AAC00076-17 Supplemental Materials

Table S1. Antimicrobial resistance genes identified in the whole genome sequences of three *K. pneumoniae* isolates, in either chromosome (Chr) or plasmids

Resistance Gene	Phenotype	CR14	NY9	CN1
aacA4	Aminoglycoside resistance	pCR14_2		
aac(3)-IIa		pCR14_2		pCN1_1
aac(3)-IVa			Chr	
aac(6')-Ib		pCR14_4, pCR14_5	pNY9_2	pCN1_2
aac(6')Ib-cr	Fluoroquinolone and aminoglycoside resistance	pCR14_2, pCR14_4, pCR14_5	pNY9_2, pNY9_3	pCN1_1, pCN1_2
aadA1	Aminoglycoside resistance		Chr, pNY9_2	
aadA2			Chr, pNY9_1	
aph(3')-Ia			pNY9_1	
aph(4)-Ia			Chr	
bla$_{\text{CTX-M-2}}$	β-lactam resistance	pCR14_2		
bla$_{\text{CTX-M-15}}$			pNY9_3	Chr (x3)
bla$_{\text{KPC-2}}$		pCR14_3		Chr
bla$_{\text{KPC-3}}$			pNY9_2	
bla$_{\text{OXA-1}}$			pNY9_3	pCN1_1
bla$_{\text{OXA-2}}$		pCR14_2		
bla$_{\text{OXA-9}}$		pCR14_3	pNY9_2	
bla$_{\text{SHV-11}}$			Chr	Chr
bla$_{\text{TEM-1A}}$		pCR14_3	pNY9_2	
bla$_{\text{TEM-1B}}$		pCR14_2	pNY9_3	
catA1	Phenicol resistance	pCR14_1		
catB3			pNY9_3	pCN1_1
cmlA1			Chr	
dfrA12	Trimethoprim resistance		pNY9_1	
dfrA14			pNY9_2	pCN1_1
fosA	Fosfomycin resistance			Chr
mph(A)	Macrolide resistance	pCR14_1	pNY9_1	
oqxAB	Quinolone resistance	Chr	Chr	Chr
QnrB66				pCN1_1
sul1	Sulfonamide resistance	pCR14_2 (x2)	pNY9_1	
sul2			pNY9_2	
sul3			Chr	
strAB	Aminoglycoside resistance		pNY9_2	
tet(A)	Tetracycline resistance		pNY9_3	pCN1_1

Table S2. Antimicrobial susceptibility profiles of the three *K. pneumoniae* isolates

Antimicrobial agent	MIC (μg/mL) (interpretation) for isolate[a]		
	CR14	NY9	CN1
Ampicillin	>16 (R)	>16 (R)	>16 (R)
Cefoxitin	>32 (R)	>32 (R)	>32 (R)
Cefotaxime	>256 (R)	>256 (R)	>256 (R)
Ceftazidime	96 (R)	>256 (R)	64 (R)
Ciprofloxacin	>2 (R)	>2 (R)	>2 (R)
Levofloxacin	>4 (R)	>4 (R)	>4 (R)
Meropenem	32 (R)	32 (R)	32 (R)
Imipenem	32 (R)	8 (R)	8 (R)
Gentamicin	>8 (R)	>8 (R)	>8 (R)
Tetracycline	<=4 (S)	>8 (R)	>8 (R)
Trimethoprim-sulfamethoxazole	>2/38 (R)	>2/38 (R)	>2/38 (R)
Tigecycline	3.0 (I)	2.0 (S)	1.5 (S)
Colistin[b]	0.19 (S)	0.125 (S)	0.094 (S)

[a] MIC: minimum inhibitory concentration; R: resistant; I: intermediate; S: susceptible.

[b] No clinical breakpoint has been defined by the Clinical and Laboratory Standards Institute; we thus utilized the European breakpoint (S ≤ 2μg/mL) published by the European Committee on Antimicrobial Susceptibility Testing for *Enterobacteriaceae*.

Table S3. Whole-genome comparison of two *K. pneumoniae* isolates, CN1 and KPNIH31

	Genome composition for isolate			
	CN1 (bp)		KPNIH31 (bp)	
Chromosome	5,237,856		5,228,295	
Plasmids	pCN1_1	182,846	pKPN-c22	178,563
	pCN1_2	15,100	pAAC154-a9e	15,100
	-	-	pKPN-852	51,622

Table S4. Characteristics of CRISPR-*cas* system along with β-lactamase-encoding genes in chromosomes of 22 *K. pneumoniae* strains with complete genome sequences available from GenBank

