# Dominant Carbapenemase-Encoding Plasmids in Clinical Enterobacterales Isolates and Hypervirulent *Klebsiella pneumoniae*, Singapore

# Appendix 2

## **Supplementary Methods**

### **Mutant Generation**

To delete genes/regions in hypervirulent *Klebsiella pneumonia*e,  $\approx$ 1,000-bp fragments upstream and downstream of the target were amplified with PCR (Appendix 2 Table 3) from genomic DNA templates with Taq polymerase and then assembled in a conditional suicide pR6KmobsacB vector by using NEBuilder HiFi DNA Assembly Master Mix (New England Biolabs). The vectors were then transformed into *Escherichia coli* S17–1 $\lambda$ pir competent cells before being conjugated into hypervirulent *K. pneumoniae* strains. Single crossover transconjugants were selected on lysogeny agar with kanamycin + fosfomycin (50 µg/mL + 40 µg/mL) and subcultured in lysogeny broth containing 15% sucrose without NaCl. Kanamycinsensitive, double crossovers were screened for successful deletion by PCR. For the generation of capsule null mutants in NUH66, TTSH25, and SGH07, genomes were first annotated with Prokka 1.13 (1) to locate the initial glycotransferase *wcaJ* gene. The *wcaJ* gene was deleted in each strain by using the same method.

## **Construction of Plasmids**

Plasmids were introduced into bacterial strains by electroporation, chemical transformation, or conjugation (Appendix 2 Table 2). Plasmids were constructed by first amplifying individual fragments from various plasmids by using Q5 High-Fidelity DNA Polymerase (New England Biolabs, NEB) in PCR and then all the fragments were assembled by using NEBuilder HiFi DNA Assembly Cloning Kit (NEB). Plasmid mutants were generated based on the Lambda Red system (2) with some modifications. First, a curable Lambda Red plasmid (pACYC-flp-Red-sacB) was generated by combining the enhanced Flp recombinase gene from pFLPe2 (3), the Lambda Red recombinase genes from pKD46 (4), and the *sac*B gene from pK18mobsacB (5) into pACYC184 (6). Electroporation-competent cells of *E. coli* MG1655 carrying pACYC-flp-Red-sacB and pKPC2 or pNDM1 were prepared in the presence of L-arabinose (0.2%). The competent cells were electroporated with PCR product of chloramphenicol resistance gene (CmR) or kanamycin resistance gene (KmR) with 40-nt extensions that are homologous to regions adjacent to the gene to be inactivated. CmR or KmR transformants were selected. Gene replacement was verified by colony PCR. The pACYC-flp-Red-sacB plasmid was cured from the strain by growing them in medium containing sucrose (15%).

#### Plasmid Genome Sequence Reference Database

Reference plasmid genome sequences for each carbapenemase gene allele were derived from 2 sources: NCBI RefSeq database (https://www.ncbi.nlm.nih.gov/refseq) or fully circularized plasmid genome sequences from hybrid assemblies based on Oxford Nanopore Technologies (ONT, https://nanoporetech.com). Complete plasmid sequences were downloaded from NCBI RefSeq database by using the search term "plasmid." The downloaded sequence data were further filtered to only include plasmid sequences with identifiers that met the keyword criteria "plasmid" AND "circular" NOT "gene" NOT "partial" NOT "incomplete" NOT "putative." Additionally, the dataset was filtered to remove exact duplicate sequences. All known alleles from ARG-ANNOT database (7) were downloaded and plasmid sequences were aligned against the carbapenemase gene allele sequences. Only plasmids that had 100% coverage and 100% identity with the carbapenemase gene allele were incorporated into the database (n = 396). Second, a set of fully circularized plasmid genome sequences (n = 542) extracted from ONTbased hybrid assemblies were added to the reference database using the same criteria for the specific carbapenemase gene allele presence. In total, the plasmid genome sequence reference database contained 938 complete plasmid sequences. Subsequently, a self-BLAST was performed and sequences that shared 99% identity, 99% query coverage, and 99% subject coverage were grouped into clusters.

#### Identification of Carbapenemase-Encoding Plasmids for Each Isolate

For each isolate, fully circularized CP sequence was identified from among the 542 hybrid assemblies described above, if available. For isolates that did not have a fully circularized carbapenemase-encoding plasmids sequence, plasmid identification was performed using the following steps: (i) PlasmidSeeker (v0.1; 2017–04–21) was run using the Illumina reads as input and with default parameters against the carbapenemase gene allele-specific reference database described above (n = 938) to obtain candidate plasmids; (ii) For each isolate, the carbapenemase gene-containing contig (carrying the carbapenemase gene with at least 99% identity and 90% gene coverage) was identified from the Illumina assembly; (iii) The carbapenemase genecontaining contig identified in step (ii) was aligned using the NCBI BLAST tool against the candidate plasmids obtained by PlasmidSeeker in step (i), only retaining candidate plasmids sharing ≥90% k-mers with the isolate as determined by PlasmidSeeker, and carbapenemase gene-containing contig BLAST alignment coverage of  $\geq 90\%$ ; (iv) Finally, the specific CPplasmid for each isolate was determined according to the following selection criteria consecutively: highest query coverage, highest k-mer coverage, and longest plasmid. For isolates that still had multiple valid candidate plasmid assignments, priority was given to the dominant plasmid that was determined for the given carbapenemase gene. Cluster assignment was derived from the cluster membership of the assigned carbapenemase-encoding plasmid. The largest cluster of carbapenemase-encoding plasmids for a given carbapenemase gene was termed as the dominant cluster.

#### Survival-Analysis Approach

We used the dataset shown in Appendix 2 Figures 8 and 9 comprising 98 donor-recipient pairs, we first classified each pair into one of 4 levels of taxonomic relatedness: same strain, same species, same genus, or different genera. The full dataset of log-conjugation frequencies for pKPC2 (522 datapoints) or pNDM1 (530 datapoints) was regressed against this variable. Unobserved plasmid conjugation events were taken to be left-censored, with the detection limit set at 1e-8 (as the minimum observed value was 5e-8). Donor-recipient pair was modeled as a random effect to account for unobserved heterogeneity specific to each pair (such as incompatible plasmids in the recipient).

Mathematically,

 $\log_{10} f = \beta_0 + \beta_1$ (same species) +  $\beta_2$ (same genus) +  $\beta_3$ (other genus),

where f is the conjugation frequency,  $\beta_0$  is the baseline log-conjugation frequency between the same strain, and  $\beta_1$ ,  $\beta_2$ , and  $\beta_3$  denote the relative change in log-conjugation frequency for transfer between the same species, same genus, or other genus, respectively. The coefficients  $\beta_0$ ,  $\beta_1$ ,  $\beta_2$ , and  $\beta_3$  were inferred using the survreg function of the R survival package (version 3.1-12) (8), with donor-recipient pair modeled as a gaussian frailty. The 95% confidence intervals were computed from 1,000 bootstrap replicates using the R boot package version 1.3-28 (9).

#### **Data Availability**

Raw sequence data from this study were uploaded to the NCBI Sequence Read Archive (SRA) Database under Bioproject accession numbers PRJNA757551 and PRJNA765801 for the Illumina short-read sequencing data, PRJNA801425 for the Oxford Nanopore Technologies (ONT) long-read sequencing data for the plasmid growth rate experiment, and PRJNA801415 for the ONT long-read sequencing data that contributed to the plasmid genome reference sequence database.

