The first report of a novel IncHIIB *bla*_{SIM-1}-carrying megaplasmid pSIM-1-BJ01 from a clinical *Klebsiella pneumoniae* isolate

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Yang Lü¹
Shulong Zhao²
Hui Liang³
Wei Zhang³
Jia Liu³
Hongyan Hu³

¹State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, People's Republic of China; ²Department of Laboratory Medicine, The Affiliated Hospital of Xuzhou Medical University, Xuzhou, Jiangsu, People's Republic of China; ³Department of Laboratory Medicine, The Third Medical Center, Chinese PLA General Hospital, Beijing, People's Republic of China

Background: A rare member of metallo-β-lactamases genes, *bla*_{SIM-1}, carried by a 316-kb plasmid designated pSIM-1-BJ01 was isolated from a clinical cephalosporins- and carbapenem-resistant *Klebsiella pneumoniae* 13624. This is the first sequence report of a transferable *bla*_{SIM-1}-carrying conjugative plasmid isolated from *K. pneumoniae*.

Purpose: The sequence analysis of pSIM-1-BJ01 will help us to identify genes responsible for conjugation, plasmid maintenance and drug resistance, to understand the evolution and control the dissemination of resistance plasmids.

Patients and methods: *K. pneumoniae* 13624 was isolated from the urine specimen of a patient. Bacterial genomic DNA was sequenced with PacBio RSII platform.

Results: Most of the pSIM-1-BJ01 backbone matches that of pRJA166a, which was isolated from a clinical hypervirulent *K. pneumoniae* ST23 strain at Shanghai, China, recently. The highly homologous backbones between the two plasmids imply the close relationship of evolution. Two different multidrug-resistant regions both carrying the class 1 integrons with different resistance genes have been assembled into the pSIM-1-BJ01. Besides, the other two resistance plasmids, pKP13624-1 carrying *bla*_{TEM-1} and *bla*_{CTX-M-15} and pKP13624-2 carrying *bla*_{CTX-M-14} and *bla*_{LAP-2} were also identified.

Conclusion: The emergence of the *bla*_{SIM-1}-carrying IncHIIB pSIM-1-BJ01 suggests the spread of *bla*_{SIM} among Enterobacteriaceae is possible. We should pay more attention to supervise and control the dissemination of hypervirulent carbapenem-resistant *K. pneumoniae* in public hospitals.

Keywords: SIM-1, carbapenemase, *Klebsiella pneumoniae*, multidrug-resistant, China

Introduction

SIM, a rare member of metallo-β-lactamases (MBLs), belongs to Class B1 MBLs. It can hydrolyze a broad array of β-lactams, including penicillin, narrow to extended spectrum cephalosporins, and carbapenems, but not monobactams. The SIM-1 protein exhibits 64–69% identity with the IMP-type MBLs, which are its closest relatives. The first reported bacteria carrying *bla*_{SIM-1} were *Acinetobacter baumannii* isolated from Korea in 2005.¹ In *bla*_{SIM-1}-harboring *Acinetobacter* isolates collected from 2003 to 2008 in Korea, the ca. 280-kb plasmid carrying the *bla*_{SIM-1} gene was the major transfer mechanism between different *Acinetobacter* spp., but these plasmids were untypable.² In 2011, an *A. baylyi* strain with a 360-kb plasmid carrying both *bla*_{SIM-1} and *bla*_{OXA-23} was isolated from a Chinese patient in Ningbo, China, who had no history of visiting Korea, but the sequence of the 360-kb plasmid was not determined.³ In 2012, a *Pseudomonas aeruginosa* strain with a 282-kb *bla*_{SIM-2}-harbouring plasmid pHN39-SIM

Correspondence: Yang Lü
State Key Laboratory of Mycology,
Institute of Microbiology, Chinese
Academy of Sciences, Building 3, Room
F717, 1st Beichen West Road, Chaoyang
District, Beijing 100101, People's Republic
of China
Tel +861 348 876 1410
Email biolyy@hotmail.com

