

**Supplementary Materials for**

**An outbreak of *bla*<sub>KPC-4</sub>- and *bla*<sub>VIM-1</sub>-producing *Klebsiella pneumoniae* and  
*Klebsiella variicola* at a single hospital in South Korea**

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## **Supplemental Methods**

### **High molecular DNA isolation**

High molecular weight DNA was extracted from the nine strains. Bacterial strains were cultured overnight in 10 mL of Todd Hewitt Broth (THB) (Difco™) broth at 37 °C and 5 % CO<sub>2</sub>. After the incubation, the cultured medium was centrifuged at 4°C, 4,000 x g for 5 min, and the supernatant was removed. The cell pellet was re-suspended in 300 ul of Tris-EDTA (TE) buffer (10 mM Tris HCl, 1 mM EDTA, pH 8) containing 10 ul of RNase (Sigma) and 20 ul of lysozyme (Sigma), and then the further incubation was performed at 37 °C for 30 min. Following the lysozyme and RNase incubation, 1.7 ml of lysis solution (10 mM Tris HCl, 1 M NaCl, 100 mM EDTA, 0.5% Brij-58, 0.5% sodium lauroylsarcosine, pH 8.0) with 20 ul of proteinase K (Qiagen) was added. The mixture was incubated at 65 °C for 10 min in a water bath. After the lysis step, 2 ml of phenol-chloroform solution (1:1, vol/vol) were added. It was centrifuged at 12,000 x g, 4 °C for 30 min. Upper aqueous phase transferred to a new 1.5 mL centrifuge tube. The aqueous part was further washed with chloroform (1:1, vol/vol), and then it was centrifuged. Following the extraction, it was precipitated with isopropanol and the precipitated pellet was washed with 70 % ethanol. After aspiration the remain ethanol, the pellet was re-solved with the solution of 10 mM Tris-HCl, pH 8.3, and the purified DNA stored at -20 °C until used.

### **Whole genome sequencing and assembly**

Short-read sequencing libraries were prepared for each bacterial DNA sample using the TruSeq Nano DNA sample preparation kit (Illumina, San Diego, CA). Subsequently, the sequencing libraries were pooled and subjected to sequencing on the Illumina NovaSeq 6000 instrument for 300 cycles (2 x 150-bp paired-end). Sequencing adapters and low-quality bases were trimmed using Trimmomatic (version 0.39) [1]. The trimmed reads were assembled using

SPAdes (version 3.15.4) with default parameters [2], and the contigs were further scaffolded using MeDuSa (version 1.6) [3]. Contigs shorter than 200 bp were removed. QUAST (version 5.2.0) was used to generate assembly statistics [4].

For the five strains selected for long-read sequencing, SMRT (single molecule, real-time) sequencing libraries were constructed using the SMRTbell Express Template Prep Kit 2.0 (PacBio, Menlo Park, CA) and sequenced on the RSII (DSMCPE25 and DSMCPE65) or Sequel (DSMCPE46, DSMCPE56, and DSMCPE136) instrument (PacBio). SMRT cell data from the RSII instrument were *de novo* assembled using the RS HGAP assembly (version 3.0) included in SMRT Portal, while those from the Sequel instrument were assembled using the microbial assembly application of SMRT Link based on the HGAP assembly version 4.0. Further corrections were conducted by using Pilon (version 1.21) together with Illumina short-reads to improve assembly quality by correcting bases, fixing mis-assemblies, and filling gaps [5]. Raw sequencing reads have been deposited in the Sequence Read Archive under the accession number PRJNA1079714.

## **Molecular typing**

Kraken2 (version 2.1.3) was used to determine the species of each strain [6]. The identified *Klebsiella* spp. genomes were further confirmed using Kleborate (version 2.3.2) [7]. Multilocus sequence typing (ST) was predicted from the sequences using the Kleborate [7] and allelic profile of the seven housekeeping genes (*gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB*, and *tonB*) [8]. Acquisition of antimicrobial resistance genes, including carbapenemase gene, and their mutations were detected using Kleborate [7] employing the Comprehensive Antibiotic Resistance Database [9]. Capsule (K-) and O- antigen biosynthesis locus typing was performed using Kaptive [10], while the virulence loci and *wzi* alleles were determined using the BIGSdb [8]. The mobilomes were identified using IntegronFinder (version 2.0.2) [11] and

