

Supplementary datas

Supplementary data 1

Fig S1. A total of 459 *K. pneumoniae* clinical isolates collected from six provinces of China with different carbapenem resistance rates.

Fig S2. The R-M units profiles (excluding 14 major clades) identified in *K. pneumoniae*.

Table S1. Oligonucleotides for PCR

Table S2. The distribution of type I R-M systems in 459 *K. pneumoniae* clinical isolates

Table S3. Example of type I R-M systems matches on clinical plasmid p187-2

Supplementary data 2

Maximum Likelihood-based phylogenetic tree (Jukes–Cantor model) based on aligned amino acid sequences of HdsM of RM positive *K. pneumoniae* genomes

Supplementary data 3

Supplementary data 3a. 217 completely sequenced *K. pneumoniae* strains used in this study

Supplementary data 3b. Type I R-M systems linked with plasmids

Supplementary data 3c. Type I R-M information of 217 completely sequenced *K. pneumoniae* strains

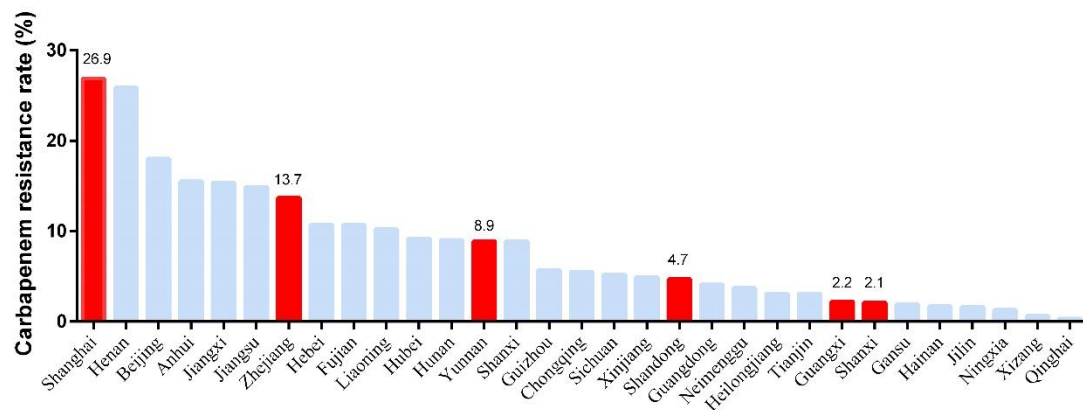


Fig S1. A total of 459 *K. pneumoniae* clinical isolates collected from six provinces of China with different carbapenem resistance rates. Carbapenem resistance rates of *K. pneumoniae* among different provinces of China in 2017 was taken from data of China Antimicrobial Resistance Surveillance System (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>

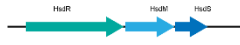
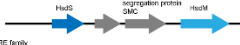


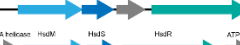




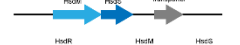






Enzyme	Clade	Schematics of R-M systems	Family	Recognition sequence*	Distance (bp)#	Accession Number
Kpn209ORF21490P	15		Type I A/B	Unknown	Unknown	CP009461
M.Kpn0158I	16		Type I 6mA	GA C NNNNNNGT G	8	CP021696
Kpn1768ORF24685P	17		Type I D	Unknown	Unknown	CP025140
M.Kpn146ORF27965P	18		Type I 6mA	Unknown	Unknown	CP021687
M.Kpn1158ORF4524P	19		Type I D	Unknown	Unknown	CP013711
Kpn1481ORF15080P	20		Type I D	Unknown	Unknown	CP020847
Kpn35657IP	21		Type I D	TTAC NNNNNTAG	8	CP015134
KpnK54ORFDP	22		Type I D	Unknown	Unknown	CP023134
KpnTGH8ORF23945P	23		Type I D	Unknown	Unknown	CP012743
Kpn5632ORF24705P	24		Type I D	Unknown	Unknown	CP025143
M.Kpn293ORF16850P	25		Type I 6mA	Unknown	Unknown	CP014008
Kpn492ORF22630P	26		Type I A/B	Unknown	Unknown	APGM01000001
Kpn139IIP	27		Type I D	GGC A NNNNNNNTNNCG	7	CP021960
KpnSGH10ORF4877P	28		Type I D	Unknown	Unknown	CP025080
Kpn440ORF27725P	29		Type I C	CA AYNNNNNCT G G	8	CP023923
KpnNIH49IP	30		Type I D	T GAYNNNNNC T	10	CP026178