Hongyan Hu
Department of Laboratory Medicine, The
Third Medical Center, Chinese PLA
General Hospital, 69 Yongding
Road, Haidian District, Beijing 100039,
People's Republic of China
Tel +86 1 369 144 6958
Email huhongyanwj@163.com

(Genbank: KU254577) was isolated from a Chinese patient in Zhengzhou, China; the 282-kb *bla*_{SIM-2}-harbouring plasmid was sequenced, showing the SIM-2 protein differs from SIM-1 due to only a single amino acid substitution Gly198Asp.⁴ Overall, the detection of *bla*_{SIM} is rare and mainly reported by Korea and China, mostly confined to *Acinetobacter* spp. and *P. aeruginosa*.

In *Acinetobacter* spp., the reported *bla*_{SIM-1} genes, derived from chromosome or plasmids, were always carried on a gene cassette inserted into a class 1 integron with three additional resistance genes (*arr-3*, *catB3* and *aadA1*);¹ the *bla*_{SIM-2} gene in the pHN39-SIM from *P. aeruginosa* was also inserted into a class 1 integron but in a different cassette array (*ereA1*, *catB3q*, *gcu161*, *arr-3*, *aadA1a*).⁴ Except for the pHN39-SIM, none of these plasmids is fully sequenced.

Here, we present a 316-kb plasmid designated pSIM-1-BJ01 carrying *bla*_{SIM-1} isolated from a clinical carbapenem-resistant *Klebsiella pneumoniae* 13624 strain harboring three resistance plasmids in Beijing, China. To the best of our knowledge, this is the first sequence report of a transferable plasmid with *bla*_{SIM-1} isolated from *K. pneumoniae*, the sequence analysis of pSIM-1-BJ01 reveals a mosaic-like structure and identifies genes responsible for conjugation, plasmid maintenance and drug resistance.

Materials and methods

Bacterial isolates and identification

K. pneumoniae 13624 was isolated in 2013 from the urine specimen of a 79-year-old female patient with acute onset of cerebral infarction and type 2 diabetes mellitus in a tertiary hospital in Beijing, China. The urinary tract infection was cured by levofloxacin after 2 weeks. The bacterial species identification was performed using BioMérieux Vitek 2, Bruker MALDI Biotyper and 16S rRNA gene sequencing.

Antimicrobial resistance

Susceptibility tests with the Vitek 2(bioMérieux, France) system were performed using AST-GN cards according to the manufacturer's instructions, and the antimicrobial susceptibility results were evaluated according to 2014 CLSI guidelines. Metallo- β -lactamase activity was detected by Modified Hodge test. The carbapenemase genes (*bla*_{GES}, *bla*_{KPC}, *bla*_{SME}, *bla*_{IMI}, *bla*_{BIC}, *bla*_{IMP}, *bla*_{VIM}, *bla*_{NDM}, *bla*_{TMB}, *bla*_{FIM}, *bla*_{SPM}, *bla*_{DIM}, *bla*_{GIM}, *bla*_{SIM}, *bla*_{AIM}, *bla*_{SMB}, *bla*_{OXA}) were screened for by PCR with specific primers described by Chen.⁵

Plasmid transfer

Conjugal transfer experiment was performed with *Escherichia coli* J53 Azi^r as the recipient strain. Overnight cultures of the bacteria was diluted to 1.5×10^8 cells/mL. Donor and recipient cells were mixed at 1:10 donor-to-recipient ratio, after 18 h of incubation of donor-recipient mixtures on blood plates at 35 °C, cells were washed by normal saline solution and diluted to be cultured selectively on MacConkey agar plates supplemented with sodium azide (100 μ g/mL) and imipenem (1 μ g/mL) for 24 h. Transconjugants were confirmed by PCR amplifying *bla*_{SIM} with primers (SIM-F: 5' TACAAGGGATTTCGGCATCG 3'; SIM-R: 5' TAATGGCCTGTTCCCATGTG 3').⁵