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Strains     Features     Vinulence factors     reference       ATCC 13047     Type strain     ND     ATCC       Escherichia coli     K-12     ND     (10)       SLC-568     MG1655 ASC:kan     ND     (12)       Nissle 1917     Probiotic strain     ND     (12)       SIT-1 Apir     Conjugation donor carrying an integrated RP4-2     ND     (14)       ATCC 70323     Quality control strain     ND     ATCC       ATCC 70323     Quality control strain     ND     ATCC       S071167306     Clinical isolate     ND     This study       8071167307     ND     (15)     Study       S071167308     Clinical isolate     ND     This study       NUH59     KL10, ST906     ND     This study       NUH59     KL24, ST29, environmental isolate     ND     (15)       NUH60     KL2, ST85     ICEKp10, <i>iuc, ab, iro, mpA</i> (15)       NUH614     KL2, ST80     ICEKp10, <i>iuc, ab, iro, mpA</i> (15)       NUH64     KL2, ST1049     ICEKp10, <i>iuc, ab, iro, mpA</i> (15) </th <th>Typervirulent Nebslena priedri</th> <th>nomae, Singapore</th> <th></th> <th>Source or</th>	Typervirulent Nebslena priedri	nomae, Singapore		Source or
Enterbalacter cloacae     Type strain     ND     ATCC       StCC 13047     Type strain     ND     (10)       Escherichia coli     K-12     ND     (10)       StC-568     M01655 hsdS:kan     ND     (11)       UTB9     Uropathogenic strain     ND     (13)       S17-1 Apir     Conjugation donor carrying an integrated RP4-2     ND     (14)       Enterobacter hormaechei     Conjugation donor carrying an integrated RP4-2     ND     (15)       Kozytoca     Conjugation donor carrying an integrated RP4-2     ND     (15)       Kozytoca     Clinical isolate     ND     This study       8071169205     Clinical isolate     ND     This study       Koricola     ND     This study     ND     (15)       Kub159     KL10, ST906     ND     This study       NUH29     KL2, ST20, environmental isolate     ND     (16)       NUH40     KL2, ST2039     ICEKp10, i.uc, cb, iro, rmpA     (15)       NUH414     KL2, ST204     ICEKp6, i.uc, iro, rmpA     (15)       NUH428     KL3, ST	Strains	Features	Virulence factors	reference
ATCC 13047     Type strain     ND     ATCC       MG1655     K-12     ND     (10)       SLC-568     MG1655 /mSG2:kan     ND     (11)       UTB9     Uropathogenic strain     ND     (12)       Nissle 1917     Conjugation donor carrying an integrade RP4-2     ND     (14)       Enterobacter hormaechei     ATCC 700323     Quality control strain     ND     ATCC       ATCC 70323     Quality control strain     ND     ATCC     ND     (15)       Koxtoca     S071169380     Clinical isolate     ND     This study       8071169380     Clinical isolate     ND     This study       NUH59     KL10, ST906     ND     This study       ATCC 703633     KL2, ST3, type strain     ND     ATCC       NUH59     KL24, ST23, type strain     ND     ATCC       NUH40     KL2, ST300     ICEKp10, uc, cb, iro, rmpA     (15)       NUH41     KL2, ST300     ICEKp10, uc, cb, iro, rmpA     (15)       NUH42     KL2, ST300     ICEKp10, uc, cb, iro, rmpA     (15) <t< td=""><td>Enterobacter cloacae</td><td></td><td></td><td></td></t<>	Enterobacter cloacae			
Escherichia coli     K-12     ND     (f0)       SLC-588     MG1655     K-12     ND     (f0)       SIT-1 Mpir     Conjugation donor carrying integrated RP4-2     ND     (f1)       SIT-1 Apir     Conjugation donor carrying integrated RP4-2     ND     (f4)       Enterobacter hormaechei     ATCC 700323     Quality control strain     ND     ATCC       ATCC 700323     Quality control strain     ND     TSH04     ND     Tris study       K varicola     ND     This study     Tris study     Tris study       8071167205     Clinical isolate     ND     This study       K varicola     ND     (f5)     NUH29     KL20, ST20     ND     (f6)       NUH29     KL210, ST20, ST	ATCC 13047	Type strain	ND	ATCC
MG1655     K-12     ND     (10)       SLC-588     MG1655 hsdS::kan     ND     (17)       UT189     Uropathogenic strain     ND     (12)       SIT-1 Apir     Conjugation donor carrying an integrated RP4-2     ND     (14)       Enterobacter hormaechei     ATCC 70033     Quality control strain     ND     ATCC 70033       Rebsella quasipneumoniae     TTSH04     KL114, ST2037     ND     (15)       Koxytoca     S071169300     Clinical isolate     ND     This study       Korotoca     KL10, ST906     ND     This study       Korotoca     KL2, ST20     ND     (15)       Kuraicola     KL2, ST20     ND     (15)       NUH53     KL2, ST20     ND     (15)       Kuraicola     KL2, ST20     ND     (15)       NUH04     KL2, ST23     ICEKp10, iuc, cb, iro, rmpA     (15)       NUH05     KL1, ST23     ICEKp10, iuc, cb, iro, rmpA     (15)       NUH06     KL2, ST203     ICEKp10, iuc, cb, iro, rmpA     (15)       NUH64     KL2, ST203	Escherichia coli			
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Clinical solution     Quality control strain     ND     ATCC       Klebsiella quasipneumoniae     TTSH04     KL114, ST2037     ND     (15)       Koytoca     S071169205     Clinical isolate     ND     This study       8071169205     Clinical isolate     ND     This study       Koytoca     ND     This study     ND     This study       Kortoca     ND     KL28, ST20     ND     (15)       Kortoca     KL28, ST20     ND     (15)     (15)       KP-1     KL24, ST20     ND     (15)     (16)       NUH03     KL2, ST2039     ICEKp10, iuc, cib, iro, mpA     (15)       NUH04     KL2, ST380     IUCEKp10, iuc, cib, iro, mpA     (15)       NUH13     KL1, ST23     ICEKp10, iuc, cib, iro, mpA     (15)       NUH46     KL2, ST1049     ICEKp10, iuc, cib, iro, mpA     (15)       NUH46     KL2, ST1049     ICEKp10, iuc, cib, iro, mpA     This study       NUH66     KL2, ST2039     ICEKp10, iuc, cib, iro, mpA     This study       NUH66     KL2, ST2049     ICEKp10, i	Enterobacter hormaechei			
Klebsielia quasipneumoniae     KL 114, ST2037     ND     (15)       K oxyloca     ND     This study       8071187205     Clinical isolate     ND     This study       NUH-59     K.L10, ST906     ND     This study       X varitoola     NUH-59     K.L10, ST906     ND     This study       X varitoola     NUH-59     K.L28, ST20     ND     (15)       K- preumoniae     ATCC 13883     KL23, ST3 type strain     ND     ND     (15)       K-Preumoniae     KL28, ST20     ND     (15)     (16)     (17)       NUH-03     KL24, ST29, environmental isolate     ND     (15)     (16)     (17)       NUH-04     KL2, ST65     ICEKp10, iuc, clb, iro, rmpA     (15)     (15)     (15)     (15)     (15)     (16)     (15)     (16)	ATCC 700323	Quality control strain	ND	ATCC
NumberKL114, ST2037ND(15)K avylocaSCH10, ST906NDThis study8071169300Clinical isolateNDThis study8071167205Clinical isolateNDThis studyK variicolaNDKL10, ST906NDThis studyNUH59K.L10, ST906NDThis studyK pneumoniaeKL28, ST20ND(15)ATCC 13883KL3, ST3, type strainND(15)KP-1KL54, ST29, environmental isolateND(15)NUH03KL2, ST65ICEKp10, luc, cib, iro, rmpA(15)NUH04KL2, ST2039ICEKp10, luc, cib, iro, rmpA(15)NUH64KL2, ST300ICEKp6, luc, iro, rmpA(15)NUH43KL1, ST214ICEKp10, luc, cib, iro, rmpA(15)NUH44KL2, ST309ICEKp6, luc, iro, rmpA(15)NUH46KL2, ST049ICEKp6, luc, iro, rmpAThis studyNUH66KL2, ST049ICEKp10, luc, cib, iro, rmpAThis studyNUH66KL2, ST039ICEKp10, luc, cib, iro, rmpAThis studyNUH66KL2, ST049ICEKp10, luc, cib, iro, rmpAThis studyNUH66KL2, ST2039ICEKp10, luc, cib, iro, rmpAThis studyNUH66KL2, ST2039ICEKp10, luc, cib, iro, rmpAThis studyNUH66KL2, ST2039ICEKp10, luc, cib, iro, rmpAThis studyNUH66KL1, ST23ICEKp10, luc, cib, iro, rmpAThis studySGH07KL5, ST60ICEKp10, luc, cib, iro, rmpAThis study<	Klebsiella quasinneumoniae	Quality control strain	NB .	71100
F1504KLTH, S1207KD(10)8071167205Clinical isolateNDThis study8071167205Clinical isolateNDThis studyK. varicolaNUH59K.110, ST906NDThis studyMUH59K.128, ST20ND(15)K. PareumoniaeKL28, ST20ND(15)KP-1KL24, ST29, environmental isolateND(16)KU103KL2, ST65ICEKp10, luc, clb, iro, rmpA(15)NUH04KL2, ST65ICEKp10, luc, clb, iro, rmpA(15)NUH05K.L1, ST23ICEKp3, luc, iro, rmpA(15)NUH06KL1, ST23ICEKp6, luc, iro, rmpA(15)NUH26KL2, ST80ICEKp6, luc, iro, rmpA(15)NUH26KL2, ST309ICEKp6, luc, iro, rmpA(15)NUH26KL2, ST80ICEKp6, luc, iro, rmpAThis studyNUH46KL2, ST86Ice, clb, iro, rmpAThis studyNUH66KL2, ST60ICEKp6, luc, iro, rmpAThis studyNUH66KL2, ST80IceKp10, luc, clb, iro, rmpAThis studySGH07KL5, ST60ICEKp10, luc, clb, iro, rmpAThis studySGH07SGH07, wcaJ deletion (capsule null; deletion of initial glycosyltransferase) </td <td></td> <td>KI 114 ST2027</td> <td>ND</td> <td>(15)</td>		KI 114 ST2027	ND	(15)
A Dypolod 8071165380Clinical isolateNDThis study8071165380Clinical isolateNDThis study8071165380Clinical isolateNDThis study8071165380KL10, ST906NDThis studyK variicolaNDKL28, ST20ND(15)KP-1KL54, ST29, environmental isolateND(16)NUH29KL28, ST20ND(16)NUH03KL2, ST65ICEKp10, iuc, clb, iro, rmpA(15)NUH04KL2, ST2039ICEKp2, iuc, iro, irmpA(15)NUH05KL1, ST23ICEKp3, iuc, iro, rmpA(15)NUH06KL1, ST234ICEKp6, iuc, iro, rmpA(15)NUH40KL2, ST1049ICEKp6, iuc, iro, rmpA(15)NUH40KL2, ST1049ICEKp6, iuc, iro, rmpAThis studyNUH66KL2, ST1049ICEKp6, iuc, iro, rmpAThis studyNUH66KL2, ST1049ICEKp6, iuc, iro, rmpAThis studyNUH66KL2, ST2039ICEKp10, iuc, clb, iro, rmpAThis studyNUH66KL2, ST2039ICEKp10, iuc, clb, iro, rmpAThis studyNUH66NUH66, wcaJ deletion (capsule null; deletion of initial glycosyltransferase)ICEKp10, iuc, clb, iro, rmpAThis studySGH10SGH10, wcaJ deletion (capsule null; deletion of initial glycosyltransferase)ICEKp10, iuc, clb, iro, rmpAThis studySGH102SGH10, wcaJ deletion (deletion of mpA abrogates the hypernucoviscous phenotype)ICEKp10, iuc, clb, iro, rmpAThis studySGH102SGH10, WcaJ de	K avutaaa	RE114, 312037	ND	(13)
60// 1165360     Clinical isolate     ND     This study       60// 1165360     Clinical isolate     ND     This study       K. varicola     NUH59     KL10, ST906     ND     This study       K. preumoniae     ATCC 13883     KL3, ST3, type strain     ND     ATCC       K.P. neumoniae     NUH29     KL28, ST20     ND     (15)       K.P.1     KL54, ST29, environmental isolate     ND     (15)       NUH03     KL2, ST2039     ICEKp10, iuc, clb, iro, mpA     (15)       NUH04     KL2, ST380     Iuc, cb, iro, mpA     (15)       NUH14     KL2, ST1049     ICEKp6, iuc, iro, mpA     (15)       NUH66     KL2, ST1049     ICEKp6, iuc, iro, mpA     This study       NUH66     KL2, ST2039     ICEKp6, iuc, iro, mpA     This study       NUH66     KL2, ST1049     ICEKp6, iuc, iro, mpA     This study       NUH66     KL2, ST2039     ICEKp10, iuc, clb, iro, mpA     This study       NUH66     KL2, ST2039     ICEKp1, iro, mpA     This study       SGH07     KL5, ST60     ICEKp1, iro, mpA     Th	N. 0XY10Ca	Oliviaal isolata	ND	This study
80/116/205     Clinical isolate     ND     This study       K variicola     NUH59     KL10, ST906     ND     This study       K pneumoniae     ATCC 13883     KL3, ST3, type strain     ND     ATCC       NUH29     KL28, ST20     ND     (16)       NUH03     KL2, ST65     ICEKp10, iuc, clb, iro, rmpA     (15)       NUH04     KL2, ST203     ICEKp10, iuc, clb, iro, rmpA     (15)       NUH05     KL1, ST23     ICEKp10, iuc, clb, iro, rmpA     (15)       NUH64     KL2, ST30     iuc, cib, iro, rmpA     (15)       NUH64     KL2, ST30     iuc, cib, iro, rmpA     (15)       NUH64     KL2, ST30     IcEKp6, iuc, iro, rmpA     (15)       NUH66     KL2, ST2039     ICEKp6, iuc, iro, rmpA     This study       NUH66     KL2, ST2039     ICEKp6, iuc, iro, rmpA     This study       NUH66     KL2, ST2039     ICEKp1, iro, rmpA     This study       NUH66     KL2, ST2039     ICEKp1, iro, rmpA     This study       NUH66     KL2, ST2039     ICEKp10, iuc, clb, iro, rmpA     This study <t< td=""><td>8071169380</td><td></td><td>ND</td><td>This study</td></t<>	8071169380		ND	This study
K. varicola     NuH59     KL10, ST906     ND     This study       K. pneumoniae     ATCC 13883     KL3, ST3, type strain     ND     ATCC       ATCC 13883     KL3, ST3, type strain     ND     ATCC       NUH29     KL28, ST20     ND     (15)       KP-1     KL54, ST29, environmental isolate     ND     (15)       NUH03     KL2, ST65     ICEKp10, iuc, clb, iro, rmpA     (15)       NUH04     KL2, ST2039     ICEKp10, iuc, clb, iro, rmpA     (15)       NUH13     KL1, ST2044     ICEKp6, iuc, iro, rmpA     (15)       NUH40     KL2, ST380     iuc, clb, iro, rmpA     (15)       NUH41     KL2, ST380     iuc, clb, iro, rmpA     This study       NUH66     KL2, ST3786     iuc, clb, iro, rmpA     This study       NUH66     KL2, ST60     ICEKp10, iuc, clb, iro, rmpA     This study       NUH66     KL2, ST2039     ICEKp10, iuc, clb, iro, rmpA     This study       NUH66     KL2, ST2039     ICEKp10, iuc, clb, iro, rmpA     This study       NUH66     KL2, ST2039     ICEKp10, iuc, clb, iro, rmpA     T	80/116/205	Clinical isolate	ND	This study
