Whole-Genome-Sequencing characterization of bloodstream infection-causing hypervirulent

*Klebsiella pneumoniae* of capsular serotype K2 and ST374

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Supplementary data

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**Figure S1.** Detection of the ICE or prophage excision by PCR.

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**Figure S9.** Sequence alignments between four ICEKp1 family ICEs.

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**Figure S11.** Diagram of the plasmid pRJF293 and the sequence alignments to four other completely sequenced *K. pneumoniae* virulence plasmids.
| Strain   | Genotype                     | Key feature                                                                 | Reference |
|----------|------------------------------|-----------------------------------------------------------------------------|-----------|
| RJA360   | ST23, Capsular serotype K1   | Isolated from sputum sample of patient in Neurology Department.             | Lab archive |
| RJF67-2  | ST23, Capsular serotype K1   | Isolated from blood sample of patient in EICU.                             | Lab archive |
| RJA2570  | ST23, Capsular serotype K1   | Isolated from abscess sample of patient underwent trauma surgery.          | Lab archive |
| RJF999   | ST23, Capsular serotype K1   | Isolated from blood sample of patient in ICU.                              | Lab archive |
| RJA166   | ST23, Capsular serotype K1   | Isolated from sputum sample of patient underwent cardiac surgery           | Lab archive |
| RJF271   | ST680, Capsular serotype K1  | Isolated from abscess sample of patient from Emergency.                    | Lab archive |
| RJA277   | Non-typable MLST, Capsular serotype K1 | Isolated from abscess sample of patient from Intervention Department. | Lab archive |
| RJA304   | ST86, Capsular serotype K2   | Isolated from sputum sample of patient in Dermatology Department.          | Lab archive |
| RJB442   | ST86, Capsular serotype K2   | Isolated from urine sample of patient in Nephrology Department.            | Lab archive |
| RJF293   | ST374, Capsular serotype K2  | Isolated from blood sample of patient in ICU.                              | Lab archive |
| RJF294   | ST374, Capsular serotype K2  | Isolated from blood sample of patient in ICU.                              | Lab archive |
| RJA898   | ST374, Capsular serotype K2  | Isolated from sputum sample of patient in ICU.                             | Lab archive |
| RJA2225  | ST375, Capsular serotype K2  | Isolated from abscess sample of patient underwent general surgery.         | Lab archive |
| RJA1385  | ST11, Non-typable capsular serotype | Isolated from drainage sample of patient in EICU.              | Lab archive |
| RJA1253  | ST412, Non-typable capsular serotype | Isolated from hydrothorax sample of patient underwent thoracic surgery. | Lab archive |
| RJA1657  | ST412, Non-typable capsular serotype | Isolated from sputum sample of patient underwent thoracic surgery.   | Lab archive |
| RJA565   | ST412, Non-typable capsular serotype | Isolated from sputum sample of patient in Hematology Department. | Lab archive |
| RJA1547  | ST412, Non-typable capsular serotype | Isolated from bile sample of patient underwent transplantation. | Lab archive |
| RJA1504  | ST412, Non-typable capsular serotype | Isolated from sputum sample of patient in Respiratory Department. | Lab archive |
| RJA1887  | Non-tyable MLST and capsular serotype | Isolated from sputum sample of patient in Respiratory Department. | Lab archive |
| RJA726   | ST374, Capsular serotype K2  | Isolated from drainage sample of patient in ICU                             | Lab archive |
| HS11286  | ST11, KL103\(^a\) | Carbapenemase producing, low virulence; Control for the virulence determination. | Lab archive |
| NTUH-K2044 | ST23, serotype K1       | Characterized as hypervirulence; Control for the virulence determination. | Wu, et al.\(^1\) |

\(^a\)The K-locus was typed by using the tool Kaptive, based on the whole genome sequence of *K. pneumoniae* HS11286.\(^2\)
Table S2. Oligonucleotides used in this study. F indicates the forward primer and R indicates the reverse primer