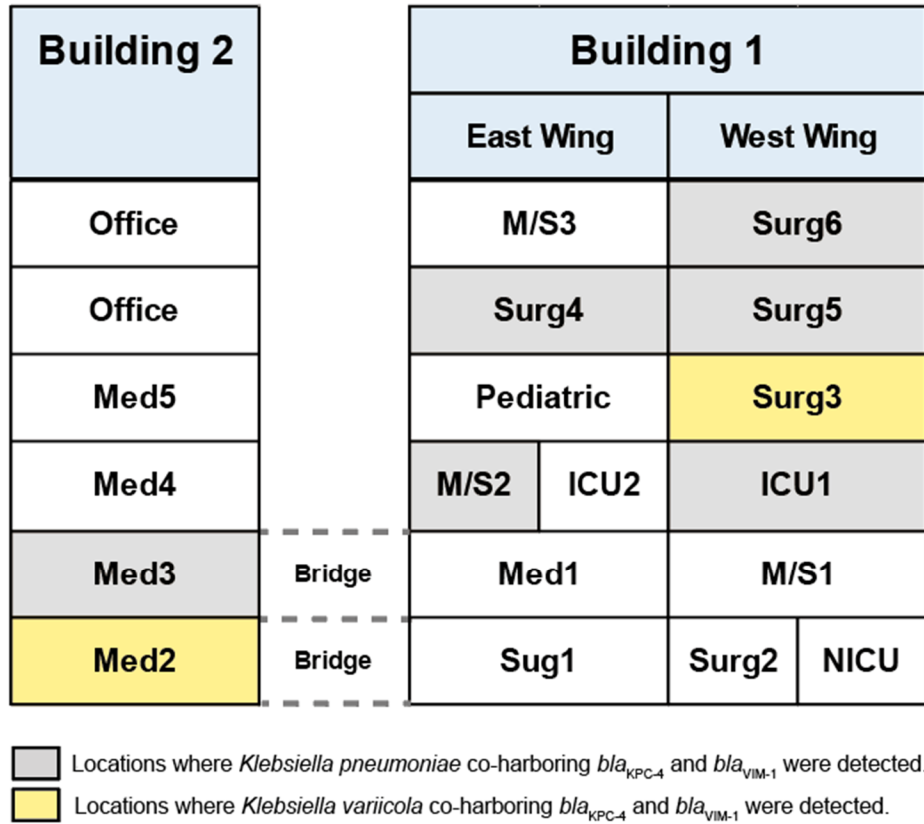
MobileElementFinder (version 1.0.6) [12]. Plasmid sequence comparison was performed using BLAST analysis and Proksee [13]. The gene cassette surrounding *bla*<sub>KPC-4</sub>, *bla*<sub>VIM-1</sub>, and *bla*<sub>OXA-2</sub> was visualized using Snapgene software (GSL Biotech, San Diego, CA) and gggenes-r-package [14]. For strains with only Illumina sequencing data, the presence of MGEs carrying *bla*<sub>KPC-4</sub> and *bla*<sub>VIM-1</sub> was confirmed by BLAST, defining a positive hit as a percent identity of  $\geq 95\%$  and percent coverage of  $\geq 95\%$ . The similarity of the plasmids was assessed by the average nucleotide identity (ANI) between the core genes of the isolates using pANito (<https://github.com/sanger-pathogens/panito>) [15]. Details of the genome-based molecular typing are listed in Supplementary Table 1.

Plasmid classification was performed based on replicon and MOB typing, using PlasmidFinder (version 2.1) [16], KpVR (version 1.0) [17], and MOB-Suite (version 3.10) [18]. Plasmid mobility was predicted according to the MOB-Suite (version 3.10.): conjugative, if plasmids contain at least one relaxase and a mate-pair formation marker; 2) mobilizable, if plasmids contain either a relaxase or an oriT but lack the mate-pair formation marker; 3) non-mobilizable, if plasmids lack both relaxase and oriT [18]. Presence of IncHI5 plasmids was further identified using repHI5B on pKOX\_R1 (accession number: CP003684) for BLASTN alignment ( $>95\%$  identity and 100% coverage) with GenBank of NCBI [19].

