

1 Supplementary Materials

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3 **Clinical evolution of ST11 carbapenem resistant and hypervirulent *Klebsiella pneumoniae***

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10 Running title: Conjugative MDR and hypervirulent plasmid

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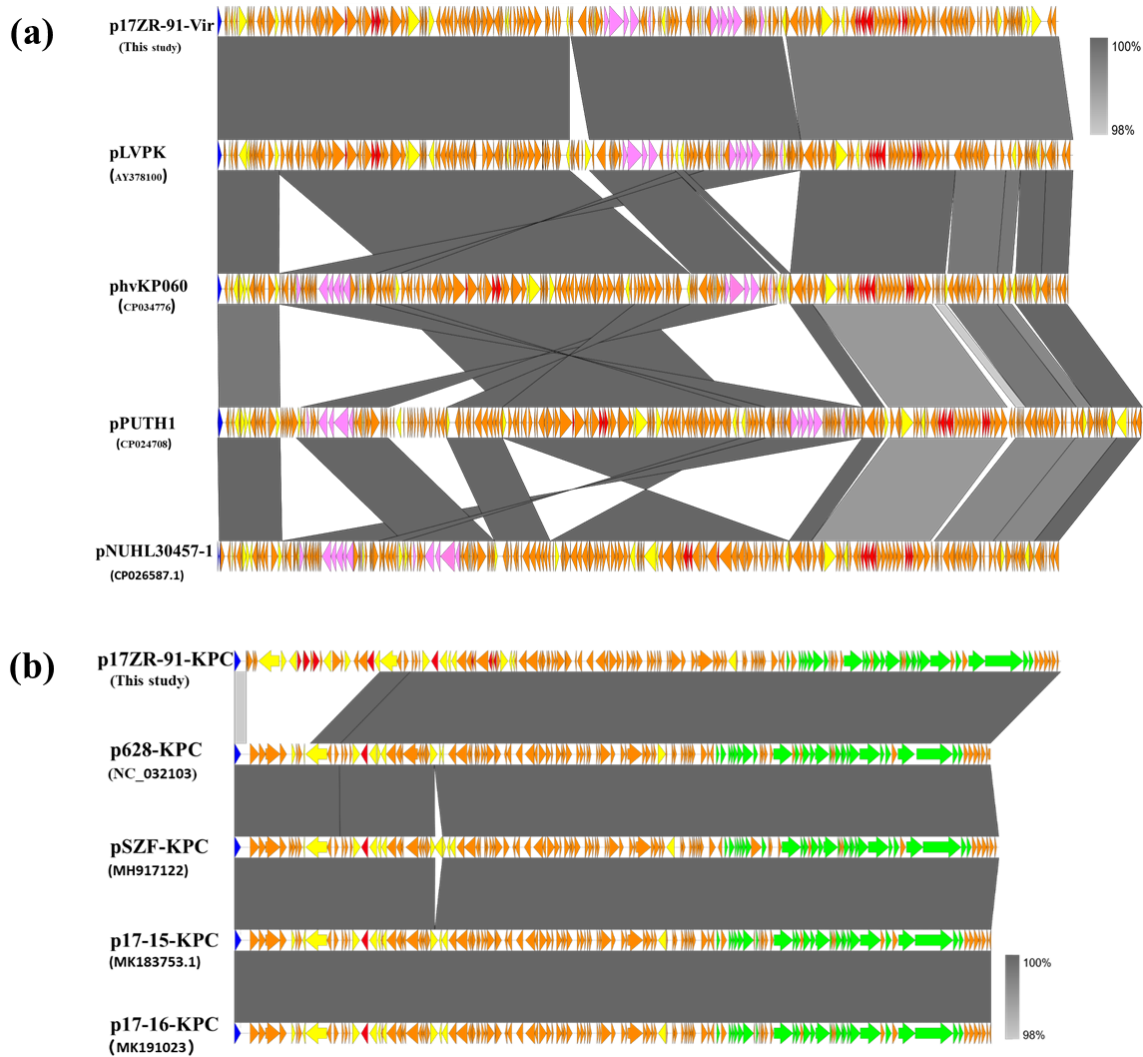
24 **Supplementary Table S1. Bacterial strains carrying *bla*_{KPC-2}-bearing plasmid p17ZR-91-KPC obtained from two hospitals in**
 25 **China and the GenBank.**