Fig S2 The R-M units profiles (excluding 14 major clades) identified in *K. pneumoniae*. Genes in R-M systems are depicted as arrows in different colors. * N, any nucleotide; R, either purine; Y, either pyrimidine. Red bold type identifies either the adenine that is the target for methylation or the thymine complementary to the target adenine. # No. base pairs between target adenines.

Table S1 Oligonucleotides for PCR

Name	Sequence (5'-3')
For R-M systems screening	
RM clade1-F	TTGTGGTCAGACAGGCGTTC
RM clade1-R	GTTGCAGGAACAGATCCGCC
RM clade2-F	TGCTGGTACTTGGATTCCACC
RM clade2-R	AGAGCGGCAGTCACAGAAG
RM clade3-F	GCGGCGTCTCCTATCAAAAC
RM clade3-R	GTGGTCGAGGTTGCCTTCAA
RM clade4-F (plasmid-borne)	TCAAGCATACGGACTTTGGC
RM clade4-R (plasmid-borne)	GTTTCCGGCTCCAACCTCCTG
RM clade5-F	CTGAGCCTCGTGTTCTCAA
RM clade5-R	GTTCGAGCATTTTCGGTCAGC
RM clade6-F (plasmid-borne)	CACCAAAGTGAAGACGCCG
RM clade6-R (plasmid-borne)	GCCCGGAGACAATTTTACGC
RM clade7-F (plasmid-borne)	CTGATTTCTAACTATGCGGC
RM clade7-R (plasmid-borne)	CATGTTTCATACGCGCCAGG
RM clade8-F	AAGATACGCTGGAGCATCCG
RM clade8-R	TGGTTTTATCGATCTCCGCC
RM clade9-F	GATTGACGGTTCGGTCTCCA
RM clade9-R	GGCATCTTAGCCGGATTGGT
RM clade10-F	CCGAGCATGAATTCGCCAAG
RM clade10-R	ACGACGTAGTTTTTCGTGGCT
RM clade11-F	TGATGACCGTCTACGCGATG
RM clade11-R	ATTCGATCAGCCACAGGCAA
RM clade12-F	CTTGCTTCCTGGGATGAGCA
RM clade12-R	TAGCTGGTTACGAAGCTCGC
RM clade13-F	ACAACCATCCCTTGCGCAAT
RM clade13-R	ACCAGTTGGTAAGCGCAAGA
RM clade14-F	AGGTCCGCAATACCAGCAAA
RM clade14-R	CGGCGGGTTGGCAAAAATTA
For <i>bla</i>_{KPC} screening	
<i>bla</i> _{KPC} -F	TCGCTAAACTCGAACAGG
<i>bla</i> _{KPC} -R	TTACTGCCCCGTTGACGCCCAATCC

Table S2 The distribution of Type I R-M systems in 459 *K. pneumoniae* clinical isolates

247 KPC-positive clinical isolates				
ID	MLST	<i>bla</i> _{KPC}	I-RM in chromosome(clades)	I-RM in plasmids (clades)
220	11	+		clade6,7
221	11	+		clade6,7
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	+		clade6
241	11	+		
242	11	+		
243	11	+		
244	11	+		
245	11	+		
246	11	+		
247	11	+		
248	11	+		
249	11	+		
250	11	+		
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	+		
272	11	+		
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	+		
289	11	+		
290	11	+		
291	11	+		
292	11	+		
293	11	+		
294	11	+		
295	11	+		
296	11	+		
297	11	+		
298	11	+		
299	11	+		