Whole genome sequencing and data analysis

Bacterial genomic DNA was sequenced with PacBio RSII platform. Raw Pacbio reads were *de novo* assembled by SMRT Portal program with default settings. The coverage of the sequences was 177. The genes were predicted with GeneMarkS and further annotated by BLASTP and BLASTN against UniPort and NR databases. The complete sequence of pSIM-1-BJ01, pKP13624-1, and pKP13624-2 was submitted to GenBank under accession number MH681289, MK158080 and MK158081, respectively.

Ethical approval

The urine specimen was part of the routine hospital laboratory procedure. The use of human specimens and all related experimental protocols was approved by the Committee on Human Research of the indicated institutions, and was carried out in accordance with the approved guidelines.

Results

Antibiotic resistance of the *bla*_{SIM-1}-carrying *K. pneumoniae*

The isolated strain was resistant to ampicillin, cephalosporin, and carbapenem, but sensitive to ciprofloxacin, levofloxacin, and colistin; see Table S1. We screened for the carbapenemase genes by PCR, only the *bla*_{SIM-1} gene was amplified.

In the conjugation experiment, we also detected the *bla*_{SIM-1} by PCR in the recipient *E. coli* J53 Azi^r, suggesting that a transferable plasmid (designated pSIM-1-BJ01) might exist in the clinically isolated strain. The transferable pSIM-1-BJ01 might give the recipient *E. coli* J53 Azi^r the carbapenem resistance, as shown in Table S1.

We sequenced the whole genome of the isolated strain and found that there were three drug-resistant plasmids in it. They are respectively the *bla*_{SIM-1}-harboring pSIM-1-BJ01 (Figure S1), pKP13624-1 (Figure S2) carrying resistance genes *bla*_{CTX-M-15} and *bla*_{TEM-1}, and pKP13624-2 (Figure S3) carrying the extended-spectrum β -lactamase (ESBL) gene *bla*_{CTX-M-14} and *bla*_{LAP-2}.

Overview and comparative analysis of pSIM-1-BJ01

The pSIM-1-BJ01 is a 316,557 bp circular plasmid containing 342 putative ORFs (153 hypothetical proteins), the average GC content is 46%. The pSIM-1-BJ01 possesses two replicons (RepFIB and RepHI1B), it is classified to IncHI1B type according to PlasmidFinder (<https://cge.cbs.dtu.dk/services/PlasmidFinder-1.0/>). The RepHI1B (encoded from 230,320 bp to 231,204 bp) identified in pSIM-1-BJ01 shared 87% amino acid identity with the RepHI1B (Protein ID AFB82850.1) encoded by the IncHI1B type pNDM-MAR (Genbank: JN420336) isolated from a clinical *K.pneumoniae* ST15.⁶

The IncH family plasmids can produce the H-pilus, a long and flexible conjugation pilus similar to the F-pilus. Here, the pSIM-1-BJ01 possess two transfer regions, carrying the H-pilus assembly and transfer related protein genes (*trhL*, *trhE*, *traK*, *traB*, *dsbC*, *traV*, *traC*, *trhZ*,

trhO, *htdA*, *htdK*, *traW*, *traU*, *traN*, *traD*, *traI*, *traF*, *traH* and *traG*). Most of these proteins have high similarity with those encoded by the IncHI1B plasmid pNDM-MAR, see Table 1; strongly suggest that the pSIM-1-BJ01 can be classified into the IncHI1B type.