<i>bla</i> _{KPC} -bearing plasmid	Virulence plasmid	Chromosome	Species	ST	K_locus	Country	Date	Source
MK262711.1	-	CP034249.1	<i>Klebsiella pneumoniae</i>	ST11	KL112	China	2018	urine
KC757417.2	-	NA	<i>Klebsiella pneumoniae</i>	NA	NA	France	2010	NA
MK158080.1	-	NA	<i>Klebsiella pneumoniae</i>	NA	NA	China	NA	urine
AP018584.1	-	GCA_003115215.1	<i>Klebsiella pneumoniae</i>	ST15	KL62	Viet Nam	2016	NA
AP018830.1	-	NA	<i>Enterobacter hormaechei</i>	NA	NA	Myanmar	2015	NA
CP014005.1	-	CP014004.1	<i>Klebsiella pneumoniae</i>	ST14	KL2	China	2014	urine
CP017386.1	-	CP017385.1	<i>Klebsiella pneumoniae</i>	ST15	KL64	Taiwan	2007	urine
CP021959.1	-	CP021960.1	<i>Klebsiella pneumoniae</i>	ST37	KL105	NA	NA	NA
CP024876.1	-	CP024874.1	<i>Klebsiella pneumoniae</i>	ST15	KL19	Thailand	2016	sputum
CP024917.1	-	CP024916.1	<i>Klebsiella pneumoniae</i>	ST147	KL10	Thailand	2016	rectal swab
CP025966.2	-	CP025963.2	<i>Klebsiella pneumoniae</i>	ST273	KL15	China	2017	NA
CP026588.1	+ (CP026587.1)	CP026586.1	<i>Klebsiella pneumoniae</i>	ST86	KL2	China	2016	NA
CP028389.2	+ (CP028390.1)	CP028391.1	<i>Klebsiella pneumoniae</i>	ST36	KL62	China	2015	NA
CP028553.1	-	CP028555.1	<i>Klebsiella variicola</i>	ST1142	KL105	China	2016	NA
CP028717.1	-	CP028716.1	<i>Klebsiella pneumoniae</i>	ST15	KL28	China	2017	Sputum
CP034085.1 (pR210-2-CTX)	+ (CP034083.1)	CP034082.1	<i>Klebsiella pneumoniae</i>	ST23	KL1	China	2015	NA
HF545434.1	-	NA	<i>Klebsiella pneumoniae</i>	NA	NA	Viet Nam	2006	nasal swab
KP987218.1	-	NA	<i>Klebsiella pneumoniae</i>	NA	NA	China	2010	cerebrospinal fluid specimen
LT968762.1	-	NA	<i>Klebsiella pneumoniae</i>	NA	NA	China	2009	specimen
MG878868.1	-	NA	<i>Klebsiella pneumoniae</i>	NA	NA	Taiwan	2013	wound
CP035124.1	-	CP035123.1	<i>Escherichia coli</i>	-	-	China	2017	urine
CP018340.1	-	CP018337.1	<i>Klebsiella pneumoniae</i>	ST23	KL57	Germany	2014	wound
CP022442.1	-	CP022444.1	<i>Klebsiella quasipneumoniae</i>	ST421	KL123	China	NA	plant
CP024510.1	-	CP024504.1	<i>Klebsiella pneumoniae</i>	ST323	KL21	NA	NA	NA
CP031262.1	-	CP031257.1	<i>Klebsiella quasipneumoniae</i>	ST367	KL1	China	2014	bronchoalveolar lavage fluid

