65		-
197	65		-
202	65		-
218	65		+
222	65		+
178	76		-

289	11		+
290	11		+
291	11		+
292	11		+
293	11		+
294	11		+
295	11		+
296	11		+
297	11		+
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314	11		+
315	11		+
316	11		+
317	11		+
318	11		+
319	11		+
320	11		+
321	11		+
322	11		+
323	11		+
324	11		+
325	11		+
326	11		+

112	48		-
113	23	I-E*	-
114	34	I-E	-
115	502	I-E*	-
116	14		-
117	420	I-E*	-
118	1		-
119	37		-
120	592	I-E	-
121	86		-
122	502		-
123	661		-
124	1783	I-E*	-
125	29		-
126	893	I-E	-
127	490		-
128	661		-
129	107		-
130	412		-
131	91	I-E*	-
132	785		-
133	54		-
134	290		-
135	54		-
136	54		-
137	54		-
138	54		-
139	20		-
140	20		-
141	705		-
142	20		-
143	705		-
144	54		-
145	54		-
146	54		-
147	20		-
148	20		-
149	54		-

184	81		-
342	81		+
343	81		+
345	81		+
7	86		-
73	86	I-E*	-
94	86		-
121	86		-
189	86		-
131	91	I-E*	-
28	101		-
70	101		-
80	101		-
129	107		-
194	111	I-E*	-
111	133		-
74	134		-
17	147		-
35	152	I-E*	-
101	152		-
87	188		-
1	218		-
45	218		-
171	254	I-E*	-
63	261		-
175	268		-
134	290		-
332	290		+
43	309		-
44	309		-
160	334		-
181	347		-
130	412		-
187	412		-
188	412		-
192	412	I-E*	-
196	412	I-E*	-
195	412		-

327	11		+
328	11		+
329	11		+
330	11		+
331	11		+
333	11		+
334	11		+
335	11		+
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337	11		+
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340	11		+
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359	11		+
360	11		+
361	11		+
362	11		+
363	11		+
364	11		+
365	11		+
366	11		+
367	11		+
371	11		+
372	11		+

150	20		-
151	54		-
152	20		-
153	20		-
154	20		-
155	20		-
156	20		-
157	64		-
158	11	I-E*	-
159	2695		-
160	334		-
161	697	I-E	-
162	697	I-E	-
163	1536		-
164	1446	I-E	-
165	2464		-
166	15	I-E*	-
167	692	I-E*	-
168	20		-
169	35	I-E*	-
170	15	I-E*	-
171	254	I-E*	-
172	814	I-E	-
173	580	I-E	-
174	65		-
175	268		-
176	876	I-E	-
177	1265	I-E*	-
178	76		-
179	1653	I-E/I-E*	-
180	1265	I-E*	-
181	347		-
182	15	I-E*	-
183	6		-
184	81		-
185	23	I-E*	-
186	23	I-E*	-
187	412		-

117	420	I-E*	-
213	423		+
214	423		+
215	423		+
216	423		+
217	423		+
103	455		-
49	461		-
127	490		-
41	494	I-E*	-
57	494	I-E*	-
58	494	I-E*	-
69	494	I-E*	-
71	494		-
100	494	I-E*	-
115	502	I-E*	-
122	502		-
173	580	I-E	-
108	584		-
48	592	I-E	-
120	592	I-E	-
204	612		-
47	626	I-E	-
64	655		-
26	660		-
123	661		-
128	661		-
23	686		-
2	690		-
46	690	I-E	-
167	692	I-E*	-
161	697	I-E	-
162	697	I-E	-
141	705		-
143	705		-
132	785		-
20	793	I-E*	-
172	814	I-E	-

373	11		+
376	11		+
377	11		+
379	11		+
380	11		+
383	11		+
384	11		+
385	11		+
386	11		+
387	11		+
388	11		+
390	11		+
391	11		+
393	11		+
394	11		+
395	11		+
396	11		+
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399	11		+
400	11		+
401	11		+
402	11		+
405	11		+
406	11		+
407	11		+
408	11		+
409	11		+
410	11		+
411	11		+
412	11		+
413	11		+
414	11		+
415	11		+
416	11		+
417	11		+
418	11		+
419	11		+

188	412		-
189	86		-
190	35	I-E*	-
191	23	I-E*	-
192	412	I-E*	-
193	23	I-E*	-
194	111	I-E*	-
195	412		-
196	412	I-E*	-
197	65		-
198	2158		-
199	ND		-
200	893	I-E	-
201	1536		-
202	65		-
203	25		-
204	612		-
205	ND	I-E	-
206	ND		-
207	ND		-
208	ND		-
209	ND	I-E*	-
210	ND		-
211	ND		-
212	ND		-
213	423		+
214	423		+
215	423		+
216	423		+
217	423		+
218	65		+
219	977		+
220	11		+
221	11		+
222	65		+
223	11		+
224	11		+
225	11		+

29	872	I-E	-
176	876	I-E	-
90	881		-
91	881		-
126	893	I-E	-
200	893	I-E	-
107	896		-
219	977		+
89	1031	I-E*	-
177	1265	I-E*	-
180	1265	I-E*	-
106	1326		-
27	1419		-
102	1419		-
104	1419		-
164	1446	I-E	-
163	1536		-
201	1536		-
179	1653	I-E/I-E*	-
83	1764		-
85	1770		-
124	1783	I-E*	-
99	1914		-
25	1933		-
33	1934	I-E*	-
75	1935		-
79	1936		-
82	1937	I-E	-
24	1938	I-E	-
40	1938	I-E	-
56	1939		-
60	1940		-
198	2158		-
165	2464		-
159	2695		-
81	ND		-
199	ND		-
205	ND	I-E	-

420	11		+
421	11		+
422	11		+
423	11		+
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425	11		+
426	11		+
427	11		+
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457	11		+

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259	11		+
260	11		+
261	11		+
262	11		+
263	11		+