NUH59KL10, ST906NDThis studyK, pneumoniaeATCC 13883KL3, ST3, type strainNDATCCNUH29KL28, ST20ND(15)NUH29KL28, ST65ICEKp10, iuc, clb, iro, rmpA(16)NUH03KL2, ST65ICEKp10, iuc, clb, iro, rmpA(15)NUH04KL2, ST2039ICEKp10, iuc, clb, iro, rmpA(15)NUH05KL1, ST23ICEKp10, iuc, clb, iro, rmpA(15)NUH06KL1, ST2044ICEKp10, iuc, clb, iro, rmpA(15)NUH13KL2, ST1049ICEKp6, iuc, iro, rmpA(15)NUH140KL2, ST1049ICEKp6, iuc, iro, rmpA(15)NUH61KL2, ST2039ICEKp10, iuc, clb, iro, rmpA(15)NUH66KL2, ST2039ICEKp10, iuc, clb, iro, rmpAThis studyNUH66KL2, ST2039ICEKp10, iuc, clb, iro, rmpAThis studyNUH66KL2, ST2039ICEKp10, iuc, clb, iro, rmpAThis studyNUH66, wcaJSGH07, wcaJ deletionICEKp1, iro, rmpAThis studySGH07KL5, ST60ICEKp1, iro, rmpAThis studySGH07KL2, ST2039ICEKp10, iuc, clb, iro, rmpAThis studySGH10KL2, ST2039ICEKp10, iuc, clb, iro, rmpAThis studyTTSH25kL2, ST2039ICEKp10, iuc, clb, iro, rmpAThis studySGH10, wcaJ deletion (capsule null; deletion of iuc, clb, iro, rmpAThis studyITSH25KL2, ST2039ICEKp10, iuc, clb, iro, rmpAThis studySGH10, Mrad Adeletion (capsule null; deletion of iuc, clb, iro, rmpA </td <td>K. variicola</td> <td></td> <td></td> <td></td>	K. variicola			
K. pneumoniae     K. J.s. ST3, type strain     ND     ATCC       ATCC 13883     KL28, ST20     ND     (15)       KP-1     KL24, ST29, environmental isolate     ND     (15)       NUH03     KL2, ST65     ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> (15)       NUH04     KL2, ST2039     ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> (15)       NUH06     KL1, ST2044     ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> (15)       NUH13     KL2, ST1049     ICEKp61, <i>iuc</i> , <i>iro</i> , <i>mpA</i> (15)       NUH46     KL2, ST1049     ICEKp6, <i>iuc</i> , <i>iro</i> , <i>mpA</i> This study       NUH66     KL2, ST1049     ICEKp6, <i>iuc</i> , <i>iro</i> , <i>mpA</i> This study       NUH66     KL2, ST1049     ICEKp6, <i>iuc</i> , <i>iro</i> , <i>mpA</i> This study       NUH66     KL2, ST60     ICEKp1, <i>iro</i> , <i>mpA</i> This study       NUH66, wcaJ deletion (capsule null; deletion of initial glycosyltransferase)     Ice Kp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> This study       SGH07     KL2, ST2039     ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> This study       TISH25     KL2, ST2039     ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> (15)       T	NUH59	KL10, ST906	ND	This study
ATCC 13883KL3, ST3, type strainNDATCCNUH29KL28, ST20ND(15)KP-1KL26, ST29, environmental isolateND(16)NUH06KL2, ST65ICEKp10, <i>iuc</i> , <i>cib</i> , <i>iro</i> , <i>mpA</i> (15)NUH06KL2, ST2039ICEKp10, <i>iuc</i> , <i>cib</i> , <i>iro</i> , <i>mpA</i> (15)NUH06KL1, ST23ICEKp10, <i>iuc</i> , <i>cib</i> , <i>iro</i> , <i>mpA</i> (15)NUH13KL1, ST2044ICEKp6, <i>iuc</i> , <i>cib</i> , <i>iro</i> , <i>mpA</i> (15)NUH26KL5, ST1049ICEKp6, <i>iuc</i> , <i>iro</i> , <i>mpA</i> (15)NUH26KL2, ST380 <i>iuc</i> , <i>cib</i> , <i>iro</i> , <i>mpA</i> (15)NUH61KL2, ST2039ICEKp10, <i>iuc</i> , <i>cib</i> , <i>iro</i> , <i>mpA</i> This studyNUH66KL2, ST2039ICEKp6, <i>iuc</i> , <i>iro</i> , <i>mpA</i> This studyNUH66KL2, ST2039ICEKp1, <i>iro</i> , <i>mpA</i> This studyNUH66KL2, ST2039ICEKp1, <i>iro</i> , <i>mpA</i> This studyNUH66KL2, ST2039ICEKp1, <i>iro</i> , <i>mpA</i> This studyTISH21KL5, ST60ICEKp1, <i>iro</i> , <i>mpA</i> This studyTISH25KL2, ST2039ICEKp10, <i>iuc</i> , <i>cib</i> , <i>iro</i> , <i>mpA</i> This studySGH10KL1, ST23ICEKp10, <i>iuc</i> , <i>cib</i> , <i>iro</i> , <i>mpA</i> This studySGH10SGH10, <i>mpA</i> GSS GH10, Geasule null; deletion of initial glycosyltransferase)ICEKp10, <i>iuc</i> , <i>cib</i> , <i>iro</i> , <i>mpA</i> This studySGH10AmrAASGH10, TrSS3 and T6SS3 deletion (deletion of microcin E492 and salmochelin)ICEKp10, <i>iuc</i> , <i>cib</i> , <i>iro</i> , <i>mpA</i> This studySGH10AGIE492SGH10, ICESS1 and T6SS3SGH10, ICESS1 and T6SS	K. pneumoniae			
NUH29     KL28, ST20     ND     (f5)       KP-1     KL54, ST29, environmental isolate     ND     (f6)       NUH03     KL2, ST65     ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> (f5)       NUH04     KL2, ST2039     ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> (f5)       NUH06     KL1, ST23     ICEKp3, <i>iuc</i> , <i>iro</i> , <i>mpA</i> (f5)       NUH13     KL1, ST2044     ICEKp6, <i>iuc</i> , <i>iro</i> , <i>mpA</i> (f5)       NUH46     KL2, ST380 <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> (f5)       NUH46     KL2, ST3049     ICEKp6, <i>iuc</i> , <i>iro</i> , <i>mpA</i> (f5)       NUH66     KL2, ST366 <i>iuc</i> , <i>iro</i> , <i>mpA</i> This study       NUH66     KL2, ST303     ICEKp6, <i>iuc</i> , <i>iro</i> , <i>mpA</i> This study       NUH66     KL2, ST303     ICEKp1, <i>iro</i> , <i>mpA</i> This study       NUH66, <i>wcaJ</i> deletion (capsule null; deletion of initial glycosyltransferase)     ICEKp1, <i>iro</i> , <i>mpA</i> This study       SGH07     KL2, ST2039     ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> This study       TTSH25     KL2, ST203     ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> (f5)       SGH10, wcaJ deletion (capsule null; deletion	ATCC 13883	KL3, ST3, type strain	ND	ATCC
KP-1KL54, ST29, environmental isolateND(16)NUH03KL2, ST65ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> (15)NUH04KL2, ST2039ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> (15)NUH05KL1, ST2044ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> (15)NUH13KL1, ST2044ICEKp61, <i>iuc</i> , <i>iro</i> , <i>mpA</i> (15)NUH14KL2, ST380 <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> (15)NUH26KL5, ST1049ICEKp61, <i>iuc</i> , <i>iro</i> , <i>mpA</i> (15)NUH61KL2, ST86 <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> This studyNUH66KL2, ST2039ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> This studyNUH66KL2, ST86 <i>iuc</i> , <i>iro</i> , <i>mpA</i> This studyNUH66KL2, ST2039ICEKp10, <i>iuc</i> , <i>ib</i> , <i>iro</i> , <i>mpA</i> This studyNUH66KL2, ST860ICEKp10, <i>iuc</i> , <i>ib</i> , <i>iro</i> , <i>mpA</i> This studyNUH66/ΔwcaJNUH66, <i>wcaJ</i> deletion (capsule null; deletion of initial glycosyltransferase)ICEKp10, <i>iuc</i> , <i>ib</i> , <i>iro</i> , <i>mpA</i> This studySGH07KL2, ST2039ICEKp10, <i>iuc</i> , <i>ib</i> , <i>iro</i> , <i>mpA</i> This studyTTSH25KL2, ST2039ICEKp10, <i>iuc</i> , <i>ib</i> , <i>iro</i> , <i>mpA</i> (15)TTSH25KL2, ST2039ICEKp10, <i>iuc</i> , <i>ib</i> , <i>iro</i> , <i>mpA</i> (16)SGH10KL1, ST23ICEKp10, <i>iuc</i> , <i>ib</i> , <i>iro</i> , <i>mpA</i> (16)SGH10KL1, ST23ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> (17)SGH10SGH10, <i>mpA</i> abrogates the hypermucoviscous phenotype)ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> (18)SGH10, CEKp10SGH10, GE42 deleti	NUH29	KL28, ST20	ND	(15)
NUH03KL2, ST65ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> (15)NUH04KL2, ST2039ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> (15)NUH06KL1, ST23ICEKp3, <i>iuc</i> , <i>iro</i> , <i>mpA</i> (15)NUH13KL1, ST2044ICEKp3, <i>iuc</i> , <i>iro</i> , <i>mpA</i> (15)NUH14KL2, ST380 <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> (15)NUH26KL5, ST1049ICEKp6, <i>iuc</i> , <i>iro</i> , <i>mpA</i> (15)NUH40KL2, ST2039ICEKp6, <i>iuc</i> , <i>iro</i> , <i>mpA</i> (15)NUH61KL2, ST2039ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> This studyNUH66 wcaJNUH66, wcaJ deletion (capsule null; deletion of initial glycosyltransferase)ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> This studySGH07KL5, ST60ICEKp1, <i>iro</i> , <i>mpA</i> This studyTISH25KL5, ST60ICEKp1, <i>iro</i> , <i>mpA</i> This studyTISH25KL2, ST2039ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> This studyTISH25KL2, ST2039ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> This studyTISH25KL1, ST23ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> This studySGH10KL1, ST23ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> (15)SGH10AmrpASGH10, mpA deletion (capsule null; deletion of initial glycosyltransferase)ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> (17)SGH10AcrpASGH10, The production of genomic island responsible for the production of genomic island responsible for the production of mericocin E492 and salmochelin)ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> This studySGH10AlCEKp10SGH10, CFKp10 deletion (deletion	KP-1	KL54, ST29, environmental isolate	ND	(16)
NUH04KL2, ST2039ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> (15)NUH06KL1, ST23ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> (15)NUH13KL1, ST2044ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> (15)NUH14KL2, ST360 <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> (15)NUH26KL5, ST1049ICEKp6, <i>iuc</i> , <i>iro</i> , <i>mpA</i> (15)NUH40KL2, ST360 <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> (15)NUH66KL2, ST2039ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> This studyNUH66KL2, ST2039ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> This studyNUH66KL2, ST2039ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> This studyNUH66KL2, ST2039ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> This studyNUH66KL5, ST60ICEKp1, <i>iro</i> , <i>mpA</i> This studyTTSH21KL5, ST60ICEKp1, <i>iro</i> , <i>mpA</i> This studyTTSH25KL2, ST2039ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> This studyTSH25KL2, ST2039ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> This studySGH10KL1, ST23ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> (17)SGH10ΔwcaJSGH10, <i>mpA</i> deletion (capsule null; deletion of initial glycosyltransferase)ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> (17)SGH10ΔGIE492SGH10, GE4202 deletion (deletion of genomic island responsible for the production of microcin E492 and salmochelin)ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> This studySGH10ΔGIE4910SGH10, ICEKp10 deletion (deletion of the two clusters of type 6 secretion system)ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> T	NUH03	KL2, ST65	ICEKp10, iuc, clb, iro, rmpA	(15)
NUH06KL1, ST23ICEKp3, <i>iuc</i> , <i>iro</i> , <i>rmpA</i> (15)NUH13KL1, ST2044ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> (15)NUH14KL2, ST380Iuc clb, <i>iro</i> , <i>rmpA</i> (15)NUH26KL5, ST1049ICEKp6, <i>iuc</i> , <i>iro</i> , <i>rmpA</i> (15)NUH40KL2, ST86Iuc, <i>clb</i> , <i>iro</i> , <i>rmpA</i> This studyNUH66KL2, ST86 <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> This studyNUH66KL2, ST86 <i>iuc</i> , <i>iro</i> , <i>rmpA</i> This studyNUH66KL2, ST86 <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> This studyNUH66KL2, ST80ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> This studyNUH66KL2, ST60ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> This studySGH07KL5, ST60ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> This studyTTSH25KL2, ST2039ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> This studyTSH25KL2, ST2039ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> (15)TSH25KL2, ST2039ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> (15)SGH10KL1, ST23ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> (16)SGH10 McaJSGH10, <i>mcJ</i> deletion (capsule null; deletion of initial glycosyltransferase)ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> (17)SGH10Δ <i>mpA</i> SGH10, TFSS1 and T6SS3 deletion (deletion of the two clusters of type 6 secretion system)ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> This studySGH10Δ <i>mrkA</i> SGH10, TFSS1 and T6SS3 deletion (deletion of the encoded for major subunit of type 3 fimbrae)ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> This study	NUH04	KL2, ST2039	ICEKp10, iuc, clb, iro, rmpA	(15)
NUH13KL1, ST2044ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> (15)NUH14KL2, ST380 <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> (15)NUH26KL5, ST1049ICEKp6, <i>iuc</i> , <i>iro</i> , <i>mpA</i> (15)NUH40KL5, ST1049ICEKp6, <i>iuc</i> , <i>iro</i> , <i>mpA</i> This studyNUH61KL2, ST86 <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> This studyNUH66KL2, ST2039ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> This studyNUH664KL2, ST60ICEKp1, <i>iuc</i> , <i>inr</i> , <i>mpA</i> This studySGH07KL5, ST60ICEKp1, <i>iuc</i> , <i>inr</i> , <i>mpA</i> This studyTTSH21KL5, ST60ICEKp1, <i>iro</i> , <i>mpA</i> This studyTTSH25KL2, ST2039ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> This studyTSH25KL2, ST2039ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> This studyTSH25KL2, ST2039ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> This studyTSH25KL1, ST23ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> This studySGH10KL1, ST23ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> (17)SGH10, <i>mpA</i> SGH10, <i>mpA</i> deletion (capsule null; deletion of initial glycosyltransferase)ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> (18)SGH10Δ <i>mpA</i> SGH10, <i>GIE492</i> deletion of genomic island responsible for the production of microcin E492 and salmochelin)ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> This studySGH10Δ <i>mpA</i> SGH10, ICEKp10 deletion (deletion of the two clusters of type 6 secretion system)ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>mpA</i> This studySGH10Δ <i>mtkA</i> SGH10, <i>InKA</i> deletionICEKp10, <i>iuc</i> , <i>clb</i> , <i>i</i>	NUH06	KL1, ST23	ICEKp3, iuc. iro, rmpA	(15)