CP035384.1	-	CP035383.1	<i>Klebsiella pneumoniae</i>	ST23	KL1	China	2017	blood
FJ876826.1	-	NA	<i>Klebsiella pneumoniae</i>	NA	NA	China	NA	NA
CP035776.1	-	CP035777.1	<i>Klebsiella pneumoniae</i>	ST37-1LV	KL14	China	2015	rabbit
CP003224.1	-	CP003200.1	<i>Klebsiella pneumoniae</i>	ST11	KL103	China	2011	sputum
CP025468.1	-	CP025466.1	<i>Klebsiella pneumoniae</i>	ST11	KL103	China	2008	NA
CP021195.1	-	CP021193.1	<i>Escherichia coli</i>	-	-	China	2012	NA
CP018999.1	-	CP019000.1	<i>Escherichia coli</i>	-	-	China	2013	NA
KU295132.1	-	NA	<i>Escherichia coli</i>	NA	NA	USA	NA	rectum swab
KY270849.1	-	NA	<i>Klebsiella pneumoniae</i>	NA	NA	China	NA	NA
KX236178.1	-	NA	<i>Klebsiella pneumoniae</i>	NA	NA	China	2009	sputum
MG764550.1	-	CP022997.1	<i>Klebsiella pneumoniae</i>	ST11	KL47	China	NA	NA
MH477636.1	-	NA	<i>Klebsiella pneumoniae</i>	NA	NA	China	2013	NA
MH909340.1	-	GCF_002751155.1	<i>Klebsiella pneumoniae</i>	ST11	KL47	China	2013	NA
MH917122.1	-	GCF_003225855.1	<i>Klebsiella pneumoniae</i>	ST628	KL2	USA	2015	sputum
MK183753.1	-	CP034076.1	<i>Klebsiella pneumoniae</i>	ST15	KL19	China	NA	blood
MK191023.1	-	CP034077.1	<i>Klebsiella pneumoniae</i>	ST15	KL19	China	2017	Sputum
LR596806.1	-	LR596806.1	<i>Klebsiella pneumoniae</i>	ST11	KL31	UK	NA	NA
17ZR-127	+	RYML00000000	<i>Klebsiella pneumoniae</i>	ST11	KL64	ZR	2017	blood
17ZR-133	-	RYKN00000000	<i>Klebsiella pneumoniae</i>	ST15	KL19	ZR	2017	blood
17ZR-141	-	RYIT00000000	<i>Klebsiella pneumoniae</i>	ST15	KL19	ZR	2017	sputum
17ZR-175	-	RYIQ00000000	<i>Klebsiella pneumoniae</i>	ST15	KL19	ZR	2017	blood
17ZR-2	-	RYIM00000000	<i>Klebsiella pneumoniae</i>	ST15	KL19	ZR	2017	sputum
17ZR-31	+	RYHQ00000000	<i>Klebsiella pneumoniae</i>	ST86	KL2	ZR	2017	sputum
17ZR-84	+	RYHP00000000	<i>Klebsiella pneumoniae</i>	ST86	KL2	ZR	2017	sputum
17ZR-91	-	RYGB00000000	<i>Klebsiella pneumoniae</i>	ST86	KL2	ZR	2017	sputum

27 **Supplementary Table S2. SNPs of *K. pneumoniae* strains carrying both p17ZR-91-Vir and**
 28 **p17ZR-91-KPC plasmid collected from a hospital in Zhejiang Province, China.**

	29				
SNP-dists 0.6	17ZR-31	17ZR-84	17ZR-91	17ZR-127	Reference
17ZR-31	0	8717	8714	20094	20377
17ZR-84	8717	0	23	28712	29094
17ZR-91	8714	23	0	28702	29083
17ZR-127	20094	28712	28702	0	4118
Reference	20377	29094	29083	4118	0



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32 **Supplementary Figure S1. Alignment of virulence plasmid p17ZR-91-Vir and *bla*_{KPC-2}-**
 33 **bearing plasmid p17ZR-91-KPC recovered from *K. pneumoniae* 17ZR-91 with similar**
 34 **plasmids in the NCBI database, respectively. (a) Alignment of virulence plasmid p17ZR-91-**
 35 **Vir with pLVPK (AY378100), phvKP060 (CP034776), pPUTh1(CP024708) and pNUHL30457-**
 36 **1(CP026587.1) using Easyfig. (b) Alignment of *bla*_{KPC-2}-bearing plasmid p17ZR-91-KPC with**
 37 **p628-kpc (NC_032103), pSZF-KPC (MH917122), p17-15-KPC (MK183753) and p17-16-**
 38 **KPC(MK191023) using Easyfig.**

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HR1 AGATCCGAAAAAAGCTAGTTCCGGATCTGCTTCAGCCCCGCCGACGCGCAGCGTCCAAAA 60
HR2 AGATCCGAAAAAAGGTAGTTCCGGATCTGCCGGAACGGCTCCGGCGCGCAGCGGCCCGGC 60
*****
***** * * * * * ***** **

HR1 AACACAAGATGTAGCGGTGTTCCAGCTGACAGTGACGCTATATGTTGTGTCTCTCCCCT 120
HR2 AACACAAGATGTAGCGGTGTTCCCAGCTGAGGATGACGCTATATGTTGTGTCCCTCCCCT 120
*****
***** ***** *****

HR1 GCCCTGCGACCGGCCTCTCACCACCCTAAAAGGACGCTTTGCTTATTCCCTGGCCGTGGT 180
HR2 CCCCCGTGACCGGCCGCTCACCACCGGAAAACGCCGCTTTGCTTATTCTCTCACCGTGGT 180
*** * ***** ***** ***** * ***** ** *****