206	ND		-
207	ND		-
208	ND		-
209	ND	I-E*	-
210	ND		-
211	ND		-
212	ND		-

458	11		+
459	11		+

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265	11		+
266	11		+
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345	81		+
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347	11		+
348	15	I-E*	+
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362	11		+
363	11		+
364	11		+
365	11		+
366	11		+
367	11		+
368	15	I-E*	+
369	15	I-E*	+
370	15	I-E*	+
371	11		+
372	11		+
373	11		+
374	15	I-E*	+
375	15	I-E*	+
376	11		+
377	11		+

378	15	I-E*	+
379	11		+
380	11		+
381	15	I-E*	+
382	15	I-E*	+
383	11		+
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388	11		+
389	15	I-E*	+
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392	15	I-E*	+
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402	11		+
403	15		+
404	23	I-E*	+
405	11		+
406	11		+
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454	11		+
455	11		+
456	11		+
457	11		+
458	11		+
459	11		+

Supplemental material legends

Fig S1. Schematics of identified CRISPR-cas systems in *K. pneumoniae.*

Fig S2 121 *bla*$_{KPC}$- positive plasmids in *K. pneumoniae.*

Fig S3. A total of 459 *K. pneumoniae* clinical isolates collected from six provinces of China with different carbapenem resistance rates.

Fig S4 (A) Characteristics of plasmid with different proto-spacers and PAMs. (B) Construction of *Escherichia coli* BW25113 without and with the CRISPR-Cas system mutant strain

Table S1. Strains and plasmids used in this study

Table S2. Oligonucleotides for cloning or PCR

Table S3. MICs for clones isolated in the plasmid stable assay.

Table S4. Proto-Spacer sequences matching KP8 CRISPR

Supplementary data1

Supplementary data 1a. 203 completely sequenced *K. pneumoniae* strains used in this study.

Supplementary data 1b. 121 *bla*$_{KPC}$- positive plasmids in *K. pneumoniae* used in this study.

Supplementary data 1c. 14 proto-spacers carried by *bla*$_{KPC}$- positive plasmids matched for the CRISPR system in *K. pneumoniae*.

Supplementary data 1d. 459 clinical isolates in China.

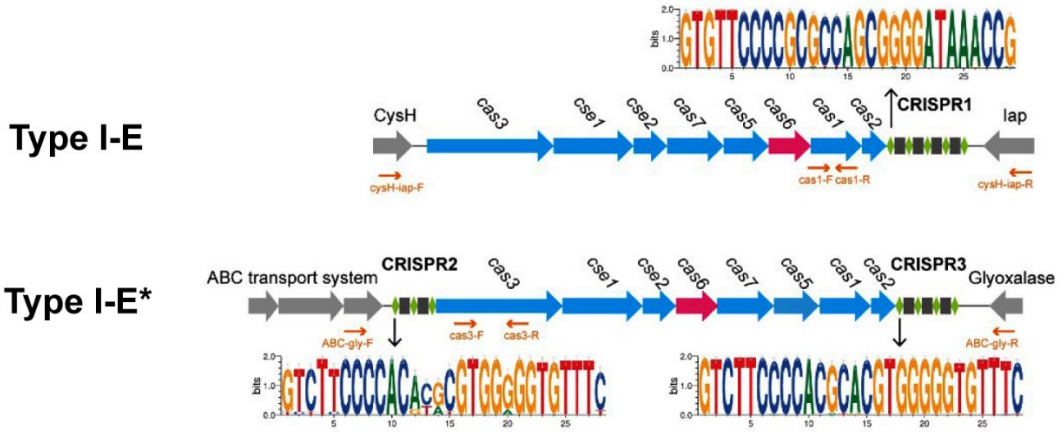


Fig S1. Schematics of identified CRISPR-cas systems in *K. pneumoniae.*

Genes are depicted as arrows in different colors. The green diamonds and dark grey rectangles indicate direct repeats (DRs) and spacers, respectively. Numbers and sizes of DRs and spacers are not to scale. The consensus sequences of the repeats were generated in WebLogo. The primers for CRISPR-Cas screen are shown as small and orange arrows.

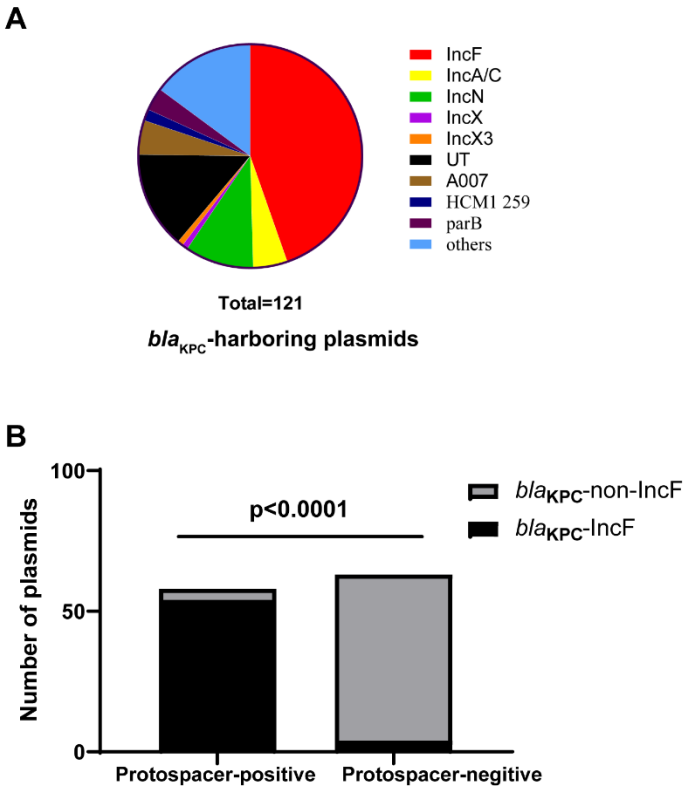


Fig S2 121 *bla*KPC- positive plasmids in *K. pneumoniae.* (A) The categories of 121 *bla*KPC- positive plasmids. (B) Plasmid incompatibility type (IncF and non-IncF) of *K. pneumoniae bla*KPC- positive plasmids in protospacer-positive and negative groups.