NUH14K12, ST380iuc, clb, iro, mpA(15)NUH26KL5, ST1049ICEKp6, iuc, iro, mpA(15)NUH40KL5, ST1049ICEKp6, iuc, iro, mpA(15)NUH40KL2, ST86iuc, clb, iro, mpAThis studyNUH66KL2, ST80ICEKp6, iuc, iro, mpAThis studyNUH66KL2, ST80ICEKp6, iuc, iro, mpAThis studyNUH66KL2, ST80ICEKp1, iro, mpAThis studyNUH66KL5, ST60ICEKp1, iro, mpAThis studySGH07KL5, ST60ICEKp1, iro, mpAThis studyTTSH21KL5, ST60ICEKp1, iro, mpAThis studyTTSH25KL1, ST23ICEKp1, iro, mpAThis studySGH10KL1, ST23ICEKp1, iro, mpA(15)SGH10_mcaJSGH10, mcaJ deletion (capsule null; deletion of initial glycosyltransferase)ICEKp1, iuc, clb, iro, mpA(17)SGH10ΔmcaJSGH10, mcaJ deletion (capsule null; deletion of initial glycosyltransferase)ICEKp10, iuc, clb, iro, mpA(17)SGH10ΔmpASGH10, GE492 deletion (deletion of mpA abrogates the hypermucoviscous phenotype)ICEKp10, iuc, clb, iro, mpAThis studySGH10ΔmrpASGH10, GE492 deletion (deletion of microcin E42 and salmochelin)ICEKp10, iuc, clb, iro, mpAThis studySGH10ΔmrkASGH10, T6SS1 and T6SS3 deletion (deletion of the encoded for major subunit of type 3 fimbrae)ICEKp10, iuc, clb, iro, mpAThis studySGH10ΔmrkASGH10, KpVP deletionICEKp10, clbThis studySGH10ΔmrkASGH10, KpVP deletionIC	NUH13	KI 1 ST2044	ICEKp10 iuc clb iro rmpA	(15)
NUH26KL5, ST1049ICEKp6, <i>iuc</i> , <i>iro</i> , <i>rmpA</i> (15)NUH40KL5, ST1049ICEKp6, <i>iuc</i> , <i>iro</i> , <i>rmpA</i> This studyNUH40KL2, ST86 <i>iuc</i> , <i>iro</i> , <i>rmpA</i> This studyNUH61KL2, ST2039ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> This studyNUH66KL2, ST2039ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> This studyNUH66KL2, ST2039ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> This studySGH07KL5, ST60ICEKp11, <i>iro</i> , <i>rmpA</i> This studyTTSH25KL2, ST2039ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> This studyTTSH25KL2, ST2039ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> This studySGH10KL1, ST23ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> This studySGH10KL1, ST23ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> (17)SGH10, <i>mcA</i> Jeletion (capsule null; deletion of initial glycosyltransferase)ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> (17)SGH10, <i>mcA</i> SGH10, <i>mcA</i> J deletion (deletion of mcrocin E492 and salmochelin)ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> (18)SGH10Δ <i>rmpA</i> SGH10, GIE492 deletion (deletion of the poduction of mcrocin E492 and salmochelin)ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> This studySGH10Δ <i>rmpA</i> SGH10, ICEKp10SGH10, ICEKp10 deletion (deletion of the gene encoded for major subunit of type 3 fimbrae)ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> This studySGH10Δ <i>mrkA</i> SGH10, KpVPSGH10, KpVP deletionICEKp10, <i>clb</i> This studySGH10Δ <i>mrkA</i> SGH10, KpVPSGH10, KpVP deletion	NUH14	KL2 ST380	iuc clb iro rmpA	(15)
NUH40KL5, ST1049ICEKp6, iuc, iro, mpAThis studyNUH61KL2, ST86iuc, iro, mpAThis studyNUH66KL2, ST2039ICEKp1, iuc, clb, iro, mpAThis studyNUH66 wcaJNUH66, wcaJ deletion (capsule null; deletion of initial glycosyltransferase)ICEKp1, iuc, clb, iro, mpAThis studySGH07KL5, ST60ICEKp1, iro, mpAThis studySGH07KL5, ST60ICEKp1, iro, mpAThis studyTTSH21KL5, ST60ICEKp1, iro, mpAThis studyTTSH25KL2, ST2039ICEKp1, iro, mpAThis studyTTSH25KL2, ST2039ICEKp1, iro, mpAThis studyTSH25 AwcaJTTSH25, wcaJ deletion (capsule null; deletion of initial glycosyltransferase)ICEKp1, iro, mpAThis studySGH10KL1, ST23ICEKp10, iuc, clb, iro, mpA(17)SGH10ArmpASGH10, mmA deletion (deletion of mmA abrogates the hypernucoviscous phenotype)ICEKp10, iuc, clb, iro, mpA(18)SGH10AT6SS1 ΔT6SS3SGH10, TGSS1 and T6SS3 deletion (deletion of the chromosomal integrative and conjugative element encoding collbactin and yersiniabactin)ICEKp10, iuc, clb, iro, mpAThis studySGH10ATrkASGH10, KpVPSGH10, KpVP and mrkA deletionICEKp10, clbThis studySGH10ArmkASGH10, KpVP and mrkA deletionICEKp10, clbThis study	NUH26	KL5, ST1049	ICEKp6 iuc iro rmpA	(15)
NUH61KL2, ST86ICEL(p, Id, Id, Id, Id, Id, Id, Id, Id, Id, Id	NUH40	KL5, ST1040	ICEKp6 iuc iro rmpA	This study
NUH66KL2, S100Ide, ino, impAThis studyNUH66KL2, S1039ICEK p10, iuc, clb, iro, rmpAThis studyNUH66wcaJ deletion (capsule null; deletion of initial glycosyltransferase)ICEK p10, iuc, clb, iro, rmpAThis studySGH07KL5, ST60ICEK p1, iro, rmpAThis studyTTSH21KL5, ST60ICEK p10, iuc, clb, iro, rmpAThis studyTTSH25KL2, ST2039ICEK p10, iuc, clb, iro, rmpAThis studyTTSH25KL2, ST2039ICEK p10, iuc, clb, iro, rmpAThis studyTTSH25KL2, ST2039ICEK p10, iuc, clb, iro, rmpAThis studySGH10KL1, ST23ICEK p10, iuc, clb, iro, rmpAThis studySGH10KL1, ST23ICEK p10, iuc, clb, iro, rmpA(17)SGH10ΔarmpASGH10, rmpA deletion (deletion of rmpA abrogates island responsible for the production of microcin E492 and salmochelin)ICEK p10, iuc, clb, iro, rmpAThis studySGH10ΔT6SS1 ΔT6SS3SGH10, ICEKp10 deletion (deletion of the chromosomal integrative and conjugative elementi- encoded for major subunit of type 6 secretion system)ICEKp10, iuc, clb, iro, rmpAThis studySGH10ΔmrkASGH10, mrkA deletion (deletion of the encoded for major subunit of type 3 finbrae)ICEKp10, iuc, clb, iro, rmpAThis studySGH10ΔmrkASGH10, mrkA deletion (deletion of the encoded for major subunit of type 3 finbrae)ICEKp10, iuc, clb, iro, rmpAThis studySGH10ΔmrkASGH10, mrkA deletion (deletion of the encoded for major subunit of type 3 finbrae)ICEKp10, iuc, clb, iro, rmpAThis study<		KL2 ST86		This study
NUH66 NUH66, wcaJNUH66, wcaJ deletion (capsule null; deletion of initial glycosyltransferase)IDERP10, 10C, 0D, 100, 100, 100, 100, 100, 100, 100,		KL2, 5100		This study
NOHoo ΔwcaJNOHoo, wcaJ deletion (capsule null, deletion of initial glycosyltransferase)Ide, cb, ind, mpAThis studySGH07KL5, ST60ICEKp1, iro, rmpA(15)SGH07ΔwcaJSGH07, wcaJ deletion KL2, ST2039iro, rmpAThis studyTTSH25KL2, ST2039ICEKp10, iuc, clb, iro, rmpA(15)TTSH25KL1, ST23ICEKp10, iuc, clb, iro, rmpAThis studySGH10SGH10, wcaJ deletion (capsule null; deletion of initial glycosyltransferase)ICEKp10, iuc, clb, iro, rmpA(17)SGH10SGH10, wcaJ deletion (deletion of minitial glycosyltransferase)ICEKp10, iuc, clb, iro, rmpA(18)SGH10ΔmpASGH10, mpA deletion (deletion of mpA abrogates the hypermucoviscous phenotype)ICEKp10, iuc, clb, iro, rmpA(18)SGH10ΔGIE492SGH10, GIE492 deletion (deletion of microcin E492 and salmochelin)ICEKp10, iuc, clb, iro, rmpAThis studySGH10ΔICEKp10SGH10, ICEKp10 deletion (deletion of the two clusters of type 6 secretion system)ICEKp10, iuc, clb, iro, rmpAThis studySGH10ΔICEKp10SGH10, GIE492 deletion (deletion of the chromosomal integrative and conjugative element encoded for major subunit of type 3 fimbrae)ICEKp10, iuc, clb, iro, rmpAThis studySGH10ΔICEKpVPSGH10, KpVP and mrkA deletionICEKp10, clbThis study		NLZ, 512039	icerpio, iuc, cib, iro, impa	This study
SGH07KL5, ST60ICEKp1, iro, rmpA(15)SGH07ΔwcaJSGH07, wcaJ deletioniro, rmpAThis studyTTSH21KL5, ST60ICEKp1, iro, rmpAThis studyTTSH25KL2, ST2039ICEKp10, iuc, clb, iro, rmpA(15)TTSH25ΔwcaJTTSH25, wcaJ deletion (capsule null; deletion of initial glycosyltransferase)ICEKp10, iuc, clb, iro, rmpA(15)SGH10KL1, ST23ICEKp10, iuc, clb, iro, rmpA(17)SGH10KL1, ST23ICEKp10, iuc, clb, iro, rmpA(18)SGH10_mrpASGH10, wcaJ deletion (deletion of initial glycosyltransferase)ICEKp10, iuc, clb, iro, rmpA(19)SGH10_ArmpASGH10, GIE492 deletion (deletion of genomic island responsible for the production of genomic island responsible for the production of microcin E492 and salmochelin)ICEKp10, iuc, clb, iro, rmpAThis studySGH10_AT6SS1SGH10, T6SS1 and T6SS3 deletion (deletion of the chromosomal integrative and conjugative element encoding colibactin and yersiniabactin)ICEKp10, iuc, clb, iro, rmpAThis studySGH10_AmrkASGH10, mrkA deletion (deletion of the gene encoded for major subunit of type 3 finbrae)ICEKp10, iuc, clb, iro, rmpAThis studySGH10_AmrkASGH10, KpVP SGH10, KpVP and mrkA deletionICEKp10, clbThis study	NUH00ΔwcaJ		iuc, cib, iro, impa	This study
SGH07KL5, S160ICE Kp1, iro, rmpA(15)SGH07ΔwcaJSGH07, wcaJ deletioniro, rmpAThis studyTTSH21KL5, ST60ICEKp1, iro, rmpAThis studyTTSH25KL2, ST2039ICEKp10, iuc, clb, iro, rmpA(15)TTSH25 wcaJTTSH25, wcaJ deletion (capsule null; deletion of initial glycosyltransferase)ICEKp10, iuc, clb, iro, rmpA(17)SGH10KL1, ST23ICEKp10, iuc, clb, iro, rmpA(17)SGH10ΔwcaJSGH10, wcaJ deletion (capsule null; deletion of initial glycosyltransferase)ICEKp10, iuc, clb, iro, rmpA(18)SGH10ΔmrpASGH10, rmpA deletion (deletion of rmpA abrogates the hypermucoviscous phenotype)ICEKp10, iuc, clb, iro, rmpA(18)SGH10ΔGIE492SGH10, GIE492 deletion (deletion of genomic island responsible for the production of microcin E492 and salmochelin)ICEKp10, iuc, clb, iro, rmpAThis studySGH10ΔICEKp10SGH10, ICEKp10 deletion (deletion of the two clusters of type 6 secretion system)ICEKp10, iuc, clb, iro, rmpAThis studySGH10ΔmrkASGH10, mrkA deletion (deletion of the encoding colibactin and yersiniabactin)ICEKp10, iuc, clb, iro, rmpAThis studySGH10ΔmrkASGH10, KpVP SGH10, KpVP deletionICEKp10, iuc, clb, iro, rmpAThis studySGH10ΔmrkAΔKpVPSGH10, KpVP and mrkA deletionICEKp10, clbThis study	0.01107	Initial glycosyltransferase)		
SGH0/ΔwcaJSGH0/, wcaJ deletioniro, rmpAThis studyTTSH21KL5, ST60ICEKp1, iro, rmpAThis studyTTSH25KL2, ST2039ICEKp10, <i>iuc, clb, iro, rmpA</i> This studyTTSH25 McaJTTSH25, wcaJ deletion (capsule null; deletion of initial glycosyltransferase)ICEKp10, <i>iuc, clb, iro, rmpA</i> This studySGH10KL1, ST23ICEKp10, <i>iuc, clb, iro, rmpA</i> (17)SGH10ΔwcaJSGH10, wcaJ deletion (capsule null; deletion of initial glycosyltransferase)ICEKp10, <i>iuc, clb, iro, rmpA</i> (18)SGH10ΔwcaJSGH10, rmpA deletion (deletion of rmpA abrogates the hypermucoviscous phenotype)ICEKp10, <i>iuc, clb, iro, rmpA</i> (19)SGH10ΔGIE492SGH10, GE492 deletion (deletion of microcin E492 and salmochelin)ICEKp10, <i>iuc, clb, iro, rmpA</i> This studySGH10ΔT6SS1 ΔT6SS3SGH10, T6SS1 and T6SS3 deletion (deletion of the two clusters of type 6 secretion system)ICEKp10, <i>iuc, clb, iro, rmpA</i> This studySGH10ΔmrkASGH10, mrkA deletion (deletion of the encoding colibactin and yersiniabactin)ICEKp10, <i>iuc, clb, iro, rmpA</i> This studySGH10ΔmrkASGH10, KpVP deletionICEKp10, <i>iuc, clb, iro, rmpA</i> This studySGH10ΔmrkAΔKpVPSGH10, KpVP deletionICEKp10, <i>iuc, clb, iro, rmpA</i> This study	SGH07	KL5, S160	ICEKp1, Iro, ImpA	(15)
TTSH21KL5, ST60ICEKp1, iro, rmpAThis studyTTSH25KL2, ST2039ICEKp10, iuc, clb, iro, rmpA(15)TTSH25ΔwcaJTTSH25, wcaJ deletion (capsule null; deletion of initial glycosyltransferase)ICEKp10, iuc, clb, iro, rmpA(17)SGH10KL1, ST23ICEKp10, iuc, clb, iro, rmpA(17)SGH10ΔwcaJSGH10, wcaJ deletion (capsule null; deletion of initial glycosyltransferase)ICEKp10, iuc, clb, iro, rmpA(18)SGH10ΔrmpASGH10, mpA deletion (deletion of genomic island responsible for the production of microcin E492 and salmochelin)ICEKp10, iuc, clb, iro, rmpAThis studySGH10ΔICEKp10SGH10, T6SS1 and T6SS3 deletion (deletion of the two clusters of type 6 secretion system)ICEKp10, iuc, clb, iro, rmpAThis studySGH10ΔmrkASGH10, mrkA deletion (deletion of the encoding colibactin and yersiniabactin)ICEKp10, iuc, clb, iro, rmpAThis studySGH10ΔmrkASGH10, mrkA deletion (deletion of the sGH10, KpVP and mrkA deletionICEKp10, iuc, clb, iro, rmpAThis study	SGH0/AwcaJ	SGH07, wcaJ deletion	iro, rmpA	This study
TTSH25KL2, ST2039ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> (15)TTSH25ΔwcaJTTSH25, wcaJ deletion (capsule null; deletion of initial glycosyltransferase)ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> (17)SGH10KL1, ST23ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> (17)SGH10ΔwcaJSGH10, wcaJ deletion (capsule null; deletion of initial glycosyltransferase)ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> (18)SGH10ΔrmpASGH10, rmpA deletion (deletion of rmpA abrogates the hypermucoviscous phenotype)ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> (19)SGH10ΔGIE492SGH10, GIE492 deletion (deletion of microcin E492 and salmochelin)ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> This studySGH10ΔICEKp10SGH10, ICEKp10 deletion (deletion of the two clusters of type 6 secretion system)ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> This studySGH10ΔmrkASGH10, ICEKp10 deletion (deletion of the gencoded for major subunit of type 3 fimbrae)ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> This studySGH10ΔmrkAΔKpVPSGH10, KpVP and mrkA deletionICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> This study	TTSH21	KL5, ST60	ICEKp1, <i>iro, rmpA</i>	This study