The backbone of pSIM-1-BJ01(18,028–35,486 bp; 36,998–51,411 bp; 107,310–168,501 bp; 173,574–196,369 bp; 197,420–222,610 bp) highly matched that of the plasmid RJA166a (Genbank: CP019048.1) isolated from a clinical hypervirulent *K. pneumoniae* ST23 strain at Shanghai, China, recently.⁷ In addition, a small part of the backbone (233,359–254,569 bp) highly matched that of the pOZ181 (Genbank: CP016764.1) isolated from *Citrobacter freundii* strain B38 at Guangzhou, China,⁸ and the other small part of the backbone (265,886–305,806 bp) highly matched that of the pRpNDM1(Genbank: JX515588.1) isolated from *Raoultella planticola* strain RpNDM1 at Gansu, China,⁹ as shown in the outermost circle of Figure S1. Besides, the pSIM-1-BJ01 also contains two class 1 integron carrying multidrug-resistant genes and one unique region related to phage invasion and assembly, see Figure S1.

A mosaic clinical class I integron harboring *bla*_{SIM-1}

The region from 34,290 bp to 84,861 bp was a mosaic structure of a novel clinical class 1 integrons,¹⁰ see

Table 1 The homology analysis for pilus assembly and transfer related proteins

ORF	Coding Region	Length (AA)	Homology or Designation	Identity %/Range	GI accession
TrhL	182,462–182,764	100	TrhL, IncHI2 plasmid p100R	70.1%/97	ASO65070.1
TrhE	182,783–183,643	286	TrhE, IncHI1B plasmid pNDM-MAR	92%/286	AFB82816.1
TraK	183,645–184,814	389	TraK, IncHI1B plasmid pNDM-MAR	93.1%/389	AFB82817.1
TraB	185,349–186,665	438	TraB, IncF plasmid pNDM-TJ11	93%/420	AXJ98221.1
DsbC	187,141–187,992	283	DsbC, IncHI1B plasmid pNDM-MAR	85.2%/283	AFB82948.1
TraV	188,002–188,739	245	TraV, IncHI1B plasmid pNDM-MAR	93.9%/245	AFB82819.1
TraC	188,788–191,505	905	TraC, IncHI1B plasmid pNDM-MAR	90.8%/905	AFB82820.1
TrhZ	191,534–191,950	138	TrhZ, IncHI plasmid unnamed	44%/98	AQT24070.1
TrhO	193,115–193,918	267	TrhO, IncHI1B plasmid pNDM-MAR	84%/267	AFB82824.1
HtdA	194,636–195,094	152	HtdA, IncHI1B plasmid pNDM-MAR	90.2%/152	AFB82826.1
HtdK	195,461–195,997	178	HtdK, IncHI1B plasmid pNDM-MAR	87.6%/104	AFB82825.1
TraW	202,082–203,443	453	TraW, IncHI1B plasmid pNDM-MAR	86.3%/453	AFB82829.1
TraU	203,440–204,477	345	TraU, IncHI1B plasmid pNDM-MAR	94.2%/345	AFB82830.1
TraN	204,492–207,668	1,058	TraN, IncHI1B plasmid pNDM-MAR	84.2%/1,058	AFB82831.1
TraD	277,644–279,770	708	TraD, IncHI1B plasmid pNDM-MAR	90.8%/708	AFB82910.1
TraI	279,760–282,696	978	TraI, IncHI1B plasmid pNDM-MAR	79.6%/978	AFB82909.1
TraF	286,999–288,036	345	TraF, IncHI1B plasmid pNDM-MAR	89.9%/345	AFB82905.1
TraH	288,026–289,435	469	TraH, IncHI1B plasmid pNDM-MAR	94.3%/459	AFB82904.1
TraG	289,437–293,375	1,312	TraG, IncHI1B plasmid pNDM-MAR	67.4%/1,312	AFB82903.1

Figure 1A. As reported in *Acinetobacter baylyi* (Genbank: JF731030) or *Acinetobacter baumannii* (Genbank: AY887066), the *bla*_{SIM-1} was embedded in the conserved *intI1-bla*_{SIM-1}-*arr3-catB3-aadA1* gene cassette array.¹ In the pSIM-1-BJ01, the *bla*_{SIM-1} is also embedded in the conserved cassette array, but the *aadA1* was replaced by *aadA17*. AadA17, displaying two amino acids substitution (Met126Thr and Thr136Ile), has 93% similarity to the AadA1, so the *aadA17* cassette seems to be hybrid of *aadA1* and *aadA2* cassettes.