No.	Strain	Collection Date	Location	CRISPR-cas	Start	End	Spacers	Chromosomal β-lactamase
1	NTUH-K2044	1996-2001	Taiwan, China	CRISPR	3001234	3002603	22	SHV-36
				cas operon	3002651	3010944		
				CRISPR	3011356	3011567	3	
2	1084	N/A	Taiwan, China	CRISPR	2319792	2320306	8	SHV-36
				cas operon	2320939	2329014		
				CRISPR	2329062	2329943	14	
3	ATCC 43816 KPPR1	N/A	USA	CRISPR	383812	384388	9	SHV-1
				cas operon	385020	393094		
				CRISPR	393142	393666	8	
4	U25	2010/10	Chennai, India	CRISPR	3265852	3266619	12	SHV-28; OXA-1
				cas operon	3266667	3274960		
				CRISPR	3275372	3275950	9	
5	PMK1	2011/8	Nepal	CRISPR	4766150	4766857	11	SHV-28; CTX-M-15
				cas operon	4766905	4775198		
				CRISPR	4775610	4776005	6	
6	PittNDM01	2013/3	PA, USA	CRISPR	1170138	1170905	12	SHV-28; OXA-1
				cas operon	1170953	1179246		
				CRISPR	1179658	1180236	9	
7	KP617	2013/5	Seoul, Korea	CRISPR	554714	555292	9	SHV-28; OXA-1
				cas operon	555704	565197		
				CRISPR	565245	566012	12	
8	J1	2014/10	Harbin, China	CRISPR	5072070	5073012	15	SHV-11
				cas operon	5073060	5081353		
				CRISPR	5081766	5082220	7	
9	NUHL24835	2014/9	Jiangxi, China	CRISPR	539827	540405	9	SHV-28
				cas operon	540817	549110		
				CRISPR	549158	550108	15	
10	RJF293	2014/9	Shanghai, China	CRISPR	2991230	2991753	8	SHV-1
				cas operon	2991801	3001158		
				CRISPR	3001570	3001903	5	

11	RJF999	2015/1	Shanghai, China	CRISPR	3052030	3053704	27	SHV-36
				cas operon	3053752	3062045		
				CRISPR	3062458	3063035	9	
12	Kp52.145	N/A	France	*cas* operon	1035623	1044066		-
				CRISPR	1044163	1044802	10	
				CRISPR	1044875	1045391	8	
13	SKGH01	2015	UAE	*cas* operon	829985	838428		CTX-M-15; SHV-11; OXA-181 (×3)
				CRISPR	840433	843082	43	
14	AATZP	2014	MD, USA	CRISPR	4233882	4236531	43	SHV-11
				cas operon	4236628	4245071		
15	MS6671	2014/4	UAE	CRISPR	4302572	4305160	43	CTX-M-15; SHV-11; OXA-181 (×3)
				cas operon	4305318	4313761		
16	KPNIH31	2013/12	MD, USA	CRISPR	4112616	4114960	39	SHV-11; KPC-2; CTX-M-15 (×2)
				cas operon	4115118	4123561		
17	CN1	2013	NY, USA	*cas* operon	1060505	1068948		SHV-11; KPC-2; CTX-M-15 (×3)
				CRISPR	1069045	1071450	39	
18	TGH10	2013/3	Greece	*cas* operon	1059346	1067789		SHV-1
				CRISPR	1067886	1069375	24	
19	KPNIH27	2012/1	MD, USA	CRISPR	4177526	4179930	39	SHV-26
				cas operon	4180027	4188469		
20	TGH8	2011/6	Greece	*cas* operon	1161154	1169597		-
				CRISPR	1169694	1171183	24	
21	CAV1344	2010/12	VA, USA	*cas* operon	3283434	3291877		SHV-11
				CRISPR	3291974	3294561	42	
22	CAV1193	2010/5	VA, USA	*cas* operon	3303580	3312023		SHV-11
				CRISPR	3312120	3314707	42	

Subtype (a): 1-11; Subtype (b): 12-22.

Table S5. Chromosomal β-lactamase genes in 42 *K. pneumoniae* strain without CRISPR-*cas* in the chromosomes based on complete genome sequences available from GenBank

Strain	Collection year	Location	Chromosomal *bla*
342	N/A[a]	N/A	LEN22
1158	2002	Taiwan	SHV-11
32192	2010	United States	SHV-11
34618	2011	United States	SHV-11
234-12	2011	Germany	SHV-43
30660	2010	United States	-
30684	2010	United States	SHV-11
500_1420	2012	United States	SHV-11
MGH 78578	1994	N/A	SHV-11
BAA-2146	2010	United States	SHV-11, CTX-M-15
blaNDM-1	2012	United States	SHV-11
CAV1392	2011	United States	SHV-11, CTX-M-15, KPC-3
CAV1596	2012	United States	SHV-11
CG43	N/A	Taiwan	SHV-1
CR14	2013	United States	-
DMC1097	2010	United States	SHV-11
HK787	N/A	N/A	SHV-1
HKUOPLC	2013	Hong Kong	OKP-B-6
HS11286	N/A	N/A	SHV-11
JM45	2010	N/A	SHV-11
KCTC 2242	N/A	N/A	SHV-99
KP-1	N/A	Australia	SHV-187
Kp13	2009	Brazil	SHV-11
KpN01	2013	Canada	SHV-27
KpN06	2013	Canada	SHV-27
Kpn223	2013	United States	SHV-11
Kpn555	2014	United States	SHV-1
KPNIH1	2011	United States	SHV-11
KPNIH10	2011	United States	SHV-11
KPNIH24	2012	United States	SHV-11
KPNIH29	2013	United States	SHV-1
KPNIH30	2013	United States	SHV-11
KPNIH32	2013	United States	SHV-11
KPNIH33	2013	United States	SHV-11, KPC-3 x2

KPNIH39	2014	United States	SHV-11
KPR0928	2012	United States	SHV-11
NY9	2013	United States	SHV-11
UHKPC07	2007	United States	SHV-11
UHKPC33	2008	United States	SHV-11
W14	2014	China	SHV-1
XH209	2013	China	SHV-11
YH43	N/A	N/A	LEN22

[a] N/A: data not available.