HR1 ACACTTCCGAAACTTCGCTAAATGTGAAAAGTCGGATCTGCCGAAACGGTCATCCGAT 240
HR2 ACACTTCCGAAACTTCGTAAAGCGCGAAAAGTCGGATCCGGACGGAACGGCCGTCCTGT 240
*****
***** * * ***** * * ***** * ** *

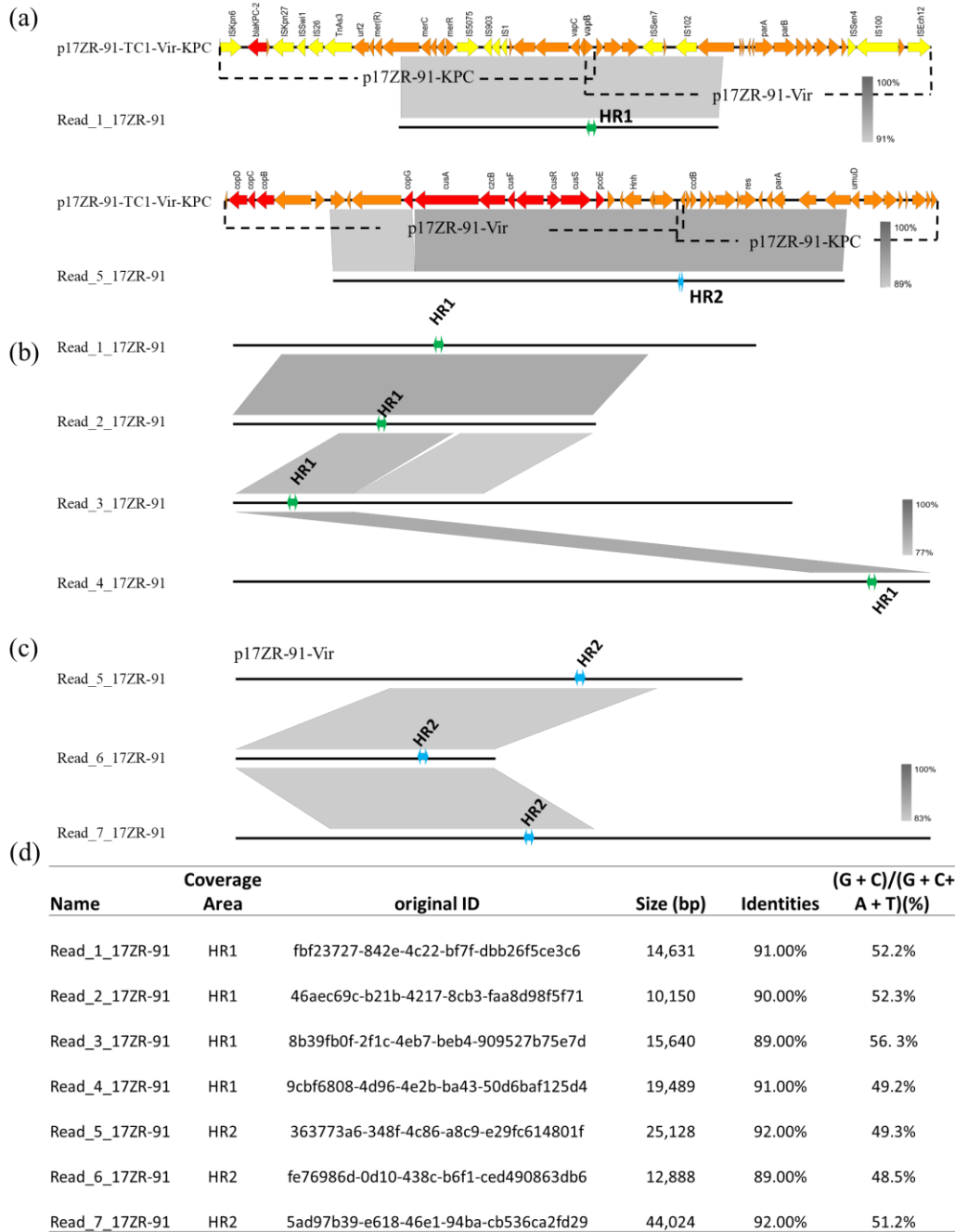
HR1 AATTTAATAGCGTGGTTATAATGCTGCTTATTATC 275
HR2 AATTTAACGGCGTGGCTGTATGGCTGCTTGTATC 275
***** ***** * ** ***** *****