| Primer name | Sequence (5’-3’) |
|-------------|-----------------|
| MLST        |                 |
| rpoB-F      | GGCGAAATGGCGWAGAACC |
| rpoB-R      | GAGTCTTCGAAAGTTGTAACC |
| gapA-F       | TGAATATGACTCCACCTCAAGG |
| gapA-R       | CTTCAGAAGCGGCTTTTGTAGGCTT |
| mdh-F        | CCAACTCGCTTACAGGTTCAG |
| mdh-R        | CGTTTTTCCCCAGCAGCAG |
| pgI1-F       | GAGAAAAACCTGCTGTACTGCTGGC |
| pgI1-R       | CGCGCCAGCCTTATAGCGGTTAAT |
| pgI2-F       | CTGCTGCGCTGATCGGCAT |
| pgI2-R       | TTATAGCGGTTAATCAGCGGCTG |
| phoE-F       | ACCCTACGCAACACCCAAGCTTCCTCG |
| phoE-R       | TGATCAGAACTCGTAGGCTAG |
| infB1-F      | CTGCTGCTGGACTATATTCG |
| infB1-R      | CGCTTTACGACTCAAGAACCT |
| infB2-F      | ACTAAGGTCCTCCGCGCAAGC |
| infB2-R      | ATTTTGTTGCTCCGCCGCAAGC |
| tonB-F       | TTTTATACCTCGGAGATCCAGT |
| tonB-R       | ATTCGCCGACCTGGRGACAG |
| Capsular serotype |         |
| wzx_K1-F | GTAGGTATTGCAAGGCCCATGC |
| wzx_K1-R | GGGACGTTAATGAAACCT |
| wzy_K1-F | GTTGCTTTTACATCATCCG |
| wzy_K1-R | GCAATTGCGATCTGCTAG |
| wzx_K2-F | GGAGCGTATTGCAAGGCCCATGC |
| wzx_K2-R | GGGACGTTAATGAAACCT |
| wzy_K2-F | GTTGCTTTTACATCATCCG |
| wzy_K2-R | GCAATTGCGATCTGCTAG |
| Ten known virulence factor genes |         |
| magA-F | GTAGGTATTGCAAGGCCCATGC |
| magA-R | GGGACGTTAATGAAACCT |
| rmpA-F | GCAATTGCGATCTGCTAG |
| rmpA-R | GTTGCTTTTACATCATCCG |
| allS-F | TCTGATTTAACCACACAT |
| allS-R | CGTTAGGAACATCCAGAC |
| mrkD-F | TATTGGCTTTAATGCGAGCGT |
| mrkD-R | TAAGTGACAGTCAATAGGGAAGACC |
| kfucF-F | GAAGTGACGCTTTGTTCG |
| kfucF-R | TTTGCGGCGGATGCTAGGCTG |
| fimH-F | GCTTGCGCGATACACACACAGG |
| fimH-R | GCGAAGTAGCTGGCCTGGAAGC |
| uge-F | GATTCATCGGTCCTCCTGTA |
| uge-R | TCTCAGCCTTCTTCCTACT |
|     |     |         |         |
|-----|-----|---------|---------|
| wabG-F |     | CGGACTGCGAGATCCATATC |         |
| wabG-R |     | ACCATCGGCCATTGATAGA  |         |
| ureA-F  |     | GCTGACTTAAGAGAACGTTATG |         |
| ureA-R  |     | GATCATGGCGCTACCTCA    |         |

| ICEKpnRJF293 & PHAGE_KpnRJF293 |     |         |         |
| ICE293-P1            |     | GGTGACGTTCAAGAGACC |         |
| ICE293-P4            |     | GTGAATTCACTACTGAGC |         |
| PHG293-P1            |     | AAGCCGAGAAAACAGGCAC |         |
| PHG293-P4            |     | GATCCGGTTACGATACGGT |         |