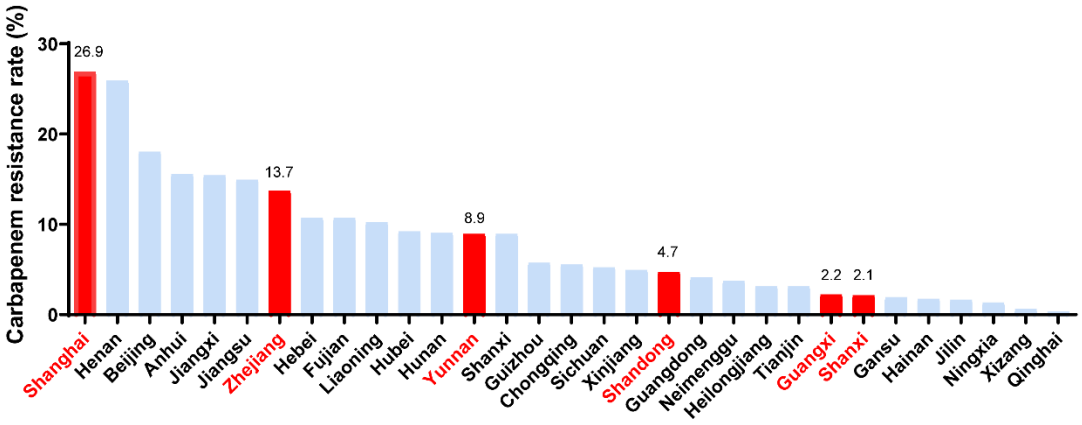


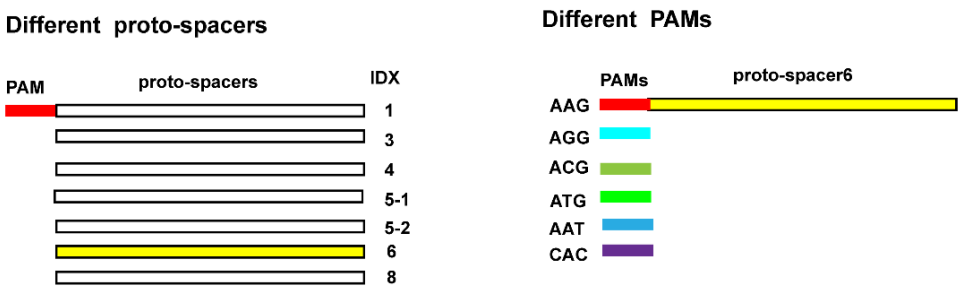
Fig S3. A total of 459 *K. pneumoniae* clinical isolates collected from six provinces of China with different carbapenem resistance rates.

Carbapenem resistance rates of *K. pneumoniae* among different provinces of China in 2017 was taken from data of China Antimicrobial Resistance Surveillance System (CARSS)*. The six red bars characterize the six provinces, from which our 459 strains collected and the resistance rate in these provinces typically represent high, medium and low levels. The variance distribution of resistance levels in the six provinces ensures the universality of our research results.

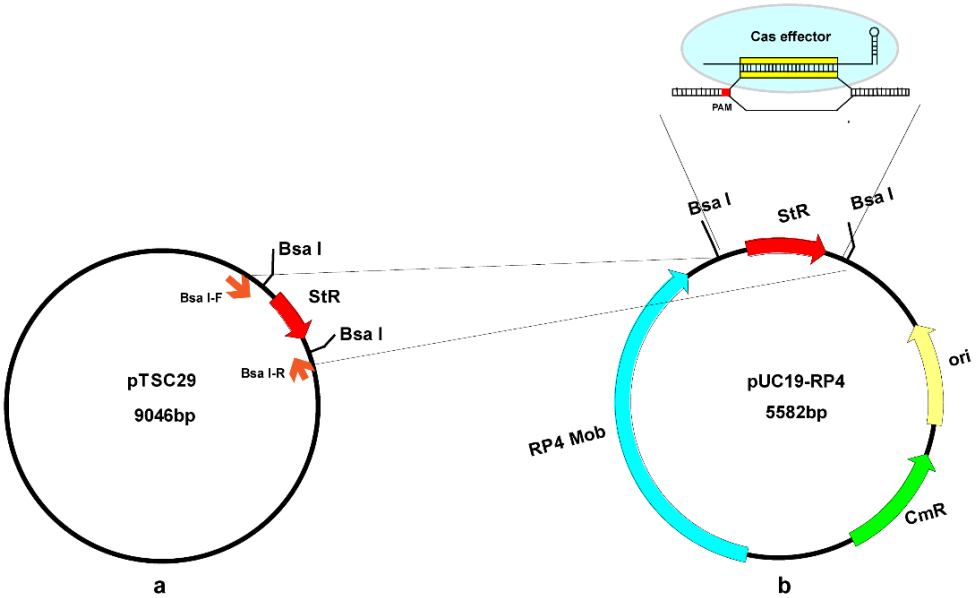
* http://www.carss.cn/Report/Details/552

A

i


Different proto-spacers
PAM
proto-spacers
IDX
1
3
4
5-1
5-2
6
8
Different PAMs
PAMs
proto-spacer6
AAG
AGG
ACG
ATG
AAT
CAC

ii


Cas effector
PAM
Bsa I
StR
Bsa I
Bsa I-F
Bsa I-R
pTSC29
9046bp
a
Bsa I
StR
Bsa I
ori
pUC19-RP4
5582bp
RP4 Mob
CmR
b