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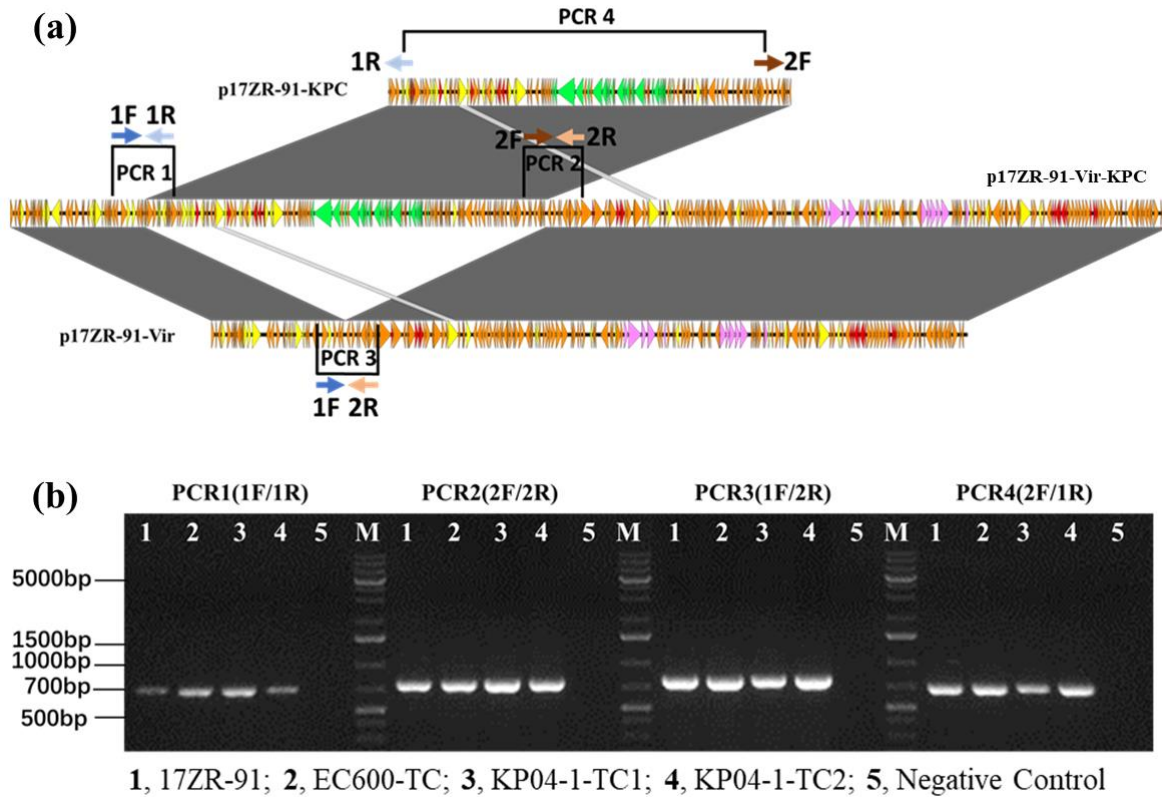
41 **Supplementary Figure S2. DNA sequence alignment for 275bp homologous regions, HR1**
42 **and HR2, located in plasmids p17ZR-91-Vir and p17ZR-91-KPC.** Sequence alignment were
43 conducted using multiple sequence alignment tool, Clustal Omega. These two sequences shared
44 82% (224/275) homology.