### **Phylogenetic analysis**

The *de novo* assemblies were annotated using Prokka (version 1.14.6) with default parameters [20]. A core gene alignment for the seven *K. pneumoniae* strains was created using Roary (version 3.13.0) [21]. A maximum likelihood phylogenetic tree based on core gene single nucleotide polymorphisms (SNPs) was constructed using RAxML (version 8.2.12) with 10,000 bootstraps and a generalized time-reversible gamma model [22]. The *K. pneumoniae subsp. pneumoniae* HS11286 (GenBank accession: CP003200) was used as an outgroup to root the

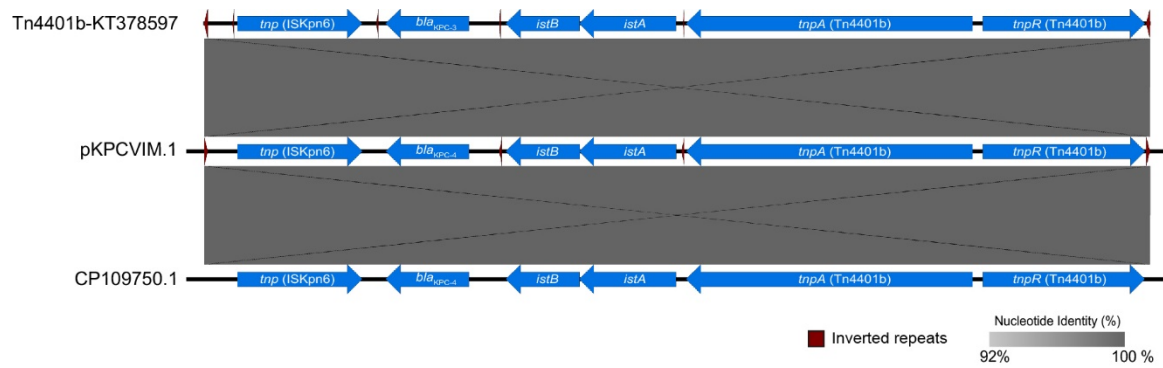
tree. The resulting phylogenetic tree was visualized in Microreact [23]. The chromosomal ANI between isolates was also calculated from the core genes using pANIto [15].



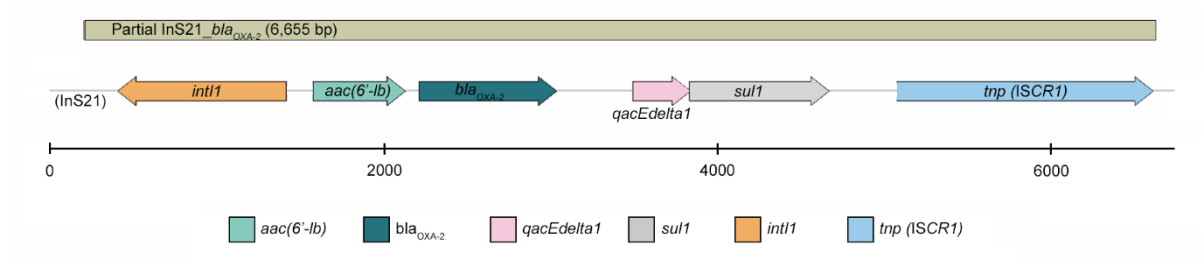
**Fig. S1.** Schematic of ward and ICU locations of the hospital. MED: medical ward, Surg: surgical ward, M/S: medical and surgical ward, ICU: intensive care unit, NICU: neonatal intensive care unit.

|                   | pDSMCPE56.1 (KP) | pDSMCPE65.1 (KP) | pDSMCPE136.1 (KP) | pDSMCPE25.1 (KV) |
|-------------------|------------------|------------------|-------------------|------------------|
| pDSMCPE56.1 (KP)  | 0                | 0                | 0                 | 11               |
| pDSMCPE65.1 (KP)  | 0                | 0                | 0                 | 11               |
| pDSMCPE136.1 (KP) | 0                | 0                | 0                 | 11               |
| pDSMCPE25.1 (KV)  | 11               | 11               | 11                | 0                |