B

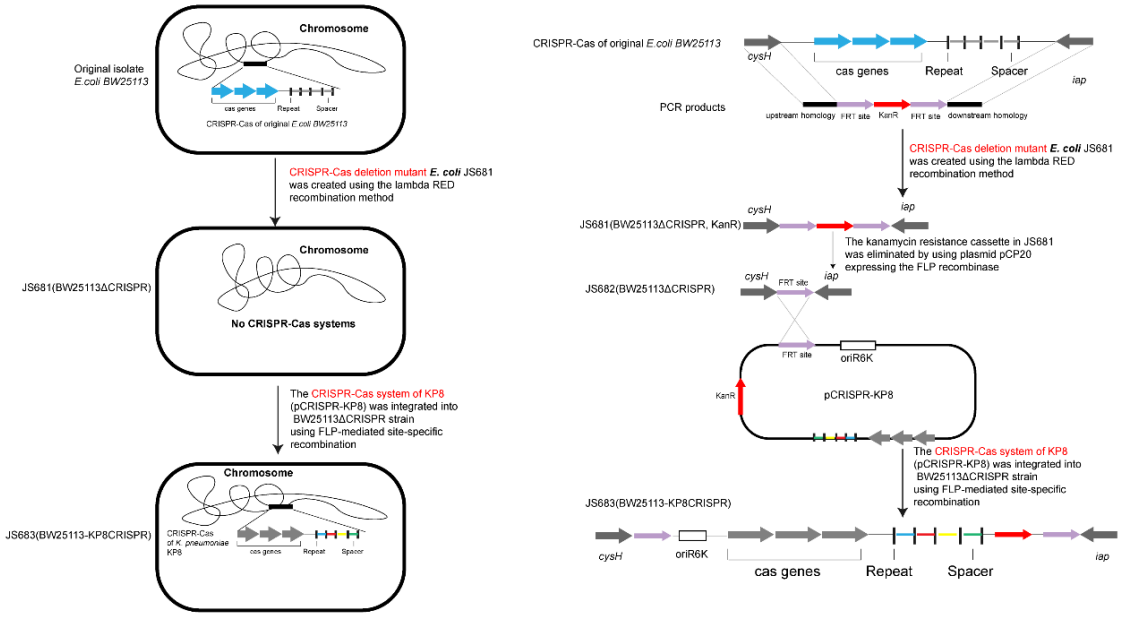

Original isolate
E.coli BW25113
Chromosome
CRISPR-Cas of original E.coli BW25113
CRISPR-Cas deletion mutant E. coli JS681 was created using the lambda RED recombination method
JS681(BW25113ΔCRISPR)
Chromosome
No CRISPR-Cas systems
The CRISPR-Cas system of KP8 (pCRISPR-KP8) was integrated into BW25113ΔCRISPR strain using FLP-mediated site-specific recombination
JS683(BW25113-KP8CRISPR)
Chromosome
CRISPR-Cas of K. pneumoniae KP8
cas genes
Repeat
Spacer
CRISPR-Cas of original E.coli BW25113
cysH
cas genes
Repeat
Spacer
iap
PCR products
upstream homology
FRT site
KanR
FRT site
downstream homology
CRISPR-Cas deletion mutant E. coli JS681 was created using the lambda RED recombination method
JS681(BW25113ΔCRISPR, KanR)
The kanamycin resistance cassette in JS681 was eliminated by using plasmid pCP20 expressing the FLP recombinase
JS682(BW25113ΔCRISPR)
FRT site
oriR6K
KanR
pCRISPR-KP8
The CRISPR-Cas system of KP8 (pCRISPR-KP8) was integrated into BW25113ΔCRISPR strain using FLP-mediated site-specific recombination
JS683(BW25113-KP8CRISPR)
cysH
oriR6K
cas genes
Repeat
Spacer
iap

Fig S4 (A) Characteristics of plasmid with different proto-spacers and PAMs. The plasmid pTSC29 was used as a template (primers are shown as small and orange arrows) for amplifying the *Bas I* restriction site, in which the proto-spacers (i)were inserted. The RP4 mob (ii) cloned from the pJTOOL-3 functioned for plasmid mobilization. **(B) Construction of *Escherichia coli* BW25113 without and with the CRISPR-Cas system mutant strain**

Table S1. Strains and plasmids used in this study

Strains/plasmids	Relevant characteristics	Source or reference
K. pneumoniae		
KP8	*K. pneumoniae* ST458(non-CC258) clinical isolates containing CRISPR-Cas system (containing spacer1, 3, 4, 5, 6 and 8).	**CP025636.1**
JS686	KP8 derivative (CAM^R) for allelic replacement of *cas3* protein with chloramphenicol resistance cassette flanked by FRT sites	This study
JS687	KP8-ΔCas3 derivative, eliminates chloramphenicol resistance cassette by using FLP recombinase and contains an FRT site only	This study
E. coli		
BW25113	DE (araD-araB)567 lacZ4787(del)(::rrnB-3) lacIp-4000(lacI^Q) LAM- rph-1 DE(rhaD-rhaB)568 rrnB-3 hsdR514	23
DH5αpir	endA1 hsdR17 glnV44 (= supE44) thi-1 recA1 gyrA96 relA1 φ80dlacΔ(lacZ)M15 Δ(lacZYA-argF) U169 zdg-232::Tn10 uidA::pir+	Lab stock
S17-1 λpir	TpR SmR *recA, thi, pro, hsd*R-M+RP4: 2-Tc: Mu: KmR Tn*7* λ*pir*	25
MG1655	F- lambda- ilvG- rfb-50 rph-1	Lab stock
JS681	BW25113 derivative (KAN^R) for allelic replacement of E. *coli* CRISPR-Cas system with kanamycin resistance cassette flanked by FRT sites	This study
JS682	BW25113-ΔCRISPR derivative, eliminates kanamycin resistance cassette by using FLP recombinase and contains an FRT site only	This study