Another three resistance genes *dfrA3b*, *aph(3')-Ia* and *qnrS1* are closely adjacent to the conserved *intI1-bla*_{SIM-1}-*arr3-catB3-aadA17-qacEΔ1-sul1* gene arrays. The *dfrA3b* is flanked by ISCR1 at its 5'-end and IS26 at its 3'-end. The *aph(3')-Ia* is flanked by directly oriented IS26 at both ends, which is most likely Tn6023(Genbank: GU562437).

Tn6023 is a 2.6 kb composite transposon, found in plasmid pSRC125 from a multiply antibiotic-resistant *Salmonella enterica* serovar Typhimurium isolate of bovine origin, it comprised of the *aph(3')-Ia* gene flanked by inversely oriented IS26 at both ends.¹¹ The *qnrS1* is flanked by IS26 at its 5'-end and ISKpn19 at its 3'-end; as shown in Figure 1A. These suggest the region is variable and might capture the different resistance genes following exposure to a sophisticated antibiotic selective environment.

The reported *bla*_{SIM-2}-harboring pHN39-SIM carries *intI1-bla*_{SIM-2}-*ereA1-catB3q-arr3-aadA1a-qacEΔ1-sul1* gene array and two different mercury resistance gene arrays a and b, respectively harbored in Tn5046 and Tn512; the CatB3q is a derivative of CatB3.⁴ The mercury resistance gene array of pSIM-1-BJ01 (from 50,597 to 54,673 bp) only has 78% homology to the *mer* gene

Figure 1 Organization and alignment of the two drug-resistant regions on the pSIM-1-BJ01. **(A)** The *bla*_{SIM-1}-containing clinical class I integron. **(B)** The Tn1696-associated *armA*-containing region. Genes are denoted by arrows and are colored based on gene function classification. Red, drug-resistance genes; green, mobile elements; orange, heavy-metal resistance genes; violet, common genes.

array b of pHN39-SIM, but has 99.3% identity to that of a truncated mercury resistance transposon in pMER610 (GenBank: Y08993.1), as shown in Figure 1A. The mer resistance transposon in pMER610 was firstly found in England in a large Inc II-2 plasmid in a wide range of strains belonging of *Pseudomonas*, *Acinetobacter*, *Alcaligenes* and *Enterobacter*.¹² In pSIM-1-BJ01, the left-flank insertion site (5'-AATAAAGCAC-3') of IS4321R is located at the 5'-end of the mer gene array, the 3'-end of the mer array is flanked by IS4321R, indicating the mer gene array in pSIM-1-BJ01 might origin from the reported mer resistance transposon in pMER610 and be recombined into the pSIM-1-BJ01 by IS4321.

The tellurium resistance gene array (from 36,998 to 51,411 bp) in the pSIM-1-BJ01 is 99% identical to that of pRJA166a (from 78,751 bp to 93,171 bp), as shown in Figure 1A. The pRJA166a was also isolated from a *K. pneumoniae* strain, which caused a fatal outbreak of hyper-virulent carbapenem-resistant *K. pneumoniae* (hv CRKP) in a Chinese hospital.⁷ In pRJA166a, the *terF* was interrupted by the insertion of two transposase genes.

The *relB* between the *tel* and *mer* arrays, belongs to type II antitoxin systems which are expected to increase the longevity of plasmids in bacterial populations even in the absence of selection pressure due to antibiotics and metals;¹³ besides, a DNA starvation and stationary phase protection protein (ferritin) is also located between the *mer* array and the drug-resistant gene array in the clinical class 1 integron, see Figure 1A.