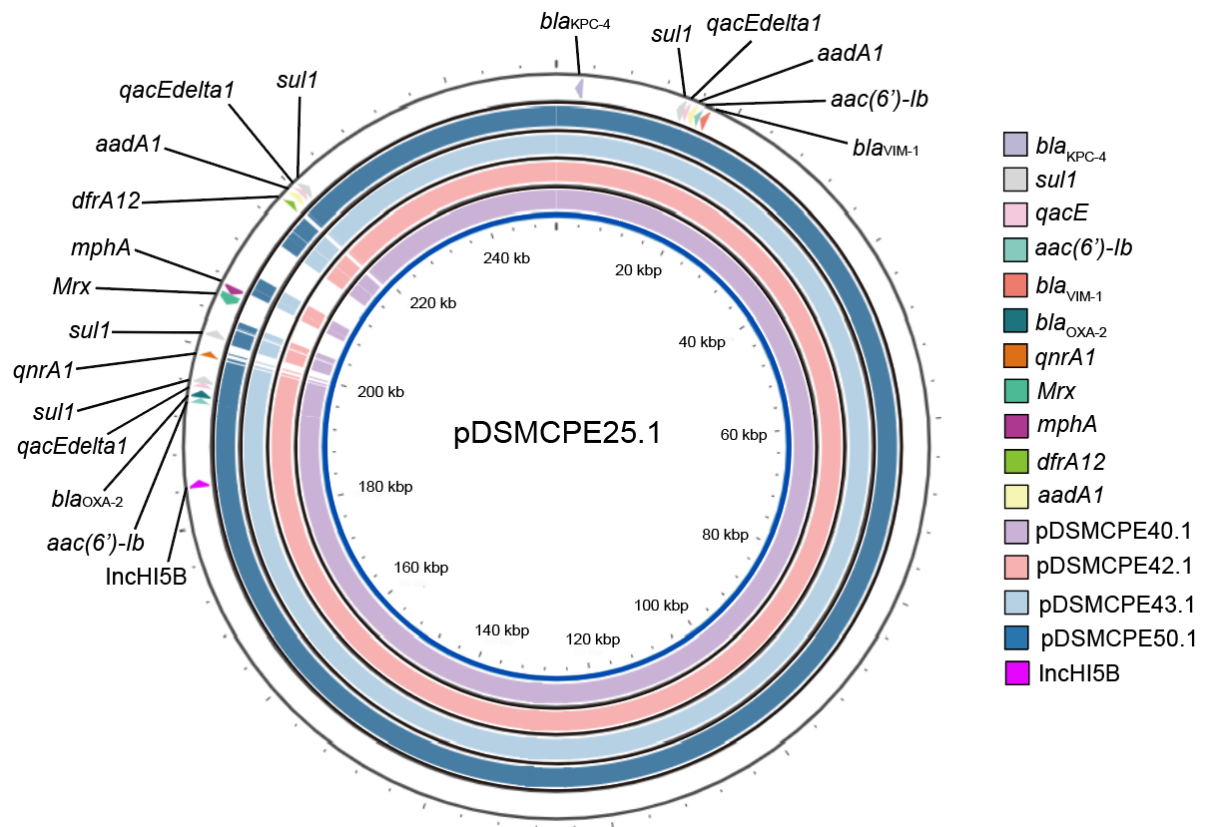
**Fig. S2.** SNP difference matrix of pKPCVIM.1 detected in *K. pneumoniae* (KP) and *K. variicola* (KV).



**Fig. S3.** Comparison of Tn4401 with previous sequence data. BLAST results against the TnCentral and NCBI nucleotide databases identified Tn4401b (GenBank accession number: KT378597) and *Enterobacter hormaechei* strain 2017-45-136-01-01 plasmid p2017-45-136-01-01\_3 (GenBank accession number: CP109750) as the most similar to Tn4401 in pKPCVIM1.1.



**Fig. S4.** A schematic diagram of gene structure of Class I integron carrying *bla*<sub>OXA-2</sub>. pKPCVIM.1 had Class I integron carrying *bla*<sub>OXA-2</sub>, *aac*(6')-Ib, *qacEdelta1*, and *sul1*, partially identical to InS21\_*bla*<sub>OXA-2</sub> [24].



**Fig. S5.** Comparison of plasmids carrying the *bla*<sub>KPC-4</sub>, *bla*<sub>VIM-1</sub>, and *bla*<sub>OXA-2</sub> genes. BLAST alignment of four *K. pneumoniae* isolates for which only Illumina sequencing data was produced are shown. The circular map was generated using Proksee, and 252-Kb sized plasmid recovered from the DSMCPE25 (pDSMCPE25.1) was used as the reference sequence.