300	11	+		
301	11	+		
302	11	+		
303	11	+		
304	11	+		
305	11	+		
306	11	+		
307	11	+		
308	11	+		
309	11	+		
310	11	+		
311	11	+		
312	11	+		
313	11	+		
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	+		clade6
327	11	+		
328	11	+		
329	11	+		clade6
330	11	+		
331	11	+		
333	11	+		
334	11	+		
335	11	+		
336	11	+		
337	11	+		
338	11	+		
339	11	+		clade6
340	11	+		clade6
341	11	+		clade6
344	11	+		
346	11	+		

347	11	+	clade8	clade6
349	11	+		
350	11	+		
351	11	+		
352	11	+		clade6
353	11	+		clade6
354	11	+		clade6
355	11	+		
356	11	+		clade6
357	11	+		
358	11	+		clade6
359	11	+		clade4
360	11	+		
361	11	+		
362	11	+		
363	11	+		
364	11	+		
365	11	+		
366	11	+		
367	11	+		
371	11	+		
372	11	+		
373	11	+		
376	11	+		
377	11	+		
379	11	+		
380	11	+		clade6
383	11	+		
384	11	+		
385	11	+		
386	11	+		
387	11	+		
388	11	+	clade3	
390	11	+		
391	11	+		
393	11	+		
394	11	+		
395	11	+		
396	11	+		clade6
397	11	+		
398	11	+		clade6
399	11	+		
400	11	+		

401	11	+		
402	11	+		
405	11	+		
406	11	+		
407	11	+		clade6
408	11	+		
409	11	+		
410	11	+		clade6
411	11	+		
412	11	+		
413	11	+		
414	11	+		
415	11	+		clade6
416	11	+		
417	11	+		clade6
418	11	+		clade6
419	11	+		
420	11	+		
421	11	+		
422	11	+		
423	11	+		
424	11	+		
425	11	+		
426	11	+		
427	11	+		
428	11	+		
429	11	+		clade6
430	11	+		
431	11	+		
432	11	+		
433	11	+		
434	11	+		
435	11	+		
436	11	+		
437	11	+		
438	11	+		
439	11	+		
440	11	+		
441	11	+		
442	11	+		
443	11	+		
444	11	+		
445	11	+		

446	11	+		
447	11	+		
448	11	+		
449	11	+		
450	11	+		
451	11	+		
452	11	+		
453	11	+		
454	11	+		
455	11	+		
456	11	+		
457	11	+		
458	11	+		
459	11	+		
348	15	+	clade2	
368	15	+		clade6
369	15	+	clade3	clade6
370	15	+	clade3,8	
374	15	+	clade8	
375	15	+	clade3	clade6
378	15	+	clade3	clade6
381	15	+	clade3	clade6
382	15	+	clade3	
389	15	+		
392	15	+	clade3	
403	15	+	clade3	
404	23	+		
218	65	+	clade10	
222	65	+	clade10	
342	81	+		
343	81	+		
345	81	+		
332	290	+		
213	423	+	clade12	clade4,7
214	423	+	clade12	clade4,7
215	423	+	clade12	clade4,7
216	423	+	clade12	clade4,7
217	423	+	clade12	clade4,7
219	977	+		

212 KPC-negative clinical isolates				
ID	MLST	<i>bla</i> _{KPC}	I-RM in chromosome(clades)	I-RM in plasmids (clades)
9	1	-		clade6,7

118	1	-	clade12	
183	6	-		
5	11	-		
15	11	-		clade4
66	11	-		clade6
67	11	-		
158	11	-		
19	14	-	clade3	
21	14	-	clade3	
22	14	-	clade3	
116	14	-	clade3	
52	15	-	clade8	
68	15	-	clade8	clade7
166	15	-	clade3	clade7
170	15	-	clade3	clade7
182	15	-	clade2,8	
10	17	-		
11	17	-		clade6
55	17	-		clade6
16	20	-		
37	20	-	clade5	
65	20	-		clade6
139	20	-		
140	20	-		
142	20	-	clade12	
147	20	-		
148	20	-		
150	20	-		
152	20	-		
153	20	-		
154	20	-		
155	20	-	clade1	
156	20	-		
168	20	-		
4	23	-	clade2	
6	23	-		clade4
12	23	-		
30	23	-		
34	23	-		
36	23	-		
39	23	-		
42	23	-		
50	23	-	clade10	