Here, the *bla*_{SIM-1}-carrying clinical class 1 integron, carrying the *mer* array, the *tel* array and different drug resistance genes, suggests the synergistic gene combination under clinical selection.

A Tn1696-associated transposon harboring *armA* and other resistance genes

The other class 1 integron in the pSIM-1-BJ01 has the *dfrA1-gcu37-aadA5-qacEΔ1-sul1* gene arrays, as shown in Figure 1B. The 16s rRNA methylase gene *armA* has been observed in a number of plasmids,¹⁴ among these plasmids, two direct repeats of IS26 and the resistance gene backbone consisting of *qacEΔ1-sul1-ISCRI-tnpU-armA-tnpD-msr(E)-mph(E)* are well conserved, here, the *armA* surrounding region is consistent with the reported conserved backbone. Compared with the Tn1548-associated *armA* backbone (GenBank: JN225877),¹⁴ as shown in Figure 1B, the sequence analysis showed that the IS26 at

the upstream of the conserved backbone was replaced by IS5075 in the pSIM-1-BJ01; IS5075 is normally found interrupting the terminal inverted repeats of Tn21-family transposons, here it is to be Tn1696 (Genbank: U12338).¹⁵

The region (from 258,498 to 265,885 bp) carrying resistance genes *cata2* and *tet(D)* with three direct repeats of IS26 is also conserved and consistent with that of the *bla*_{IMP-4}-carrying pMS7884A (Genbank: CP022533.1, from 143,213 to 135,889 bp) isolated from an *Enterobacter cloacae*, see Figure 1B. The antitoxin gene *higA*, toxin gene *higB* as well as the DNA protection protein (ferritin) gene, flanked by IS26 and ISKpn26, are also captured here to help bacteria to tolerate various antibiotic treatments.¹⁶

Overview of pKPI3624-1

The pKPI3624-1 is a 95,324 bp circular plasmid containing 124 putative ORFs (54 hypothetical proteins), and the average GC content is 52%, as shown in Figure S2. The pKPI3624-1 carrying *bla*_{TEM-1} and *bla*_{CTX-M-15} belongs to the IncFIIK type according to the PubMLST website and it is almost identical to the pL22-5 (Genbank: CP031262.1) isolated from a hypermucoviscous strain *Klebsiella quasi* ST367 and the pKF3-94 (Genbank: FJ876826) isolated from a clinical drug-resistant strain *K. pneumoniae* KF3.¹⁷ The *bla*_{CTX-M-15} was flanked by ΔTn2 at the downstream and ISEcp1 (interrupted by a truncated IS1) at the upstream, along with the *bla*_{TEM-1} carried by ΔTn2 transposon, which increasing the mobility of the two resistance genes.

Overview of pKPI3624-2

The pKPI3624-2 is a 38,669 bp circular plasmid containing 29 putative ORFs (8 hypothetical proteins), and the average GC content is 49%, as shown in Figure S3. The pKPI3624-2 belongs to IncFIA type and has never been reported before. The region (from 12,339 to 27,055 bp) containing three resistance genes *bla*_{CTX-M-14}, *bla*_{LAP-2}, and *qnrS1* flanked by ISEcp1 and ΔISKpn19, is 99% identical to that of pE66An (Genbank: HF545433.1, from 79,443 to 64,716 bp) isolated from *E. coli* in Vietnam. The *bla*_{CTX-M-14} is flanked by ISEcp1 at its 5'-end and IS903B at its 3'-end, while the *bla*_{LAP-2} is flanked by IS26 at its 5'-end and ΔIS2 at its 3'-end and the *qnrS1* is flanked by ΔIS2 at the upstream and ΔISKpn19 at the downstream, as shown in Figure S3.