JS683	BW25113-ΔCRISPR: FRT derivative (KAN^R), contains CRISPR-Cas of KP8 integrated in the FRT site	This study
JS531	DH5α contains plasmid p187-2, an IncFII conjugative plasmid harbouring *bla*KPC and matches for spacer1, 3, 4, 5 and 6 recovered in K. pneumoniae. Accession number of p187-2: CP025468.1	This study
Plasmids		
p187-2	an IncFII conjugative plasmid harbouring bla_{KPC} and matches for spacer1, 3, 4, 5 and 6 recovered in *K. pneumoniae*	**CP025468.1**
pKD4	AMP^R KAN^R, kanamycin cassette flanked by FRT sites template, pir-dependent	23
pKD3	AMP^R CAM^R, chloramphenicol cassette flanked by FRT sites template, pir-dependent	23
pKD46	AMP^R, λ Red recombinase expression, 30°C	23
pKOBEG	Apr^R, λ Red recombinase expression, 30°C	Lab stock
pCP20	AMP^R CAM^R, FLP recombinase expression, 30°C	25
pCP20-KAN	AMP^R KAN^R, FLP recombinase expression, 30°C	This study
pUC19	AMP^R, ori pMB1	Lab stock
pUC19-CAM	CAM^R, ori pMB1	This study
pUC19-Apr	Apr^R, ori pMB1	This study
pJTOOL-3	CAM^R, RP4-specific Mob site template	25
PTSC29	STR^R CAM^R, *Bsa I* restriction site template	Lab stock
pCRISPR-KP8	KAN^R, pKD4 derivative containing CRISPR-Cas system of KP8 and one FRT site, pir-dependent	This study
pUC-RP4	CAM^R, ori pMB1, pUC19 derivative, inserts *Bsa I* restriction site and RP4-specific Mob site by In-Fusion Cloning	This study

Table S2. Oligonucleotides for cloning or PCR

Name	Sequence[a]
For CRISPR-Cas screening	
cysH-iap-F	CGGTTCTTCGGGCTTAAACG
cysH-iap-R	CTGCTGCAATGACGCCAG
ABC-gly-F	TGTTCGCCGCTGAGTTTATG
ABC-gly-R	TACCACGCCAGTTACTACGC
cas1-F	CTTTTGGCACGACGGAATCA
cas1-R	TGGCGCTGGATGATGATTTG
cas3-F	GTCCCGACTAAAATGCGTCC
cas3-R	CGTTGATGGCGGTGATGAAT
For *bla*$_{KPC}$ Screening	
bla$_{KPC}$-F	TCGCTAAACTCGAACAGG
bla$_{KPC}$-R	TTACTGCCCGTTGACGCCCAATCC
For constructing PUC-protospacer	
PUC-bsaI-F	GTTGGCGGGTGTCGGGGCGCAGCCA CTTGCCACCAGTGATGCGG
PUC-bsaI-R	TGCAGGTCGACTCTAGAGGATCCCC ATCCGTTTCCACGGTGTGC
RP4-F	GCCAGTGAATTCGAGCTCGGTACCC GATCCAGCCGACCAGGCTTT
RP4-R	TGGCTGCGCCCCGACACCCG
proto-spacer1-F	TTCG AAGCAGACAGACAGCAGGCAGCAAACAGGGAAGACGCGGA
proto-spacer1-R	CAAG TCCGCGTCTTCCCTGTTTGCTGCCTGCTGTCTGTCTGCTT
proto-spacer3-F	TTCG AAGGTGGTTTGTTACCGTGTTGTGTGGCAAAAAGCAGAAA
proto-spacer3-R	CAAG TTTCTGCTTTTTGCCACACAACACGGTAACAAACCACCTT
proto-spacer4-F	TTCG AAGGAACGGAGGAATATAAGAACAAAAGCCCGCAGAGAAA

proto-spacer4-R	CAAG TTTCTCTGCGGGCTTTTGTTCTTATATTCCTCCGTTCCTT
proto-spacer5-1-F	TTCG GAAAGTTATATCCAGGGGGCAGGTTCAGCAGGTCCCCGCACA
proto-spacer5-1-R	CAAG TGTGCGGGGACCTGCTGAACCTGCCCCCTGGATATAACTTTC
proto-spacer5-2-F	TTCG AAGTTAATACCAGGGGGCAGGTTCAGCAGGTCCCCGCA
proto-spacer5-2-R	CAAG TGCGGGGACCTGCTGAACCTGCCCCCTGGTATTAACTT
proto-spacer6-F	TTCG AAGCGATAACAGGGCGTTTCGACTGAACTCACCTCCCCCT
proto-spacer6-R	CAAG AGGGGGAGGTGAGTTCAGTCGAAACGCCCTGTTATCGCTT
proto-spacer8-F	TTCG AAGTCGTCTGAGTTCCGGCTTACGCCGTGCCGACACGA
proto-spacer8-R	CAAG TCGTGTCGGCACGGCGTAAGCCGGAACTCAGACGACTT
none-proto-spacer6-F	TTCGCGATAACAGGGCGTTTCGACTGAACTCACCTC
none-proto-spacer6-R	CAAG GAGGTGAGTTCAGTCGAAACGCCCTGTTATCG
AAT-proto-spacer6-F	TTCGAATCGATAACAGGGCGTTTCGACTGAACTCACCTC
AAT-proto-spacer6-R	CAAG GAGGTGAGTTCAGTCGAAACGCCCTGTTATCGATT
AGG-proto-spacer6-F	TTCGAGGCGATAACAGGGCGTTTCGACTGAACTCACCTC
AGG-proto-spacer6-R	CAAG GAGGTGAGTTCAGTCGAAACGCCCTGTTATCGCCT
ATG-proto-spacer6-F	TTCGATGCGATAACAGGGCGTTTCGACTGAACTCACCTC
ATG-proto-spacer6-R	CAAG GAGGTGAGTTCAGTCGAAACGCCCTGTTATCGCAT
ACG-proto-spacer6-F	TTCGACGCGATAACAGGGCGTTTCGACTGAACTCACCTC
ACG-proto-spacer6-R	CAAG GAGGTGAGTTCAGTCGAAACGCCCTGTTATCGCGT
CAC-proto-spacer6-F	TTCGCACCGATAACAGGGCGTTTCGACTGAACTCACCTC
CAC-proto-spacer6-R	CAAG GAGGTGAGTTCAGTCGAAACGCCCTGTTATCGGTG
For constructing PUC-two-protospacer	
Xba I -HindIII-proto-spacer5-1-F	CTAG GAAGGTTATATCCAGGGGGCAGGTTCAGCAGGTCCCCGCACA
Xba I -HindIII-proto-spacer5-1-R	AGCT TGTGCGGGGACCTGCTGAACCTGCCCCCTGGATATAACCTTC
Xba I -HindIII-proto-spacer4-F	CTAG GGAAGGAACGGAGGAATATAAGAACAAAAGCCCGCAGAGAAA
Xba I -HindIII-proto-spacer4-R	AGCT TTTCTCTGCGGGCTTTTGTTCTTATATTCCTCCGTTCCTTCC