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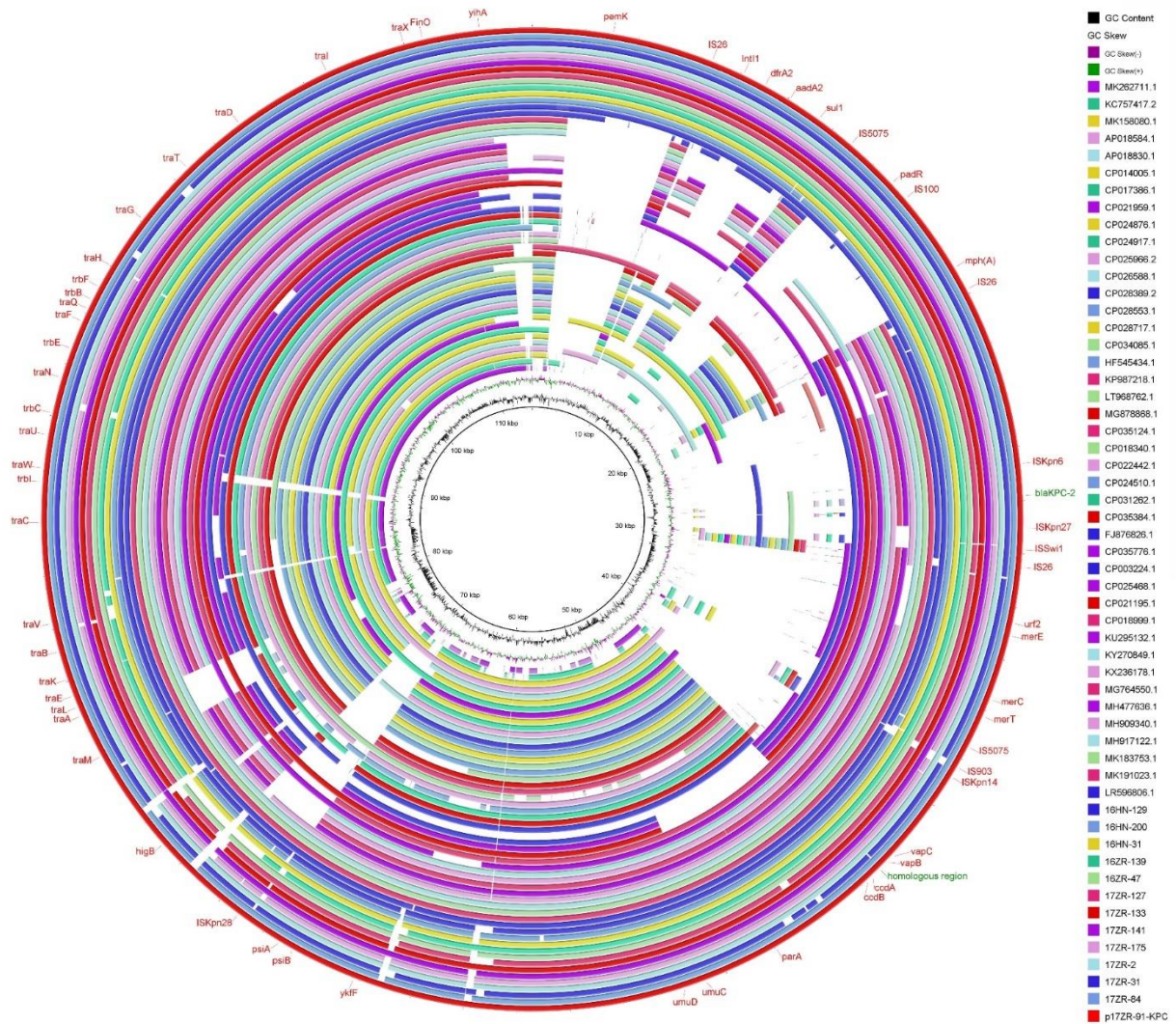
47 **Supplementary Figure S3. Nanopore reads covering the fusion region of fusion plasmid**
 48 **p17ZR-91-Vir-KPC.** (a) Linear alignment between part of p17ZR-91-Vir-KPC and the Nanopore
 49 long-read Read_1_17ZR-91 at HR1 region and Read_5_17ZR-91 at HR2 region. Linear alignment
 50 among different Nanopore reads that containing HR1 (b) or HR2 (c) parts of fusion plasmid. (d)
 51 detail information of 7 reads.