Table S6. Spacer sequences in the CRISPR-*cas* array of *K. pneumoniae* CN1 and the number of their targeting plasmids from BLASTn homology search as of June 2016

No.	Position	Length	Spacer sequence	No. of Target Plasmids
1	1069074	32	GAGCAGGCACCCGCCGCAACGACGAAGAGCGC	210
2	1069135	32	AAATCAGCCAGCACCACGATTCTGGGAAATTT	
3	1069196	32	ACAGGCTTACCCGTATTGAGACGGTTGCTGAA	
4	1069257	33	GAAACCCCATCAGATGACCCTCCCCATGTTGGC	
5	1069319	32	TTGCCTGGTCTGCTTGGTGATGATCCGTGGTA	
6	1069380	32	TACAGAACGACTGAGGCGGCGTGTATTGCATA	
7	1069441	32	GATCTTAACTCTATTGCCAATGGCGCAATTCA	
8	1069502	32	GGCGATGCGCGCTCTGCTGGCTATCGGTAAAA	
9	1069563	32	AATGCAGCAACCGGCAAATATATCGCCGGTAA	
10	1069624	32	GGGCTGCCGCACGCCTGGGACGAGTCGAGCCC	
11	1069685	32	CCGCAATAACAAAAATAAATGAGGGTTAAAGT	
12	1069746	32	GTAAATGGGAATGAGTAGAAGAGCGTCATTGG	
13	1069807	32	CCCCCGCGCACATGCTTAAACGCGCTATCACG	1 (*E. coli* pV139-c)
14	1069868	32	GGCATCTGTTGTGTAATGTTGAGTTTTTTTCA	
15	1069929	32	CAGGTTAAACATGTAAAAAATGACCGTCGCCG	
16	1069990	32	CACATTGCCCGGTCTGAAAAGTATTTGAAAAT	
17	1070051	32	TCCGCACAGTCAAACGCTCCAGACACCAACCC	
18	1070112	32	CCGGAACACCACCAGTAACAGCTACTGTAGGC	
19	1070173	32	TGACCCTGTTGATTTTGTTCCAGGTAATACGT	
20	1070234	32	TTAACCTCGTCGTTCTGGTTTCCGCCCAGGAT	
21	1070295	32	GAACCTGAATTCGAAGGGTGGGTCATCCTTCC	
22	1070356	32	GGACCCCGAGCGACCCGGTCACCCTCCGACCT	
23	1070417	32	CCGTCGAACGGCGGTTATATCCATCTTGAGTC	
24	1070478	32	ACCGATCCCACAATTGCGGCGGTTGAGATTGA	
25	1070539	32	GTTGGTAATTACTGCTGTGTGTTACGGATAAA	
26	1070600	32	CCGATTGTCTGGCGGTCGAGCGCCATTTGCTC	
27	1070661	32	TGCCGGACGTTGTACCTGTGAGTTAATTCTTC	
28	1070722	32	CGATAACCGGGCGTTTCGACTGAACTCACCTC	71
29	1070783	32	TTAATACCAGGGGGCAGGTTCAGCAGGTCCCC	31
30	1070844	32	CCGCTTTAACCCGCTCCGGCAGATCCGGGTGA	
31	1070905	32	CCCTCCGCTTTCAGGGTGTGGCTGATATCACC	
32	1070966	32	CGCGCTGCGAATTTGTTGGTCGATTTCGATCT	
33	1071027	32	TGGGTAGAGGTTAACTGGTTATTGGTCATTGA	
34	1071088	32	ATCGCGGAGGCCTTCGGTGTGTCTCTTTCCTG	
35	1071149	32	CCGTTGTCAATATCTCCCGGCGTCCGCGCCAG	
	AATZP SKGH01 MS6671	32	CACGTCCGGAAACCACGGGTTATCCGTGTAAT	
		32	CAGAGGTCCTTATCTTTTCAACGTCAAAGTCG	
		32	GCAATCCCAGAGCGCGAATATCTTGGGCTCTC	
		32	GACATGGCGCGCGAGTTTATCGACGCCTGCGC	
36	1071210	32	GGGATGAGCGTTTTCCGGTGGATTCTGATGTG	
37	1071271	32	GTGATCGTCATGGATATCACTGCCGTTCCGTC	
38	1071332	32	CAGACAGACAGCAGGCAGCAAACAGGGAAGAC	112
39	1071393	29	GGGTTCACTTGGGTGAAACTGAACTAACT	