For constructing KP8 Cas3-deletion	
Upstream of Cas3-F	GCGCGGTTGTCTCCTCTATT
Upstream of Cas3-R	GAAGCAGCTCCAGCCTACAC TGCGGCGACATCAAGGGAAT
Downstream of Cas3-F	GGACCATGGCTAATTCCCAT GCAGCTAACTACATGAATAC
Downstream of Cas3-R	CGGGCCAAACTGAAAGGCAG
Cam-FRT-F	GTGTAGGCTGGAGCTGCTTC
Cam-FRT-R	ATGGGAATTAGCCATGGTCC
For constructing *E. coli* BW25113 CRISPR-deletion	
upstream of CRISPR-F	GGAATGGATGATAACGCCGC
upstream of CRISPR-R	GAAGCAGCTCCAGCCTACACTCATGCCAGCTATTTCCCGC
Kan with FRT-F	GTGTAGGCTGGAGCTGCTTC
Kan with FRT-R	CCATATGAATATCCTCCTT
downstream of CRISPR-F	AAGGAGGATATTCATATGGCAGCACCGGTAAATTGGCAC
downstream of CRISPR-R	TCGATCTAAACGCCCTGAACG
For constructing pCRISPR-KP8	
PKD4-F	AAATATCGCTACCTGCCGCTCTAGCGATAT ACTGGGCTATCTGGACAAGG
PKD4-R	GGGATTAAATAATAGAGGAGACAACCGCGC GCAAGATCCGCAGTTCAACC
KP8-CRISPR1-F	ATATCGCTAGAGCGGCAGG
KP8-CRISPR1-R	GAGGTACTTCGCCAGGCTTA
KP8-CRISPR2-F	GCTGTCGCAGAGAAAGCCTC
KP8-CRISPR2-R	GCGCGGTTGTCTCCTCTATT

[a] underlines sequences were the overlaps used for SOE-PCR or plasmid construction; the red bases were the PAM seque

Table S3. MICs for clones isolated in the plasmid stable assay.

Strains	Passage Number	MIC (mg/L)	
		Imipenem	Chloramphenicol
[1]p187-2-JS683	10	⩽0.25	——
p187-2-JS681	10	⩾16	——
[2]pUC-proto-spacer6-KP8	3	——	8
pUC-proto-spacer6-JS687(KP8 Δ Cas3)	3	——	＞128
pUC-Empty-KP8	3	——	＞128
pUC-Empty-JS687	3	——	＞128
pUC-proto-spacer6-JS683	6	——	8
pUC-proto-spacer6-JS681	6	——	＞128
pUC-Empty-JS683	6	——	＞128
pUC Empty-JS681	6	——	＞128
Control			
JS683	——	⩽0.25	8
JS681	——	⩽0.25	8
KP8	——	——	8
JS687	——	——	8

Note

[1]these results indicated the imipenem sensitivity in both two strains (JS683 and JS681) after 10 times passages in LB broth, which consistent with the Fig 3A

[2]these results indicated the chloramphenicol sensitivity in *K. pneumonia* (KP8 and JS687) after 3 times passages (Fig 3Bi) and corresponding MICs in *E. coli* (JS683 and JS681) after 6 times passages (Fig.3Bii)

Table S4. Proto-Spacer sequences matching KP8 CRISPR

Name of proto-spacers	Sequence (5' to 3') [a]	Plasmid	Accession number	GC Content
proto-spacer1	CAGACAGACAGCAGGCAGCAAACAGGGAAGAC	p187-2	CP025468.1	56%
proto-spacer3	GTGGTTTGTTACCGTGTTGTGTGGCAAAAAGC	p187-2	CP025468.1	47%
protospacer4	GAACGGAGGAATATAAGAACAAAAGCCCGCAG	p187-2	CP025468.1	47%
proto-spacer5-1	TT**ATAT**CCAGGGGGCAGGTTCAGCAGGTCCCC	p187-2	CP025468.1	59%
proto-spacer5-2	TT**AATA**CCAGGGGGCAGGTTCAGCAGGTCCCC	p0716-KPC	KY270849.1	59%
proto-spacer6	CGATAACAGGGCGTTTCGACTGAACTCACCTC	p187-2	CP025468.1	56%
proto-spacer8	TCGTCTGAGTTCCGGCTTACGCCGTGCCGACA	p12139-KPC-2	MF168403.1	63%

[a] red bases represent the mutations.

Table S1. Strains and plasmids used in this study

Strains/plasmids	Relevant characteristics	Source or reference
K. pneumoniae		
KP8	*K. pneumoniae* ST458(non-CC258) clinical isolates containing CRISPR-Cas system (containing spacer1, 3, 4, 5, 6 and 8).	**CP025636.1**
JS686	KP8 derivative (CAM^R) for allelic replacement of *cas3* protein with chloramphenicol resistance cassette flanked by FRT sites	This study
JS687	KP8-ΔCas3 derivative, eliminates chloramphenicol resistance cassette by using FLP recombinase and contains an FRT site only	This study
E. coli		
BW25113	DE (araD-araB)567 lacZ4787(del)(::rrnB-3) lacIp-4000(lacI^Q) LAM- rph-1 DE(rhaD-rhaB)568 rrnB-3 hsdR514	23
DH5αpir	endA1 hsdR17 glnV44 (= supE44) thi-1 recA1 gyrA96 relA1 φ80dlacΔ(lacZ)M15 Δ(lacZYA-argF) U169 zdg-232::Tn10 uidA::pir+	Lab stock
S17-1 λpir	TpR SmR *recA, thi, pro, hsd*R-M+RP4: 2-Tc: Mu: KmR Tn*7* λ*pir*	25
MG1655	F- lambda- ilvG- rfb-50 rph-1	Lab stock
JS681	BW25113 derivative (KAN^R) for allelic replacement of E. *coli* CRISPR-Cas system with kanamycin resistance cassette flanked by FRT sites	This study
JS682	BW25113-ΔCRISPR derivative, eliminates kanamycin resistance cassette by using FLP recombinase and contains an FRT site only	This study