**Table S2.** Antimicrobial susceptibility profile of *Klebsiella* spp. harboring *bla*<sub>KPC-4</sub> and *bla*<sub>VIM-1</sub>

| Isolate ID | Antimicrobial susceptibility test (MIC, µg/mL) |     |     |     |     |     |     |      |     |       |     |     |     |     |       |     |     |     |     |     |     |
|------------|------------------------------------------------|-----|-----|-----|-----|-----|-----|------|-----|-------|-----|-----|-----|-----|-------|-----|-----|-----|-----|-----|-----|
|            | MER                                            | ERT | IPM | COL | GEN | AN  | TOB | CIP  | LVX | SXT   | TET | TGC | FOS | AMP | SAM   | PIP | TZP | AZT | CTX | CAZ | FEP |
| DSMCPE25   | 64                                             | 64  | 32  | 2   | 8   | ≤16 | >8  | ≤1   | ≤2  | >2/38 | ≤4  | ≤1  | ≤32 | >16 | >16/8 | >64 | >64 | >16 | >32 | >16 | >16 |
| DSMCPE46   | 64                                             | 32  | 32  | 4   | 8   | ≤16 | >8  | ≤1   | ≤2  | >2/38 | ≤4  | ≤1  | ≤32 | >16 | >16/8 | >64 | >64 | >16 | >32 | >16 | >16 |
| DSMCPE40   | 8                                              | 8   | 16  | 64  | 8   | ≤8  | >8  | 2    | 2   | ≤2/38 | ≤4  | ≤1  | ≤32 | >16 | >16/8 | >64 | >64 | >16 | >32 | >16 | >16 |
| DSMCPE42   | 32                                             | 16  | 16  | 32  | 8   | ≤8  | >8  | >2   | 2   | ≤2/38 | ≤4  | ≤1  | ≤32 | >16 | >16/8 | >64 | >64 | >16 | >32 | >16 | >16 |
| DSMCPE43   | 32                                             | 16  | 16  | 64  | 8   | ≤8  | >8  | >2   | 4   | ≤2/38 | ≤4  | ≤1  | ≤32 | >16 | >16/8 | >64 | >64 | >16 | >32 | >16 | >16 |
| DSMCPE50   | 32                                             | 16  | 16  | 0.5 | 8   | ≤8  | >8  | ≤0.5 | ≤1  | ≤2/38 | ≤4  | ≤1  | ≤32 | >16 | >16/8 | >64 | >64 | >16 | >32 | >16 | >16 |
| DSMCPE56   | 32                                             | 16  | 32  | 16  | 8   | ≤8  | >8  | >2   | 2   | ≤2/38 | ≤4  | ≤1  | ≤32 | >16 | >16/8 | >64 | >64 | >16 | >32 | >16 | >16 |
| DSMCPE65   | 64                                             | 32  | 32  | 32  | 8   | ≤8  | >8  | >2   | 4   | ≤2/38 | ≤4  | ≤1  | ≤32 | >16 | >16/8 | >64 | >64 | >16 | >32 | >16 | >16 |
| DSMCPE136  | 64                                             | 32  | 64  | 128 | 8   | ≤8  | >8  | >2   | 4   | ≤2/38 | ≤4  | ≤1  | ≤32 | >16 | >16/8 | 64  | >64 | >16 | >32 | >16 | >16 |

MIC: minimum inhibitory concentration, MER: meropenem, ERT: ertapenem, IPM: imipenem, COL: colistin, GEN: gentamicin, AN: amikacin, TOB: tobramycin, CIP: ciprofloxacin, LVX: levofloxacin, SXT: trimethoprim/sulfamethoxazole, TET: tetracycline, TGC: tigecycline, FOS: fosfomycin, AMP: ampicillin, SAM: ampicillin/sulbactam, PIP: piperacillin, TZP: piperacillin/tazobactam, AZT: aztreonam, CTX: cefotaxime, CAZ: ceftazidime, FEP: cefepime.

**Table S3.** Characteristics of plasmid characteristics harboring *bla*<sub>KPC-4</sub> and *bla*<sub>VIM-1</sub> (pKPCVIM.1 and pKPCVIM.2) and *bla*<sub>CTX-M-15</sub> (pCTXM15).