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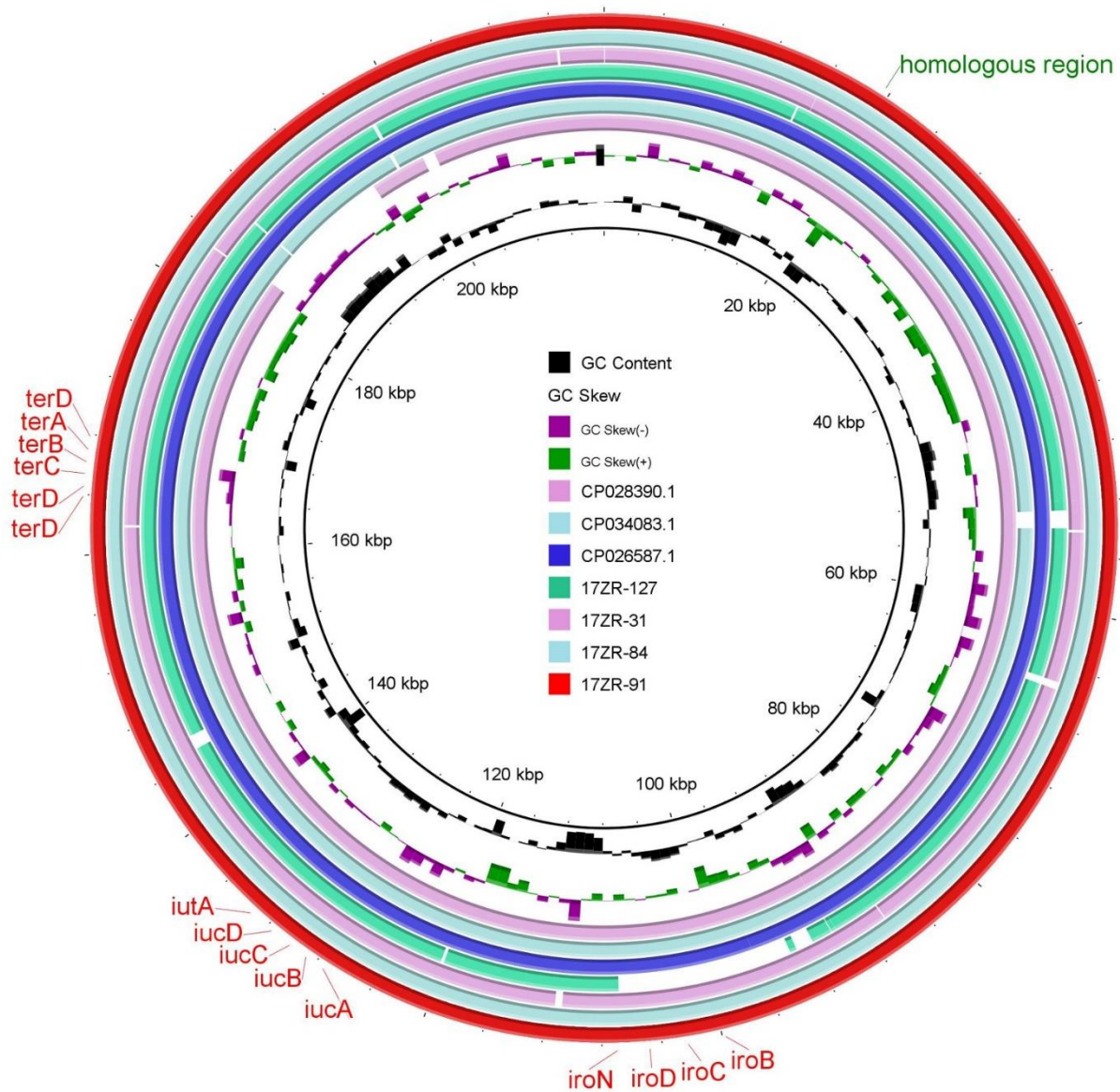
53 **Supplementary Figure S4. PCR assays to confirm the presence of fusion plasmid p17ZR-91-**
 54 **Vir-KPC in different *K. pneumoniae* strains and transconjugants.** (a) Design of PCR assays to
 55 test the presence of fusion plasmid and two non-fusion plasmids in *K. pneumoniae*. PCR1 and
 56 PCR2 were designed to confirm the presence of fusion plasmid p17ZR-91-Vir-KPC; PCR3 was
 57 designed to confirm the presence of p17ZR-91-Vir; PCR4 was designed to confirm the presence
 58 of p17ZR-91-KPC. (b) PCR results for different *K. pneumoniae* strains and transconjugants. Being
 59 positive in all the four PCR tests indicates the presence of fusion plasmid and two non-fusion
 60 plasmids in the same strain.