*a The PCR primers were also used for DNA sequencing, except for the gene *infB*, for which the primer *infB2-F* was used instead of the forward PCR primer, and for *pgi*, for which the primers *pgi2-F* and *pgi2-R* are used.*
| Strain | MLST type * | Accession no. of chromosome genome | Strain | MLST type * | Accession no. of chromosome genome |
|--------|-------------|-----------------------------------|--------|-------------|-----------------------------------|
| 1084   | ST23        | NC_018522                         | Kp-Goe | ST395       | CP018364                          |
| 1158   | ST65        | NZ_CP006722                       | Kp-Goe | ST101       | CP018450                          |
| 1756   | ST2549      | CP019219                          | Kp-Goe | ST11        | CP018692                          |
| 234-12 | ST514       | NZ_CP011313                       | Kp-Goe | ST147       | CP018140                          |
| 32192  | ST258       | NZ_CP010361                       | Kp-Goe | ST11        | CP018438                          |
| 342    | ST146       | NC_011283                         | Kp-Goe | ST147       | CP018701                          |
| 34618  | ST258       | NZ_CP010392                       | Kp-Goe | ST147       | CP018707                          |
| 500-1420 | ST258      | NZ_CP011980                       | Kp-Goe | ST147       | CP018719                          |
| AATZP  | ST147       | CP014755                          | KpN01  | ST278       | NZ_CP012987                       |
| AR-0049| ST11        | CP018816                          | KpN06  | ST279       | NZ_CP012992                       |
| ATCC35657 | ST505     | CP015134                          | KpN223 | ST273       | CP015025                          |
| BAA-2146 | ST11       | NZ_CP006659                       | KpN555 | not defined | CP015130                          |
| blaNDM-1 | ST395    | NZ_CP009114                       | KPNH1  | ST258       | NZ_CP008827                       |
| BR     | ST15        | CP015990                          | KPNH10 | ST258       | NZ_CP007727                       |
| CAV1016| ST45        | CP017934                          | KPNH24 | ST258       | NZ_CP008797                       |
| CAV1042| ST244       | CP018671                          | KPNH17 | ST34        | NZ_CP007731                       |
| CAV1193| ST941       | NZ_CP013322                       | KPNH29 | ST1518      | NZ_CP009863                       |
| CAV1217| ST340       | CP018676                          | KPNH30 | ST258       | NZ_CP009872                       |
| CAV1344| ST941       | NZ_CP0011664                      | KPNH31 | ST392       | NZ_CP009876                       |
| CAV1392| ST11        | NZ_CP0011578                      | KPNH32 | ST258       | NZ_CP009775                       |
| CAV1417| ST340       | CP018352                          | KPNH33 | ST258       | NZ_CP009771                       |
| CAV1453| ST258       | CP018356                          | KPNH36 | ST258       | CP014647                          |
| CAV1596| ST258       | NZ_CP0011647                      | KPNH39 | ST37        | CP014762                          |
| CG43   | ST86        | NC_022566                         | KPR1   | ST493       | NZ_CP009208                       |
| CN1    | ST392       | CP015382                          | KPR0928| ST258       | NZ_CP008831                       |
| CR14   | ST258       | CP015932                          | MGH-78578 | ST38   | NC_009648                         |
| DHQP1002001 | ST34   | CP016811                          | MNCRE53| ST258       | CP018437                          |
| DMC1097| ST258       | NZ_CP0011976                      | MNCRE69| ST258       | CP018427                          |
| ED2    | ST23        | CP016813                          | MNCRE78| ST258       | CP018428                          |
| ED23   | ST23        | CP016814                          | MS671  | ST147       | NZ_LN824133                       |
| GCA-001705385 | ST512 | CP015822                          | NJST258-1| ST258      | NZ_CP006923                       |
| GCA-001709275 | ST14 | CP016923                          | NJST258-2| ST258      | NZ_CP006918                       |
| GCA-001709295 | ST14   | CP016926                          | NTUH-K2044 | ST23    | NC_012731                         |
| HK787  | ST86        | NZ_CP006738                       | NUHL24835 | ST14   | NZ_CP014004                       |
| HKUOPLC| not defined | NZ_CP012300                       | NY9    | ST340       | CP015385                          |
| HS11286| ST11        | NC_016845                         | PittNDM1| ST14    | NZ_CP006798                       |
| J1     | ST111       | NZ_CP013711                       | PMK1   | ST15        | NZ_CP008929                       |
| JM45   | ST11        | NC_022082                         | RJF293 | ST374       | NZ_CP014008                       |
| KCTC-2242| ST375     | NC_017540                         | RJF999 | ST23        | NZ_CP014010                       |
| KP-1   | ST29        | NZ_CP012883                       | SKGH01 | ST147       | CP015500                          |
| Sample ID       | ST   | Accession     | Multilocus   | ST   | Accession     |
|-----------------|------|---------------|--------------|------|---------------|
| Kp13            | ST442| NZ_CP003999   | SWU01        | ST11 | CP018454      |
| KP36            | ST15 | CP017385      | TGH10        | ST383| NZ_CP012744   |
| KP5             | ST147| CP012426      | TGH13        | ST147| CP012745      |
| KP5-1           | not defined | CP008700 | TGH8         | ST383| NZ_CP012743   |
| Kp52.145        | ST66 | NZ_FO834906   | TH1          | ST1536| CP016159     |
| Kp617           | ST14 | NZ_CP012753   | UCLAOX232KP | ST16 | CP012561      |
| Kp-Goe-121641   | ST101| CP018735      | UCLAOX232KP1 | ST16 | CP012568      |
| Kp-Goe-149473   | ST147| CP018686      | UCLAOX232KP-Pt0 | ST16 | CP012560      |
| Kp-Goe-149832   | ST147| CP018695      | UHKPC07      | ST258 | NZ_CP011985  |
| Kp-Goe-152021   | ST147| CP018713      | UHKPC33      | ST258 | NZ_CP011989  |
| Kp-Goe-1544414  | ST23 | CP018337      | W14          | ST1536| CP015753      |
| Kp-Goe-33208    | ST101| CP018447      | XH209        | ST17 | NZ_CP009461   |
| Kp-Goe-39795    | ST15 | CP018458      | YH43         | not defined | NZ_AP014950 |