54	23	-		
72	23	-		
105	23	-	clade12	
109	23	-	clade12	
113	23	-	clade12	clade7
185	23	-		
186	23	-		
191	23	-		
193	23	-	clade3	
203	25	-	clade3,10	clade4
13	29	-		
32	29	-	clade3,12	
38	29	-		
53	29	-		clade6
92	29	-	clade13	
93	29	-	clade13	
97	29	-	clade13	
98	29	-	clade13	
125	29	-	clade12	
114	34	-		
61	35	-		
88	35	-		clade6,7
96	35	-	clade14	clade4,6
169	35	-	clade14	
190	35	-		
59	36	-		
76	36	-		
77	36	-		
78	36	-		
31	37	-		
51	37	-		
95	37	-	clade2,9	
119	37	-	clade12	
14	39	-		
62	39	-		
86	39	-		
84	43	-		
110	48	-	clade12	clade6
112	48	-	clade12	clade6
133	54	-	clade1	clade6,7
135	54	-	clade1,12	clade6,7
136	54	-	clade1,12	clade6,7
137	54	-	clade1,12	clade6,7

138	54	-	clade1	clade6,7
144	54	-		clade6
145	54	-	clade1	clade7
146	54	-	clade1	clade7
149	54	-	clade1	clade7
151	54	-	clade1	clade7
157	64	-	clade1	clade6,7
3	65	-	clade10	clade4
8	65	-	clade10	clade4
18	65	-	clade10	clade4
174	65	-		clade4
197	65	-	clade10	clade4
202	65	-	clade3	
178	76	-	clade1	clade6
184	81	-	clade1	
7	86	-	clade11	
73	86	-		clade7
94	86	-	clade11	
121	86	-	clade11,12	
189	86	-	clade11	
131	91	-	clade3	
28	101	-		
70	101	-		
80	101	-		
129	107	-		
194	111	-		
111	133	-		
74	134	-	clade11	
17	147	-	clade1,3,5	
35	152	-		
101	152	-	clade5	
87	188	-	clade13	clade7
1	218	-	clade1,14	
45	218	-	clade1,10	
171	254	-		clade6
63	261	-		
175	268	-		
134	290	-		
43	309	-		
44	309	-		
160	334	-	clade8	clade6,7
181	347	-		
130	412	-	clade12	

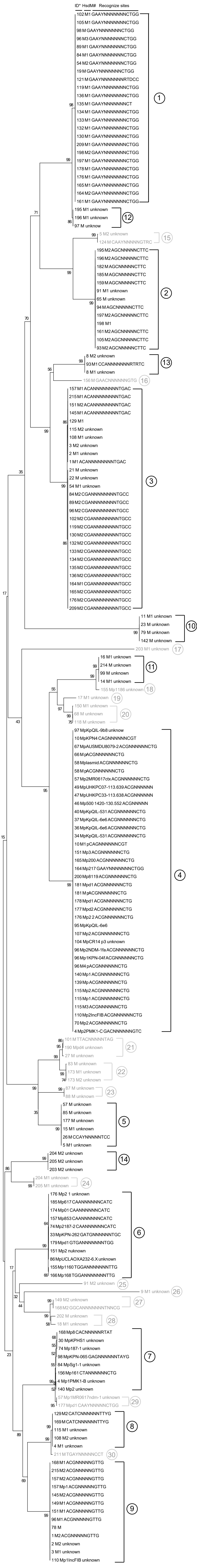
187	412	-		
188	412	-	clade11	
192	412	-		
195	412	-		
196	412	-		
117	420	-	clade3	
103	455	-		
49	461	-	clade8,10	clade6
127	490	-	clade1,5	
41	494	-		clade4,7
57	494	-		clade6
58	494	-		clade6
69	494	-		clade4
71	494	-		clade4,7
100	494	-	clade5	clade6
115	502	-		clade7
122	502	-		clade7
173	580	-		clade6
108	584	-	clade12	
48	592	-		
120	592	-		
204	612	-		
47	626	-	clade10	clade6
64	655	-	clade2,8	
26	660	-	clade1,3	
123	661	-	clade3,12	
128	661	-	clade3,12	
23	686	-		
2	690	-		clade7
46	690	-		
167	692	-	clade3	clade7
161	697	-		
162	697	-		
141	705	-	clade12	clade6,7
143	705	-	clade1,12	clade6,7
132	785	-		
20	793	-		
172	814	-	clade10	clade4,7
29	872	-	clade2	clade7
176	876	-		clade7
90	881	-	clade10	clade4
91	881	-	clade10	clade4
126	893	-	clade11,12	