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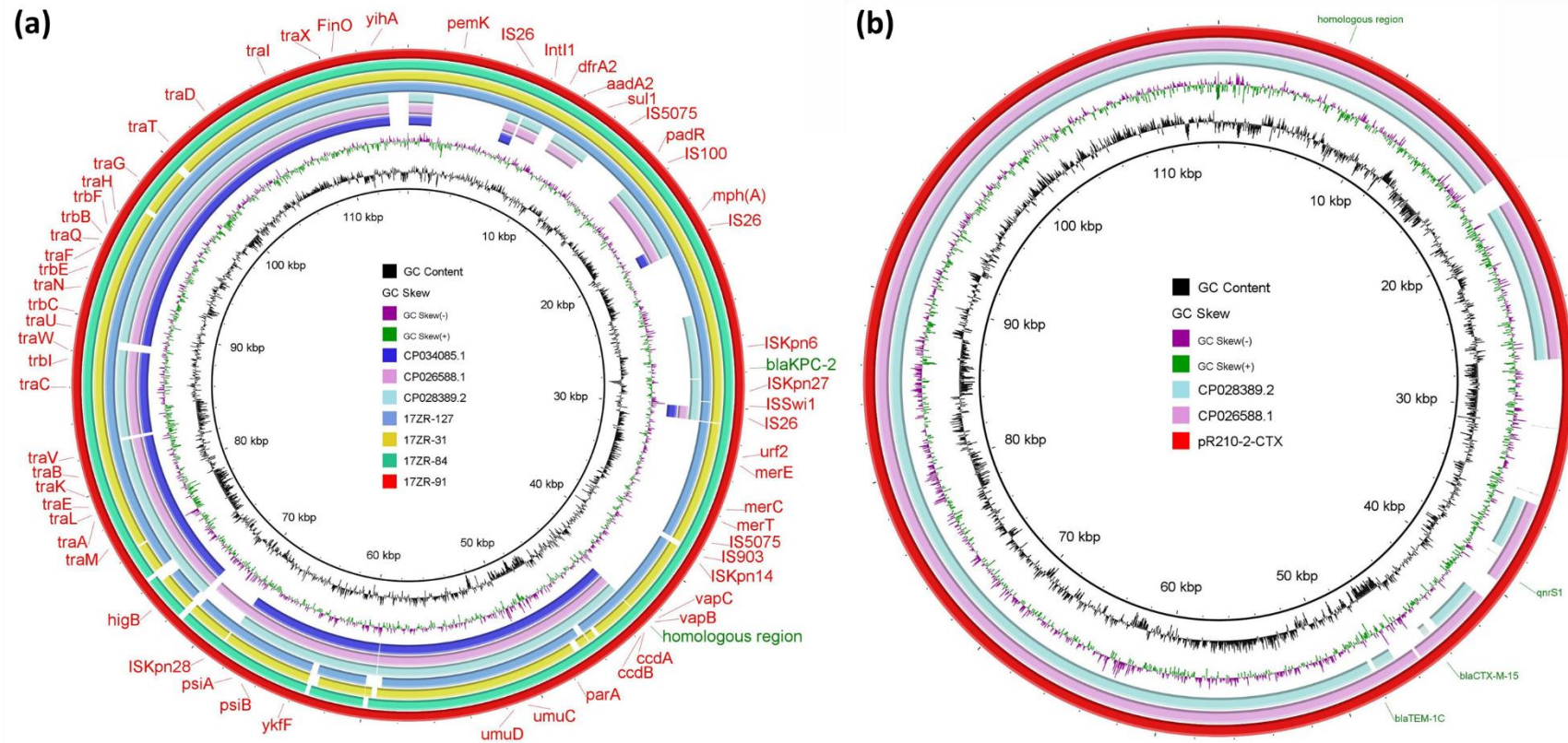
64 **Supplementary Figure S5. Alignment of the *bla*_{KPC-2}-bearing plasmid harbored by 17ZR-91,**
 65 **p17ZR-91-KPC, with other related plasmids recovered from strains in two hospitals in China,**
 66 **and those retrieved from the GenBank. The alignment was performed using BLAST Ring Image**
 67 **Generator (BRIG). Other information of these strains was shown in Supplementary Table S1.**
 68 **Homologous region (HR) was labelled.**



69

70 **Supplementary Figure S6. Alignment of virulence plasmid from 17ZR-91, p17ZR-91-Vir,**
 71 **with other virulence plasmids from bacterial strains that contained p17ZR-91-KPC,**
 72 **obtained from one hospital in China and the GenBank, respectively.** The alignment was
 73 performed using BLAST Ring Image Generator (BRIG). Homologous region (HR) was labelled.

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76 **Supplementary Figure S7. Alignment of *bla*_{KPC-2}-bearing plasmid from *K. pneumoniae* strains carrying both p17ZR-91-Vir like and**
 77 **p17ZR-91-KPC like genes. (a) Alignment of *bla*_{KPC-2}-bearing plasmid from six *K. pneumoniae* strains carrying both p17ZR-91-Vir-like and**
 78 **p17ZR-91-KPC-like genes. (b) Alignment of pR210-2-CTX like plasmid with those of the other two *K. pneumoniae* strains documented in the**
 79 **Genbank which also carried the virulence plasmid. The alignment was performed using BLAST Ring Image Generator (BRIG). Four strains from**
 80 **the same hospital exhibited highly similar p17ZR-91-KPC-like plasmids, whereas three strains from the GenBank carried a pR210-2-CTX**

- 81 (NZ_CP034085.1)-like *bla*_{CTX-M}-bearing plasmid with a backbone similar to that of p17ZR-91-KPC, but contain different MDR regions.
- 82 Homologous region (HR) was labelled.