TTSH25ΔwcaJTTSH25, wcaJ deletion (capsule null; deletion of initial glycosyltransferase)iuc, clb, iro, rmpAThis studySGH10KL1, ST23ICEKp10, iuc, clb, iro, rmpA(17)SGH10ΔwcaJSGH10, wcaJ deletion (capsule null; deletion of initial glycosyltransferase)ICEKp10, iuc, clb, iro, rmpA(18)SGH10ΔrmpASGH10, rmpA deletion (deletion of rmpA abrogates the hypermucoviscous phenotype)ICEKp10, iuc, clb, iro(19)SGH10ΔGIE492SGH10, GIE492 deletion (deletion of genomic island responsible for the production of microcin E492 and salmochelin)ICEKp10, iuc, clb, iro, rmpAThis studySGH10ΔT6SS1 ΔT6SS3SGH10, T6SS1 and T6SS3 deletion (deletion of the two clusters of type 6 secretion system)ICEKp10, iuc, clb, iro, rmpAThis studySGH10ΔmrkASGH10, ICEKp10SGH10, ICEKp10 deletion (deletion of the chromosomal integrative and conjugative element encoding colibactin and yersiniabactin)ICEKp10, iuc, clb, iro, rmpAThis studySGH10ΔmrkASGH10, mrkA deletion (type 3 fimbrae)ICEKp10, iuc, clb, iro, rmpAThis studySGH10ΔmrkAΔKpVPSGH10, KpVP and mrkA deletionICEKp10, clbThis study	TTSH25	KL2, ST2039	ICEKp10, <i>iuc, clb, iro, rmpA</i>	(15)
initial glycosyltransferase)SGH10KL1, ST23ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> (17)SGH10ΔwcaJSGH10, wcaJ deletion (capsule null; deletion of initial glycosyltransferase)ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> (18)SGH10ΔrmpASGH10, rmpA deletion (deletion of rmpA abrogates the hypermucoviscous phenotype)ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> (19)SGH10ΔGIE492SGH10, GIE492 deletion (deletion of genomic island responsible for the production of microcin E492 and salmochelin)ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> This studySGH10ΔT6SS1 ΔT6SS3SGH10, T6SS1 and T6SS3 deletion (deletion of the two clusters of type 6 secretion system)ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> This studySGH10ΔmrkASGH10, ICEKp10 deletion (deletion of the encoded for major subunit of type 3 fimbrae)ICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> This studySGH10ΔmrkAΔKpVPSGH10, KpVP and mrkA deletionICEKp10, <i>iuc</i> , <i>clb</i> , <i>iro</i> , <i>rmpA</i> This study	TTSH25∆ <i>wcaJ</i>	TTSH25, <i>wcaJ</i> deletion (capsule null; deletion of	iuc, clb, iro, rmpA	This study
SGH10KL1, ST23ICEKp10, iuc, clb, iro, rmpA(17)SGH10ΔwcaJSGH10, wcaJ deletion (capsule null; deletion of initial glycosyltransferase)ICEKp10, iuc, clb, iro, rmpA(18)SGH10ΔrmpASGH10, rmpA deletion (deletion of rmpA abrogates the hypermucoviscous phenotype)ICEKp10, iuc, clb, iro(19)SGH10ΔGIE492SGH10, GIE492 deletion (deletion of microcin E492 and salmochelin)ICEKp10, iuc, clb, iro, rmpAThis studySGH10ΔT6SS1 ΔT6SS3SGH10, T6SS1 and T6SS3 deletion (deletion of the two clusters of type 6 secretion system)ICEKp10, iuc, iro, rmpAThis studySGH10ΔICEKp10SGH10, ICEKp10 deletion (deletion of the chromosomal integrative and conjugative element encoded for major subunit of type 3 fimbrae)ICEKp10, iuc, clb, iro, rmpAThis studySGH10ΔmrkASGH10, MrkA deletionSGH10, KpVP SGH10, KpVP and mrkA deletionICEKp10, clbThis study		initial glycosyltransferase)		
SGH10ΔwcaJ   SGH10, wcaJ deletion (capsule null; deletion of initial glycosyltransferase)   ICEKp10, iuc, clb, iro, rmpA   (18)     SGH10ΔrmpA   SGH10, rmpA deletion (deletion of rmpA abrogates the hypermucoviscous phenotype)   ICEKp10, iuc, clb, iro   (19)     SGH10ΔGIE492   SGH10, GIE492 deletion (deletion of genomic island responsible for the production of microcin E492 and salmochelin)   ICEKp10, iuc, clb, rmpA   This study     SGH10ΔT6SS1 ΔT6SS3   SGH10, T6SS1 and T6SS3 deletion (deletion of the two clusters of type 6 secretion system)   ICEKp10, iuc, clb, iro, rmpA   This study     SGH10ΔICEKp10   SGH10, ICEKp10 deletion (deletion of the chromosomal integrative and conjugative element encoding collbactin and yersiniabactin)   ICEKp10, iuc, clb, iro, rmpA   This study     SGH10ΔmrkA   SGH10, MrkA deletion (deletion of the genoded for major subunit of type 3 fimbree)   ICEKp10, iuc, clb, iro, rmpA   This study     SGH10ΔmrkA   SGH10, KpVP   SGH10, KpVP and mrkA deletion   ICEKp10, clb   This study	SGH10	KL1, ST23	ICEKp10, iuc, clb, iro, rmpA	(17)
SGH10ΔrmpASGH10, rmpA deletion (deletion of rmpA abrogates the hypermucoviscous phenotype)ICEKp10, iuc, clb, iro(19)SGH10ΔGIE492SGH10, GIE492 deletion (deletion of genomic island responsible for the production of microcin E492 and salmochelin)ICEKp10, iuc, clb, rmpAThis studySGH10ΔT6SS1 ΔT6SS3SGH10, T6SS1 and T6SS3 deletion (deletion of the two clusters of type 6 secretion system)ICEKp10, iuc, clb, iro, rmpAThis studySGH10ΔICEKp10SGH10, ICEKp10 deletion (deletion of the chromosomal integrative and conjugative element encoding colibactin and yersiniabactin)ICEKp10, iuc, clb, iro, rmpAThis studySGH10ΔmrkASGH10, mrkA deletion (deletion of the chromosomal integrative and conjugative element encoded for major subunit of type 3 fimbrae)ICEKp10, iuc, clb, iro, rmpAThis studySGH10ΔmrkASGH10, mrkA deletion (deletionICEKp10, iuc, clb, iro, rmpAThis studySGH10ΔmrkASGH10, KpVP SGH10, KpVP and mrkA deletionICEKp10, clbThis study	SGH10∆ <i>wcaJ</i>	SGH10, wcaJ deletion (capsule null; deletion of	ICEKp10, iuc, clb, iro, rmpA	(18)
SGH10ΔrmpASGH10, rmpA deletion (deletion of rmpA abrogates the hypermucoviscous phenotype)ICEKp10, iuc, clb, iro(19)SGH10ΔGIE492SGH10, GIE492 deletion (deletion of genomic island responsible for the production of microcin E492 and salmochelin)ICEKp10, iuc, clb, rmpAThis studySGH10ΔT6SS1 ΔT6SS3SGH10, T6SS1 and T6SS3 deletion (deletion of the two clusters of type 6 secretion system)ICEKp10, iuc, clb, iro, rmpAThis studySGH10ΔICEKp10SGH10, ICEKp10 deletion (deletion of the chromosomal integrative and conjugative element encoding colibactin and yersiniabactin)ICEKp10, iuc, clb, iro, rmpAThis studySGH10ΔmrkASGH10, mrkA deletion (deletion of the chromosomal integrative and conjugative element encoded for major subunit of type 3 fimbrae)ICEKp10, iuc, clb, iro, rmpAThis studySGH10ΔmrkASGH10, mrkA deletion (deletion of the chromosomal integrative and conjugative element encoded for major subunit of type 3 fimbrae)ICEKp10, iuc, clb, iro, rmpAThis studySGH10ΔmrkASGH10, KpVP SGH10, KpVP and mrkA deletionICEKp10, clbThis study		initial glycosyltransferase)		( )
SGH10ΔGIE492SGH10, GIE492 deletion (deletion of genomic island responsible for the production of microcin E492 and salmochelin)ICEKp10, <i>iuc, clb, rmpA</i> This studySGH10ΔT6SS1 ΔT6SS3SGH10, T6SS1 and T6SS3 deletion (deletion of the two clusters of type 6 secretion system)ICEKp10, <i>iuc, clb, iro, rmpA</i> This studySGH10ΔICEKp10SGH10, ICEKp10 deletion (deletion of the chromosomal integrative and conjugative element encoding colibactin and yersiniabactin)ICEKp10, <i>iuc, clb, iro, rmpA</i> This studySGH10ΔmrkASGH10, mrkA deletion (deletion of the gene encoded for major subunit of type 3 fimbrae)ICEKp10, <i>iuc, clb, iro, rmpA</i> This studySGH10ΔmrkAΔKpVPSGH10, KpVP and mrkA deletionICEKp10, <i>clb</i> This study	SGH10ArmpA	SGH10 <i>rmpA</i> deletion (deletion of <i>rmpA</i> abrogates	ICEKp10 iuc clb iro	(19)
SGH10ΔGIE492   SGH10, GIE492 deletion (deletion of genomic island responsible for the production of microcin E492 and salmochelin)   ICEKp10, <i>iuc, clb, rmpA</i> This study     SGH10ΔT6SS1 ΔT6SS3   SGH10, T6SS1 and T6SS3 deletion (deletion of the two clusters of type 6 secretion system)   ICEKp10, <i>iuc, clb, iro, rmpA</i> This study     SGH10ΔICEKp10   SGH10, ICEKp10 deletion (deletion of the chromosomal integrative and conjugative element encoding colibactin and yersiniabactin)   ICEKp10, <i>iuc, clb, iro, rmpA</i> This study     SGH10ΔmrkA   SGH10, mrkA deletion (deletion of the encoded for major subunit of type 3 fimbrae)   ICEKp10, <i>iuc, clb, iro, rmpA</i> This study     SGH10ΔmrkAΔKpVP   SGH10, KpVP and mrkA deletion   ICEKp10, <i>iuc, clb, iro, rmpA</i> This study		the hypermucoviscous phenotype)		()
SGH10ΔGIC452   SGH10, GIC452 deletion (deletion of genomic of gen	SGH10AGIE492	SGH10 GIE492 deletion (deletion of genomic	ICEKn10 iuc clb rmnA	This study
Isiand responsible for the production of microcitiE492 and salmochelin)SGH10ΔT6SS1 ΔT6SS3SGH10, T6SS1 and T6SS3 deletion (deletion of the two clusters of type 6 secretion system)SGH10ΔICEKp10SGH10, ICEKp10deletion (deletion of the chromosomal integrative and conjugative element encoding colibactin and yersiniabactin)SGH10ΔmrkASGH10, mrkA deletion (deletion of the gene encoded for major subunit of type 3 fimbrae)SGH10ΔmrkASGH10, KpVPSGH10, KpVPSGH1	0011102012432	island responsible for the production of microsin		This study
SGH10ΔT6SS1 ΔT6SS3   SGH10, T6SS1 and T6SS3 deletion (deletion of the two clusters of type 6 secretion system)   ICEKp10, iuc, clb, iro, rmpA   This study     SGH10ΔICEKp10   SGH10, ICEKp10 deletion (deletion of the chromosomal integrative and conjugative element encoding colibactin and yersiniabactin)   ICEKp10, iuc, clb, iro, rmpA   This study     SGH10ΔICEKp10   SGH10, ICEKp10 deletion (deletion of the chromosomal integrative and conjugative element encoding colibactin and yersiniabactin)   ICEKp10, iuc, clb, iro, rmpA   This study     SGH10ΔmrkA   SGH10, mrkA deletion (deletion of the gene incoded for major subunit of type 3 fimbrae)   ICEKp10, iuc, clb, iro, rmpA   This study     SGH10ΔKpVP   SGH10, KpVP deletion   ICEKp10, clb   This study     SGH10ΔmrkAΔKpVP   SGH10, KpVP and mrkA deletion   ICEKp10, clb   This study		E402 and salmachalin)		
SGH10Δ16SS1 Δ16SS3   SGH10, 16SS1 and 16SS3 deletion (deletion of the inception (deletion (deletion (deletion of the inception (deletion (deletion (deletion of the inception (deletion (		E492 and Samochemin)		This study
SGH10ΔICEKp10SGH10, ICEKp10 deletion (deletion of the chromosomal integrative and conjugative element encoding colibactin and yersiniabactin)iuc, iro, rmpAThis studySGH10ΔmrkASGH10, mrkA deletion (deletion of the gene encoded for major subunit of type 3 fimbrae)ICEKp10, iuc, clb, iro, rmpAThis studySGH10ΔmrkASGH10, kpVP SGH10, kpVP deletionSGH10, kpVP deletionICEKp10, clbThis studySGH10ΔmrkAΔKpVPSGH10, KpVP and mrkA deletionICEKp10, clbThis study	SGH10210551 210553	SGH10, 16SS1 and 16SS3 deletion (deletion of the	ICERPIO, IUC, CID, Iro, IMPA	This study
SGH10/LICEKp10   SGH10, ICEKp10 deletion (deletion of the chromosomal integrative and conjugative element encoding colibactin and yersiniabactin)   IUC, Iro, rmpA   This study     SGH10/LICEKp10   SGH10, ICEKp10 deletion (deletion of the encoding colibactin and yersiniabactin)   ICEKp10, iuc, clb, iro, rmpA   This study     SGH10/LiceKp10   SGH10, mrkA deletion (deletion of the gene encoded for major subunit of type 3 fimbrae)   ICEKp10, iuc, clb, iro, rmpA   This study     SGH10/LiceKpVP   SGH10, KpVP deletion   ICEKp10, clb   This study     SGH10/LiceKpVP   SGH10, KpVP and mrkA deletion   ICEKp10, clb   This study		two clusters of type 6 secretion system)	ture the A	<b>T</b> 1.1. ( )
Chromosomal Integrative and conjugative element encoding colibactin and yersiniabactin)SGH10ΔmrkASGH10, mrkA deletion (deletion of the gene encoded for major subunit of type 3 fimbrae)ICEKp10, iuc, clb, iro, rmpAThis studySGH10ΔKpVPSGH10, KpVP deletionICEKp10, clbThis studySGH10ΔmrkAΔKpVPSGH10, KpVP and mrkA deletionICEKp10, clbThis study	SGH1UAICEKp10	SGH10, ICEKp10 deletion (deletion of the	iuc, iro, rmpA	This study
encoding colibactin and yersiniabactin)SGH10ΔmrkASGH10, mrkA deletion (deletion of the gene encoded for major subunit of type 3 fimbrae)ICEKp10, iuc, clb, iro, rmpAThis studySGH10ΔKpVPSGH10, KpVP deletionICEKp10, clbThis studySGH10ΔmrkAΔKpVPSGH10, KpVP and mrkA deletionICEKp10, clbThis study		chromosomal integrative and conjugative element		
SGH10ΔmrkASGH10, mrkA deletion (deletion of the gene encoded for major subunit of type 3 fimbrae)ICEKp10, iuc, clb, iro, rmpAThis studySGH10ΔKpVPSGH10, KpVP deletionICEKp10, clbThis studySGH10ΔmrkAΔKpVPSGH10, KpVP and mrkA deletionICEKp10, clbThis study		encoding colibactin and yersiniabactin)		
encoded for major subunit of type 3 fimbrae)SGH10ΔKpVPSGH10, KpVP deletionICEKp10, clbThis studySGH10ΔmrkAΔKpVPSGH10, KpVP and mrkA deletionICEKp10, clbThis study	SGH10∆ <i>mrkA</i>	SGH10, mrkA deletion (deletion of the gene	ICEKp10, iuc, clb, iro, rmpA	This study
SGH10ΔKpVP     SGH10, KpVP deletion     ICEKp10, clb     This study       SGH10ΔmrkAΔKpVP     SGH10, KpVP and mrkA deletion     ICEKp10, clb     This study		encoded for major subunit of type 3 fimbrae)		
SGH10Δ <i>mrk</i> AΔKpVP SGH10, KpVP and <i>mrkA</i> deletion ICEKp10, <i>clb</i> This study	SGH10∆KpVP	SGH10, KpVP deletion	ICEKp10, <i>clb</i>	This study
	SGH10Δ <i>mrkA</i> ΔKpVP	SGH10, KpVP and mrkA deletion	ICEKp10, clb	This study