* Multilocus Sequence Typing (MLST) was determined by BIGSdb.3
Table S4. General features of the *K. pneumoniae* RJF293 genome and others be compared

| Parameter | RJF293 | CG43 | Kp52:145 | KPPR1 | NTUH-K2044 | 1084 | ED23 | RJF999 | HS11286 | NJST258-1 |
|-----------|--------|------|----------|-------|------------|------|------|--------|---------|-----------|
| hvKP/cKP | hvKP   | hvKP | hvKP     | hvKP  | hvKP       | hvKP | hvKP | hvKP   | cKP,    | cKP,     |
| Isolation source | Human, blood | Human, Liver pus | -       | Human, Liver pus | -   | Human, blood | Human, blood | Human, urine |          |
| Capsular serotype | K2      | K2   | K2       | K1    | K1         | K1   | K1   | K1     | KL103   | -         |
| ST no.    | 374    | 86   | 66       | 493   | 23         | 23   | 23   | 23     | 11      | 258       |
| Chromosome size (bp) | 5,226,330 | 5,166,857 | 5,438,894 | 5,374,834 | 5,248,520 | 5,386,705 | 5,374,626 | 5,461,919 | 5,333,942 | 5,263,229 |
| No. of annotated CDSs | 4,995  | 4,979 | 5,230    | 5,136  | 5,021      | 5,109 | 5,172 | 5,211  | 5,316   | 5,192     |
| No. of virulence factors | 32     | 30    | 29       | 29     | 42         | 39   | 42   | 43     | 25      | 24        |
| No. of putative prophages | 1      | 2     | 4        | 2      | 1          | 1    | 3    | 4      | 7       | 9         |
| No. of putative ICEs | 1      | 0     | 1        | 1      | 2          | 1    | 1    | 2      | 2       | 0         |
| No. of putative T4SS | 1      | 0     | 1        | 1      | 2          | 2    | 2    | 2      | 2       | 1         |
| No. of putative T6SS | 3      | 2     | 3        | 2      | 2          | 2    | 2    | 2      | 2       | 2         |
| No. of putative CRISPR arrays | 2      | 0     | 2        | 2      | 2          | 2    | 1    | 2      | 0       | 0         |
| No. of plasmids | 1      | 1     | 2        | 0      | 1          | 0    | 1    | 1      | 6       | 5         |

* ST, sequence type; CDS, protein coding sequences; ICE, integrative and conjugative elements; T4SS, type IV secretion system; T6SS, type VI secretion system; CRISPR, clustered regularly interspaced short palindromic repeats; hvKP, hypervirulent *Klebsiella pneumoniae*; MDR, multiple drug resistance.

b The classifications of hvKP or cKP strains were taken from the corresponding publications. In general, they exhibited three typical features, including (i) causing severe infection, (ii) metastatic spread of infection, and (iii) hypermucoviscous phenotype.

c The 45 *K. pneumoniae* virulence genes (clusters) under analysis are shown in Figure 5. The description of virulence genes are also listed in Table S4.

d The putative T6SS gene clusters with was detected by VRprofile (http://bioinfo-mml.sjtu.edu.cn/VRprofile/).

e The putative CRISPR arrays was identified by PILERCR (http://www.drive5.com/pilercr/).