Discussion

The infection rate of carbapenem-resistant *K. pneumoniae* (CRKP) has increased substantially in the past 10 years, even a fatal outbreak of hypervirulent carbapenem-resistant *K. pneumoniae* (hv CRKP) in a Chinese hospital.^{18–20} Here, we presented a clinically isolated *K. pneumoniae* with three resistance plasmids. Among them, the pSIM-1-BJ01 carried the rare carbapenemase gene *bla*_{SIM-1}. This is the first report of the *bla*_{SIM-1}-carrying megaplasmid isolated from *K. pneumoniae*. Most of the pSIM-1-BJ01 backbone matches that of pRJA166a, which was isolated from a clinical hypervirulent *K. pneumoniae* ST23 strain at Shanghai, China, recently.⁷ The highly homologous backbones between the two plasmids imply the close relationship of evolution. Two different multi-drug-resistant regions both carrying the class 1 integrons with different resistance genes have been assembled into the pSIM-1-BJ01 under the continuous selection exerted by human antibiotic use, made the novel pSIM-1-BJ01 become a significant environmental contaminant.²¹ Now there was one case report about the *bla*_{SIM-1} carrying *E. coli* from India.²² Except for the pSIM-1-BJ01, the pKP13624-2 carrying *bla*_{CTX-M-14} and *bla*_{LAP-2} has never been reported before. We should pay more attention to supervise and control the dissemination of carbapenem-resistant *K. pneumoniae* in public hospitals.

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

Table SI Antimicrobial drug susceptibility profiles

Antibiotic	MIC (mg/L)		
	<i>K. pneumoniae</i> 13624	<i>E. coli</i> J53 (pSIM-1-BJ01)	<i>E. coli</i> J53
Ampicillin	≥256	≥256	≤2
Piperacillin	≥128	≥128	≤2
Cephalothin	≥256	≥256	≤2
Cefoxitin	≥256	≥256	≤1
Cefotaxime	≥256	≥256	≤2
Cefuroxime	≥256	≥256	≤2
Ceftazidime	≥256	≥256	≤2
Aztreonam	≥128	≥128	≤1
Cefepime	≥64	≥32	≤1
Ertapenem	≥16	≥8	≤0.5
Imipenem	≥16	≥8	≤0.5
Meropenem	≥16	≥8	≤0.5
Gentamycin	≥128	≥128	≤0.5
Amikacin	≥64	≥64	≤0.5
Ciprofloxacin	≤0.5	≤0.5	≤0.5
Levofloxacin	≤0.5	≤0.5	≤0.5
Colistin	≤0.5	≤0.5	≤0.5

Figure S1 Schematic map of the pSIM-1-BJ01. The innermost circle presents GC-Skew [Green, GC skew+/Violet, GC skew-]. Starting from the inside, the second circle (black) presents GC content. The open reading frames are annotated in the third circle with arrows representing the direction of transcription and colored based on gene function classification [red, antibiotic resistance genes; yellow, replication initiation protein genes; blue, plasmid partition and conjugal transfer genes; green, transposon genes; orange, heavy-metal resistance genes]. The most outside circle indicates the homologue of pSIM-1-BJ01 to the related plasmids pRJA166a, pRNDM1 and pOZ181.

Figure S2 Schematic map of pKP13624-1. The innermost circle presents GC-Skew [Green, GC skew+/Violet, GC skew -]. Starting from the inside, the second circle (black) presents GC content. The open reading frames (ORFs) are annotated in the third circle with arrows representing the direction of transcription and colored based on gene function classification [red, antibiotic resistance genes; yellow, replication initiation protein genes; blue, plasmid partition and conjugal transfer genes; green, transposon genes].

Figure S3 Schematic map of pKP13624-2. The innermost circle presents GC-Skew [Green, GC skew+/Violet, GC skew -]. Starting from the inside, the second circle (black) presents GC content. The open reading frames (ORFs) are annotated in the third circle and colored based on gene function classification [red, antibiotic resistance genes; yellow, replication initiation protein genes; blue, plasmid partition and conjugal transfer genes; green, transposon genes].

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