Appendix 2 Table 1. Description of *Enterobacterales* strains used for carbapenemase-encoding plasmids in clinical isolates and hypervirulent *Klebsiella pneumoniae*, Singapore\*

\*ATCC, American Type Culture Collection (https://www.atcc.org); ND, not done.

Appendix 2 Table 2. Description of carbapenemase-encoding plasmids used in a study of clinical Enterobacterales isolates and
hypervirulent Klebsiella pneumoniae. Singapore

Plasmid	Features	Source
pACYC184	Low copy number plasmid with a p15A replicon, CmR, TcR	(6)
pFLPe2	Source of zeocin resistance gene and FIp recombinase gene driven by PrhaB promoter, ZnR	(3)
pK18mobsacB	Source of sucrose counter-selection gene sacB, KmR	(5)
pR6KmobsacB	Conditional replicative plasmid carrying <b>sacB</b> and oriT, KmR	(18)
pKD46	Source of Red recombinases driven by Pbad promoter, ApR	(4)
pACYC-flp-Red-sacB	A p15A plasmid carrying <i>Flp</i> gene, Lambda Red recombinase genes and <i>sacB</i> gene, TcR	This study
pKD3	Source of chloramphenicol resistance gene flanked by FRT sites (FRT-Cm), CmR	(4)
pKD4	Source of kanamycin resistance gene flanked by FRT sites (FRT-Km), KmR	(4)
pKPC2	Conjugative plasmid from ENT494 carrying a <i>bla</i> <sub>KPC-2</sub> gene, CbR	(19)
pKPC2 <sup><i>KmR</i></sup>	pKPC2 containing a kanamycin resistance gene from pKD4, CbR, KmR	This study
pKPC2 <sup>KmR</sup> ΔhigB	pKPC2, higB was replaced with a kanamycin resistance gene, KmR	This study
pKPC2∆ <i>parB</i>	pKPC2, <i>parB</i> was replaced with a chloramphenicol resistance gene, CmR	This study
pNDM1	Conjugative plasmid from ENT448 carrying a <i>bla</i> NDM-1 gene, CbR	This study
pNDM1 <sup><i>KmR</i></sup>	pNDM1 containing a kanamycin resistance gene from pKD4, CbR, KmR	This study
pRK2-AraE	IncP plasmid encoding trfA replication protein	(20)