200	893	-	clade10	clade4
107	896	-		clade7
89	1031	-		clade7
177	1265	-		
180	1265	-	clade14	
106	1326	-	clade2	clade6
27	1419	-		
102	1419	-		
104	1419	-		
164	1446	-	clade3	
163	1536	-		
201	1536	-		
179	1653	-	clade5	
83	1764	-		
85	1770	-		clade7
124	1783	-		
99	1914	-		
25	1933	-	clade1	
33	1934	-		
75	1935	-		
79	1936	-		
82	1937	-		
24	1938	-	clade2	
40	1938	-		
56	1939	-	clade1	clade7
60	1940	-		clade7
198	2158	-		
165	2464	-		
159	2695	-	clade5	
81	ND	-		
199	ND	-		
205	ND	-	clade3	
206	ND	-	clade3,14	
207	ND	-		
208	ND	-	clade10	clade4
209	ND	-		
210	ND	-	clade10	clade4
211	ND	-	clade3,10	clade4
212	ND	-	clade3	

Table S3. Example of type I R-M systems matches on clinical plasmid p187-2

plasmid p187-2, an IncFII plasmid harboring <i>bla</i> _{KPC} , CP025468.1			
Recognition Sequence		Matches on plasmid (start to end)	Matches elements on plasmid
Type	Numbers		
I A/B(calde1) GAAYNNNNNNNCTGG	12	11424-11438	ISKpn24
		20832-20846	DUF262 domain-containing protein
		23032-23046	DUF262 domain-containing protein
		28958-28972	YecA family protein
		60344-60358	TraC
		70553-70567	conjugal transfer protein TrbJ
		71992-72006	RelE/ParE
		73030..73044	RelE/ParE
		80820-80834	RelE/ParE
		84208-84222	RelE/ParE
		89032-89046	DNA methylase
		122617-122631	ISKpn6
IC (clade4) ACGNNNNNNCTG	> 23	35198-35209	traX
		35446-35457	traI
		37477-37488	traI
		38155-38166	traI
		38362-38373	traI
		39073-39084	traI
		39368-39379	traI
		40100-40111	traI
		40471-40482	traI

		42456-42467	traD
		46787-46798	traG
		49543-49554	traH
		51635-51646	traF
		53612-53623	traN
		57225-57236	traW
		58124-58135	traC
		58312-58323	traC
		59461-59472	traC
		60259-60270	traC
		62737-62748	traV
		62911-62922	traV
		64060-64071	traB
		65422-65433	traE
<hr/>			
ID (clade8)	9	2060-2073	bacteriocin immunity protein
CATCNNNNNNTTYG		11248-11261	bacteriocin immunity protein
		15029-15042	DUF4011 domain-containing protein
		20863-20876	DUF262 domain-containing protein
		26504-26517	HsdR
		67022-67035	conjugal transfer protein TrbJ
		92846-92859	ltrA
		118675-118688	adenine-specific DNA-methyltransferase
		129537-129550	ISKpn6
<hr/>			



Note: *All the numbers are consistent with the strain ID in Supplementary data 3.

All the numbers of HsdM proteins(Abbreviated as M) are consistent with the number in Supplementary data 3c