| Plasmid type   | Replicase       | Relaxase | Mobility        | Plasmids of Isolates | Size (bp) | Antimicrobial Resistance Genes                                                                |                                                                                    |              |                                                                                                              |
|----------------|-----------------|----------|-----------------|----------------------|-----------|-----------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|--------------|--------------------------------------------------------------------------------------------------------------|
|                |                 |          |                 |                      |           | Beta-lactam                                                                                   | Aminoglycoside                                                                     | Quinolone    | Other antibiotics                                                                                            |
| pKPCVIM.1      | IncHI5B         | MOBH     | Conjugative     | pDSMCPE25.1          | 251,794   | <i>bla</i> <sub>KPC-4</sub> , <i>bla</i> <sub>VIM-1</sub> , <i>bla</i> <sub>OXA-2</sub>       | <i>aac</i> (6')-Ib8, <i>aac</i> (6')-Ib9, <i>ant</i> (3'')-Ia, <i>ant</i> (3'')-Ia | <i>qnrA1</i> | <i>qacEdelta1</i> , <i>sul1</i> , <i>qacEdelta1</i> , <i>sul1</i> , <i>mrx</i> , <i>mphA</i> , <i>dfrA12</i> |
|                |                 |          |                 | pDSMCPE56.1          | 226,907   | <i>bla</i> <sub>KPC-4</sub> , <i>bla</i> <sub>VIM-1</sub> , <i>bla</i> <sub>OXA-2</sub>       | <i>aac</i> (6')-Ib8, <i>aac</i> (6')-Ib9, <i>ant</i> (3'')-Ia                      |              | <i>qacEdelta1</i> , <i>sul1</i> , <i>qacEdelta1</i> , <i>sul1</i>                                            |
|                |                 |          |                 | pDSMCPE65.1          | 227,264   | <i>bla</i> <sub>KPC-4</sub> , <i>bla</i> <sub>VIM-1</sub> , <i>bla</i> <sub>OXA-2</sub>       | <i>aac</i> (6')-Ib8, <i>aac</i> (6')-Ib9, <i>ant</i> (3'')-Ia                      |              | <i>qacEdelta1</i> , <i>sul1</i> , <i>qacEdelta1</i> , <i>sul1</i>                                            |
|                |                 |          |                 | pDSMCPE136.1         | 228,870   | <i>bla</i> <sub>KPC-4</sub> , <i>bla</i> <sub>VIM-1</sub> , <i>bla</i> <sub>OXA-2</sub>       | <i>aac</i> (6')-Ib8, <i>aac</i> (6')-Ib9, <i>ant</i> (3'')-Ia                      |              | <i>qacEdelta1</i> , <i>sul1</i> , <i>qacEdelta1</i> , <i>sul1</i>                                            |
| pKPCVIM.2      | IncR            | MOBF     | Non-mobilizable | pDSMCPE25.2          | 145,583   | <i>bla</i> <sub>KPC-4</sub> , <i>bla</i> <sub>VIM-1</sub>                                     | <i>aac</i> (6')-Ib9, <i>ant</i> (3'')-Ia                                           |              | <i>qacEdelta1</i> , <i>sul1</i>                                                                              |
|                |                 |          |                 | pDSMCPE46.2          | 145,582   | <i>bla</i> <sub>KPC-4</sub> , <i>bla</i> <sub>VIM-1</sub>                                     | <i>aac</i> (6')-Ib9, <i>ant</i> (3'')-Ia                                           |              | <i>qacEdelta1</i> , <i>sul1</i>                                                                              |
| pCTXM15        | IncFII(K), IncR | MOBF     | Mobilizable     | pDSMCPE56.3          | 91,678    | <i>bla</i> <sub>CTX-M-15</sub> , <i>bla</i> <sub>TEM-1</sub>                                  |                                                                                    | <i>qnrS1</i> |                                                                                                              |
|                |                 |          |                 | pDSMCPE65.3          | 94,729    | <i>bla</i> <sub>CTX-M-15</sub> , <i>bla</i> <sub>CTX-M-15</sub> , <i>bla</i> <sub>TEM-1</sub> |                                                                                    | <i>qnrS1</i> |                                                                                                              |
|                |                 |          |                 | pDSMCPE136.3         | 94,729    | <i>bla</i> <sub>CTX-M-15</sub> , <i>bla</i> <sub>CTX-M-15</sub> , <i>bla</i> <sub>TEM-1</sub> |                                                                                    | <i>qnrS1</i> |                                                                                                              |
| Other plasmids | IncFIB          | -        | Non-mobilizable | pDSMCPE56.3          | 168,415   |                                                                                               |                                                                                    |              |                                                                                                              |
|                |                 |          |                 | pDSMCPE65.3          | 168,415   |                                                                                               |                                                                                    |              |                                                                                                              |
|                |                 |          |                 | pDSMCPE136.3         | 168,415   |                                                                                               |                                                                                    |              |                                                                                                              |

## Supplemental References

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