Appendix 2 Table 3. Primers used in mutant generation for carbapenemase-encoding plasmids in clinical *Enterobacterales* isolates and hypervirulent *Klebsiella pneumoniae*, Singapore\*

Name	Sequence	
K2_wcaJ Up F	TATGACATGATTACGAATTCAGCTCTGGCTGGTCCACTTA	
K2_wcaJ Up R	AGTTTTTCTCACATTTAAGCTGCGAACG	
K2 wcaJ Down F	AAATGTGAGAAAAACTCTTAGTGTTGCCATGA	
K2_wcaJ Down R	CTTGCATGCCTGCAGACGCGAGAATGGAATTGTTC	
K2_wcaJ Check F	GGTTGAAAACGGAGACGGTA	
K2_wcaJ Check R	CGTTGTTGCGAGTCATTCAG	
K5_wcaJ Up F	TATGACATGATTACGAATTCTATCTGGAAGACTGGCACGA	
K5_wcaJ Up_R	CGCGGAATGACATGCATAACCTCAAATCAATC	
K5_wcaJ Down_F	TGCATGTCATTCCGCGTTGTTCATTTCT	
K5_wcaJ Down_R	CTTGCATGCCTGCAGAGCTCTGGCTGGTCCACTTA	
K5_wcaJ Check_F	TTTTAGTTTTGTAACCTATGGCAGTT	
K5_wcaJ_Check_R	CCGATCTGTTGCTTGGACAT	
*E forward: R reverse		

\*F, forward; R, reverse.