JS683	BW25113-ΔCRISPR: FRT derivative (KAN^R), contains CRISPR-Cas of KP8 integrated in the FRT site	This study
JS531	DH5α contains plasmid p187-2, an IncFII conjugative plasmid harbouring *bla*KPC and matches for spacer1, 3, 4, 5 and 6 recovered in K. pneumoniae. Accession number of p187-2: CP025468.1	This study
Plasmids		
p187-2	an IncFII conjugative plasmid harbouring bla_{KPC} and matches for spacer1, 3, 4, 5 and 6 recovered in *K. pneumoniae*	**CP025468.1**
pKD4	AMP^R KAN^R, kanamycin cassette flanked by FRT sites template, pir-dependent	23
pKD3	AMP^R CAM^R, chloramphenicol cassette flanked by FRT sites template, pir-dependent	23
pKD46	AMP^R, λ Red recombinase expression, 30°C	23
pKOBEG	Apr^R, λ Red recombinase expression, 30°C	Lab stock
pCP20	AMP^R CAM^R, FLP recombinase expression, 30°C	25
pCP20-KAN	AMP^R KAN^R, FLP recombinase expression, 30°C	This study
pUC19	AMP^R, ori pMB1	Lab stock
pUC19-CAM	CAM^R, ori pMB1	This study
pUC19-Apr	Apr^R, ori pMB1	This study
pJTOOL-3	CAM^R, RP4-specific Mob site template	25
PTSC29	STR^R CAM^R, *Bsa I* restriction site template	Lab stock
pCRISPR-KP8	KAN^R, pKD4 derivative containing CRISPR-Cas system of KP8 and one FRT site, pir-dependent	This study
pUC-RP4	CAM^R, ori pMB1, pUC19 derivative, inserts *Bsa I* restriction site and RP4-specific Mob site by In-Fusion Cloning	This study

Table S2. Oligonucleotides for cloning or PCR

Name	Sequence[a]
For CRISPR-Cas screening	
cysH-iap-F	CGGTTCTTCGGGCTTAAACG
cysH-iap-R	CTGCTGCAATGACGCCAG
ABC-gly-F	TGTTCGCCGCTGAGTTTATG
ABC-gly-R	TACCACGCCAGTTACTACGC
cas1-F	CTTTTGGCACGACGGAATCA
cas1-R	TGGCGCTGGATGATGATTTG
cas3-F	GTCCCGACTAAAATGCGTCC
cas3-R	CGTTGATGGCGGTGATGAAT
For *bla*$_{KPC}$ Screening	
bla$_{KPC}$-F	TCGCTAAACTCGAACAGG
bla$_{KPC}$-R	TTACTGCCCGTTGACGCCCAATCC
For constructing PUC-protospacer	
PUC-bsaI-F	GTTGGCGGGTGTCGGGGCGCAGCCA CTTGCCACCAGTGATGCGG
PUC-bsaI-R	TGCAGGTCGACTCTAGAGGATCCCC ATCCGTTTCCACGGTGTGC
RP4-F	GCCAGTGAATTCGAGCTCGGTACCC GATCCAGCCGACCAGGCTTT
RP4-R	TGGCTGCGCCCCGACACCCG
proto-spacer1-F	TTCG AAGCAGACAGACAGCAGGCAGCAAACAGGGAAGACGCGGA
proto-spacer1-R	CAAG TCCGCGTCTTCCCTGTTTGCTGCCTGCTGTCTGTCTGCTT
proto-spacer3-F	TTCG AAGGTGGTTTGTTACCGTGTTGTGTGGCAAAAAGCAGAAA
proto-spacer3-R	CAAG TTTCTGCTTTTTGCCACACAACACGGTAACAAACCACCTT
proto-spacer4-F	TTCG AAGGAACGGAGGAATATAAGAACAAAAGCCCGCAGAGAAA