**Appendix 2 Figure 1.** Distribution of *bla*<sub>KPC</sub> and *bla*<sub>NDM</sub> plasmids in the Carbapenemase-Producing *Enterobacterales* in Singapore collection, 2010–2015. A) Percentage of isolates belonging to *bla*<sub>KPC</sub> dominant cluster or *bla*<sub>NDM</sub> dominant cluster in each year. B) Distribution of *bla*<sub>KPC</sub> dominant cluster (pKPC2), *bla*<sub>KPC</sub> nondominant cluster (other non-pKPC2 *bla*<sub>KPC</sub> plasmids), *bla*<sub>NDM</sub> dominant cluster (pNDM1), and *bla*<sub>NDM</sub> nondominant cluster (other non-pNDM1 *bla*<sub>NDM</sub> plasmids) in numerous ST sequence types of *Klebsiella pneumoniae* strains (I–IV), *Escherichia coli* (V–VIII), and *Enterobacter cloacae* (IX–XII). Each slice in pie chart indicates 1 unique ST. Undefined STs indicate strains that could not be assigned to an ST. ST, sequence type.



**Appendix 2 Figure 2.** Downstream *trfA* gene sequence detected in carbapenemase-encoding plasmids in clinical *Enterobacterales* isolates and hypervirulent *Klebsiella pneumoniae*, Singapore. The *trfA* gene was detected in pKPC2 containing 9 17-bp repeats (blue highlighting) with similarity to the 5 17-bp repeats in the pRK2 oriV sequence. The 17-bp repeats of  $T/cGACAT^A/TT^G/AAGGTACG^C/T$  were detected downstream of *trfA* gene, which resembled the pRK2 oriV repeats of TGACAC<sup>/</sup>AT/GTGAGGGGC<sup>A</sup>/G<sup>G</sup>/C. Image was generated by using SnapGene (https://www.snapgene.com).



Appendix 2 Figure 3. Replicative pKPC2 fragment detected in carbapenemase-encoding plasmids in clinical *Enterobacterales* isolates and hypervirulent *Klebsiella pneumoniae*, Singapore. The *trfA* gene and the iteron (a total of 2,018 bp) was cloned by PCR by using forward primer TAAGCAGGATCCGATTACATTGCTGAGAATA TCCAGTATTTAAA TAC; and reverse primer TGCTTAGGATCCAATTTAGAACATTCGAAAAATGATCCAA TTTCGCAT and then inserted into pR6K after *Bam*HI digestion. The fusion plasmid was transformed into *Escherichia coli* Stellar–competent cells. The insertion sequence was verified by Sanger sequencing by using isolated plasmids from successfully transformed cells.

Amino acid identity (%)



**Appendix 2 Figure 4.** Heatmap comparing the conjugative genes of pKPC2 to the distinct conjugative genes from IncP in carbapenemase-encoding plasmids from clinical *Enterobacterales* isolates and hypervirulent *Klebsiella pneumoniae*, Singapore. The protein sequence of the pKPC2 conjugative genes was compared with the IncP plasmids' conjugative genes (*tra1* and *tra2* cores) by using BlastP (https://blast.ncbi.nlm.nih.gov). The heatmap shows amino acid percent identity from each comparison.



**Appendix 2 Figure 5.** Growth curve of carbapenemase-encoding plasmids in clinical *Enterobacterales* isolates and hypervirulent *Klebsiella pneumoniae*, Singapore. A) Representative growth curve of *K. pneumoniae* SGH10. B) Representative growth curve of *E. coli* MG1655. SGH10 and MG1655 were grown with or without plasmids (pKPC2, pKPC2<sup>*KmR*</sup>, pNDM1, pNDM1<sup>*KmR*</sup>) in M9 media supplemented with 0.4% glucose and 0.4% casamino acids for 24 hours at 37°C. Symbols indicate mean; error bars denote standard deviation.



**Appendix 2 Figure 6.** Sequence comparison between carbapenemase-encoding plasmids in clinical *Enterobacterales* isolates and hypervirulent *Klebsiella pneumoniae*, Singapore. Multiple pKPC2<sup>*KmR*</sup> were isolated from *K. pneumoniae* SGH10 grown over 300 generations (pKPC2<sup>*KmR*</sup>\_Gen300) and compared with the original pKPC2<sup>*KmR*</sup>. A) Sequence alignments of pKPC2<sup>*KmR*</sup> and pKPC2<sup>*KmR*</sup>\_Gen300. White gaps on the red bars indicate single nucleotide mismatch. B) Identified nucleotide mismatches over multiple comparisons. Red highlights indicate positions of the nucleotide mismatch identified in pKPC2<sup>*KmR*</sup>\_Gen300.



**Appendix 2 Figure 7.** Sequence comparison between pKPC2, pKPC2<sup>*KmR*</sup>, and pKPC2\_sg1 (GenBank accession no. MN542377) detected in carbapenemase-encoding plasmids in clinical *Enterobacterales* isolates and hypervirulent *Klebsiella pneumoniae*, Singapore. A) Comparison between the pKPC2 and pKPC2<sup>*KmR*</sup> plasmids from this study with the pKPC2\_sg1 plasmid from clinical isolate ENT494. Plasmid alignment showed 2 mismatches (white lines on the red and blue bars) on *bla*<sub>TEM-1</sub> gene of pKPC2 and pKPC2<sup>*KmR*</sup> compared with pKPC2\_sg1. One gap (indicated by the blue arrowhead) was identified on pKPC2<sup>*KmR*</sup> corresponding to the kanamycin gene insertion. B) Nucleotide sequence of the 3 plasmids showing the position of the mismatches (T>C at position 5151 and C>A at position 5398 of the pKPC2\_sg1 sequence). Both mismatches were on *bla*<sub>TEM-1</sub> gene.



**Appendix 2 Figure 8.** Conjugation frequency and stability of pKPC2<sup>*KmR*</sup> and pNDM1<sup>*KmR*</sup> among various clinical *Enterobacterales* isolates and hypervirulent *Klebsiella pneumoniae*, Singapore. A–C) Conjugation frequency of pKPC2<sup>*KmR*</sup> and pNDM1<sup>*KmR*</sup> from *E. coli* MG1655 donor strain (A) and from *K. pneumoniae* SGH10 donor strain (B) to a panel of *Enterobacterales* recipient strains; and C) from the panel of *Enterobacterales* donor strains to *K. pneumoniae* SGH10 recipient strain. Each symbol represents 1 experimental replicate with a total of 12 replicates. Data points that are not seen on the graphs indicate no detectable transconjugant. D,E) Stability of pKPC2<sup>*KmR*</sup> (D) and pNDM1<sup>*KmR*</sup> (E) in various *Enterobacterales* strains grown in lysogeny broth up till generation 90. Symbols indicate mean; error bars indicate SD from 3 independent experiments.



**Appendix 2 Figure 9.** Conjugation frequency of pKPC2<sup>*KmR*</sup> and pNDM1<sup>*KmR*</sup> to hypervirulent *Klebsiella pneumoniae* clinical isolates in a study of dominant carbapenemase-encoding plasmids in clinical *Enterobacterales* isolates, Singapore. A,B) Conjugation frequency of pKPC2<sup>*KmR*</sup> (A) or pNDM1<sup>*KmR*</sup> (B) from *Escherichia coli* MG1655 donor to hypervirulent *K. pneumoniae* clinical isolates. C) Conjugation frequency of pKPC2<sup>*KmR*</sup> from *E. coli* MG1655 donor to *K. pneumoniae* SGH10 and its isogenic mutants (Appendix 2 Table 1) based on 1-way ANOVA statistical analysis. D) Conjugation frequency of pNDM1<sup>*KmR*</sup> from *E. coli* MG1655 donor to *K. pneumoniae* SGH10 and its capsule null mutant ( $\Delta wcaJ$ ) based on unpaired Student *t*-test. Error bars indicate SD from 3 independent experiments. \*\*p<0.01; \*\*\*\*p<0.0001.



**Appendix 2 Figure 10.** Plasmid stability in low conjugating hypervirulent *Klebsiella pneumoniae* strains in a study of carbapenemase-encoding plasmids in clinical *Enterobacterales* isolates and hypervirulent *Klebsiella pneumoniae*, Singapore. A,B) Stability of pKPC2<sup>*KmR*</sup> (A) or pNDM1<sup>*KmR*</sup> (B) in low conjugating hypervirulent *K. pneumoniae* A-KLASS isolates via continuous culture in lysogeny broth for 90 generations. Error bars indicate mean <u>+</u>SD from 3 independent experiments.



**Appendix 2 Figure 11.** Conjugation frequency of carbapenemase-encoding plasmids in clinical *Enterobacterales* isolates and hypervirulent *Klebsiella pneumoniae*, Singapore. A) Conjugation of pKPC2<sup>*KmR*</sup>, pKPC2<sup>*KmR*</sup>, and pNDM1<sup>*KmR*</sup> to capsule null mutant of low conjugating hypervirulent *K. pneumoniae* strains. B) Conjugation of pNDM1<sup>*KmR*</sup> from *Escherichia coli* MG1655 to NUH66, TTSH25, or SGH07 wildtype strains and capsule null mutant ( $\Delta wcaJ$ ). Error bars indicate mean <u>+</u>SD from 3 independent experiments. \*p<0.05; \*\*p<0.01; \*\*\*p<0.001.