proto-spacer4-R	CAAG TTTCTCTGCGGGCTTTTGTTCTTATATTCCTCCGTTCCTT
proto-spacer5-1-F	TTCG GAAAGTTATATCCAGGGGGCAGGTTCAGCAGGTCCCCGCACA
proto-spacer5-1-R	CAAG TGTGCGGGGACCTGCTGAACCTGCCCCCTGGATATAACTTTC
proto-spacer5-2-F	TTCG AAGTTAATACCAGGGGGCAGGTTCAGCAGGTCCCCGCA
proto-spacer5-2-R	CAAG TGCGGGGACCTGCTGAACCTGCCCCCTGGTATTAACTT
proto-spacer6-F	TTCG AAGCGATAACAGGGCGTTTCGACTGAACTCACCTCCCCCT
proto-spacer6-R	CAAG AGGGGGAGGTGAGTTCAGTCGAAACGCCCTGTTATCGCTT
proto-spacer8-F	TTCG AAGTCGTCTGAGTTCCGGCTTACGCCGTGCCGACACGA
proto-spacer8-R	CAAG TCGTGTCGGCACGGCGTAAGCCGGAACTCAGACGACTT
none-proto-spacer6-F	TTCGCGATAACAGGGCGTTTCGACTGAACTCACCTC
none-proto-spacer6-R	CAAG GAGGTGAGTTCAGTCGAAACGCCCTGTTATCG
AAT-proto-spacer6-F	TTCGAATCGATAACAGGGCGTTTCGACTGAACTCACCTC
AAT-proto-spacer6-R	CAAG GAGGTGAGTTCAGTCGAAACGCCCTGTTATCGATT
AGG-proto-spacer6-F	TTCGAGGCGATAACAGGGCGTTTCGACTGAACTCACCTC
AGG-proto-spacer6-R	CAAG GAGGTGAGTTCAGTCGAAACGCCCTGTTATCGCCT
ATG-proto-spacer6-F	TTCGATGCGATAACAGGGCGTTTCGACTGAACTCACCTC
ATG-proto-spacer6-R	CAAG GAGGTGAGTTCAGTCGAAACGCCCTGTTATCGCAT
ACG-proto-spacer6-F	TTCGACGCGATAACAGGGCGTTTCGACTGAACTCACCTC
ACG-proto-spacer6-R	CAAG GAGGTGAGTTCAGTCGAAACGCCCTGTTATCGCGT
CAC-proto-spacer6-F	TTCGCACCGATAACAGGGCGTTTCGACTGAACTCACCTC
CAC-proto-spacer6-R	CAAG GAGGTGAGTTCAGTCGAAACGCCCTGTTATCGGTG
For constructing PUC-two-protospacer	
Xba I -HindIII-proto-spacer5-1-F	CTAG GAAGGTTATATCCAGGGGGCAGGTTCAGCAGGTCCCCGCACA
Xba I -HindIII-proto-spacer5-1-R	AGCT TGTGCGGGGACCTGCTGAACCTGCCCCCTGGATATAACCTTC
Xba I -HindIII-proto-spacer4-F	CTAG GGAAGGAACGGAGGAATATAAGAACAAAAGCCCGCAGAGAAA
Xba I -HindIII-proto-spacer4-R	AGCT TTTCTCTGCGGGCTTTTGTTCTTATATTCCTCCGTTCCTTCC

For constructing KP8 Cas3-deletion	
Upstream of Cas3-F	GCGCGGTTGTCTCCTCTATT
Upstream of Cas3-R	GAAGCAGCTCCAGCCTACAC TGCGGCGACATCAAGGGAAT
Downstream of Cas3-F	GGACCATGGCTAATTCCCAT GCAGCTAACTACATGAATAC
Downstream of Cas3-R	CGGGCCAAACTGAAAGGCAG
Cam-FRT-F	GTGTAGGCTGGAGCTGCTTC
Cam-FRT-R	ATGGGAATTAGCCATGGTCC
For constructing *E. coli* BW25113 CRISPR-deletion	
upstream of CRISPR-F	GGAATGGATGATAACGCCGC
upstream of CRISPR-R	GAAGCAGCTCCAGCCTACACTCATGCCAGCTATTTCCCGC
Kan with FRT-F	GTGTAGGCTGGAGCTGCTTC
Kan with FRT-R	CCATATGAATATCCTCCTT
downstream of CRISPR-F	AAGGAGGATATTCATATGGCAGCACCGGTAAATTGGCAC
downstream of CRISPR-R	TCGATCTAAACGCCCTGAACG
For constructing pCRISPR-KP8	
PKD4-F	AAATATCGCTACCTGCCGCTCTAGCGATAT ACTGGGCTATCTGGACAAGG
PKD4-R	GGGATTAAATAATAGAGGAGACAACCGCGC GCAAGATCCGCAGTTCAACC
KP8-CRISPR1-F	ATATCGCTAGAGCGGCAGG
KP8-CRISPR1-R	GAGGTACTTCGCCAGGCTTA
KP8-CRISPR2-F	GCTGTCGCAGAGAAAGCCTC
KP8-CRISPR2-R	GCGCGGTTGTCTCCTCTATT

[a] underlines sequences were the overlaps used for SOE-PCR or plasmid construction; the red bases were the PAM seque

Table S3. MICs for clones isolated in the plasmid stable assay.

Strains	Passage Number	MIC (mg/L)	
		Imipenem	Chloramphenicol
[1]p187-2-JS683	10	⩽0.25	——
p187-2-JS681	10	⩾16	——
[2]pUC-proto-spacer6-KP8	3	——	8
pUC-proto-spacer6-JS687(KP8 Δ Cas3)	3	——	＞128
pUC-Empty-KP8	3	——	＞128
pUC-Empty-JS687	3	——	＞128
pUC-proto-spacer6-JS683	6	——	8
pUC-proto-spacer6-JS681	6	——	＞128
pUC-Empty-JS683	6	——	＞128
pUC Empty-JS681	6	——	＞128
Control			
JS683	——	⩽0.25	8
JS681	——	⩽0.25	8
KP8	——	——	8
JS687	——	——	8

Note

[1]these results indicated the imipenem sensitivity in both two strains (JS683 and JS681) after 10 times passages in LB broth, which consistent with the Fig 3A

[2]these results indicated the chloramphenicol sensitivity in *K. pneumonia* (KP8 and JS687) after 3 times passages (Fig 3Bi) and corresponding MICs in *E. coli* (JS683 and JS681) after 6 times passages (Fig.3Bii)

Table S4. Proto-Spacer sequences matching KP8 CRISPR

Name of proto-spacers	Sequence (5' to 3') [a]	Plasmid	Accession number	GC Content
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proto-spacer3	GTGGTTTGTTACCGTGTTGTGTGGCAAAAAGC	p187-2	CP025468.1	47%
protospacer4	GAACGGAGGAATATAAGAACAAAAGCCCGCAG	p187-2	CP025468.1	47%
proto-spacer5-1	TT**ATAT**CCAGGGGGCAGGTTCAGCAGGTCCCC	p187-2	CP025468.1	59%
proto-spacer5-2	TT**AATA**CCAGGGGGCAGGTTCAGCAGGTCCCC	p0716-KPC	KY270849.1	59%
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proto-spacer8	TCGTCTGAGTTCCGGCTTACGCCGTGCCGACA	p12139-KPC-2	MF168403.1	63%

[a] red bases represent the mutations.