f The K-locus was typed by using the tool Kaptive, based on the whole genome sequence of *K. pneumoniae* HS11286.
Table S5. Antimicrobial susceptibility tests\(^a\) of the hypermucoviscous *K. pneumoniae* isolates\(^b\) with ultra-long viscous string (> 20 mm)

| Strain | AMP  | SAM  | TZP  | KZ   | CTT  | CAZ  | TX   | FEP  | ATM  | ETP  | IPM  | AMK  | CN   | TOB  | CIP  | LEV  | F   | SXT |
|--------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|----|-----|
| RJA360 | 16/R | 4/S  | ≤4/S | ≤4/S | ≤1/S | ≤1/S | ≤1/S | ≤0.5/S | ≤1/S | ≤2/S | ≤1/S | ≤1/S | ≤0.25/S | ≤0.25/S | ≤16/S | ≤20/S |
| RJF67-2 | ≥32/R | 4/S  | ≤4/S | ≤4/S | ≤1/S | ≤1/S | ≤1/S | ≤0.5/S | ≤1/S | ≤2/S | ≤1/S | ≤1/S | ≤0.25/S | ≤0.25/S | 64/I | ≤20/S |
| RJA2570 | 16/R | 4/S  | ≤4/S | ≤4/S | ≤1/S | ≤1/S | ≤1/S | ≤0.5/S | ≤1/S | ≤2/S | ≤1/S | ≤1/S | ≤0.25/S | ≤0.25/S | 32/S | ≤20/S |
| RJF999  | 16/R | 4/S  | ≤4/S | ≤4/S | ≤1/S | ≤1/S | ≤1/S | ≤0.5/S | ≤1/S | ≤2/S | ≤1/S | ≤1/S | ≤0.25/S | ≤0.25/S | 32/S | ≤20/S |
| RJA166  | ≥32/R | ≥32/R | ≥64/R | 16/S | ≥64/R | 8/R  | ≤1/S | ≤0.5/S | ≤1/S | ≤2/S | ≤1/S | ≤1/S | ≤0.25/S | ≤0.25/S | 64/I | ≤20/S |
| RJF271  | 16/R | 4/S  | ≤4/S | ≤4/S | ≤1/S | ≤1/S | ≤1/S | ≤0.5/S | ≤1/S | ≤2/S | ≤1/S | ≤1/S | ≤0.25/S | ≤0.25/S | 64/I | ≤20/S |
| RJA277  | ≥32/R | ≥32/R | ≥64/R | 4/S  | ≤1/S | 32/R | 2/S  | 2/S  | ≤0.5/S | ≤1/S | 4/S  | ≤1/S | ≤0.25/S | ≥4/R | ≥8/R | ≤16/S | ≤20/S |
| RJA304  | 16/R | 4/S  | ≤4/S | ≤4/S | ≤1/S | ≤1/S | ≤1/S | ≤0.5/S | ≤1/S | ≤2/S | ≤1/S | ≤1/S | ≤0.25/S | ≤0.25/S | ≤16/S | ≤20/S |
| RJB442  | 16/R | 4/S  | ≤4/S | ≤4/S | ≤1/S | ≤1/S | ≤1/S | ≤0.5/S | ≤1/S | ≤2/S | ≤1/S | ≤1/S | ≤0.25/S | ≤0.25/S | ≤16/S | ≤20/S |
| RJA283  | 16/R | 4/S  | ≤4/S | ≤4/S | ≤1/S | ≤1/S | ≤1/S | ≤0.5/S | ≤1/S | ≤2/S | ≤1/S | ≤1/S | ≤0.25/S | ≤0.25/S | 128/R | ≤20/S |
| RJF294  | 16/R | 4/S  | ≤4/S | ≤4/S | ≤1/S | ≤1/S | ≤1/S | ≤0.5/S | ≤1/S | ≤2/S | ≤1/S | ≤1/S | ≤0.25/S | ≤0.25/S | 128/R | ≤20/S |
| RJA898  | 16/R | 4/S  | ≤4/S | ≤4/S | ≤1/S | ≤1/S | ≤1/S | ≤0.5/S | ≤1/S | ≤2/S | ≤1/S | ≤1/S | ≤0.25/S | ≤0.25/S | 64/I | ≤20/S |
| RJA2225 | 16/R | 4/S  | ≤4/S | ≤4/S | ≤1/S | ≤1/S | ≤1/S | ≤0.5/S | ≤1/S | ≤2/S | ≤1/S | ≤1/S | ≤0.25/S | ≤0.25/S | ≤16/S | ≤20/S |
| RJA1385 | 16/R | 4/S  | ≤4/S | ≤4/S | ≤1/S | ≤1/S | ≤1/S | ≤0.5/S | ≤1/S | ≤2/S | ≤1/S | ≤1/S | ≤0.25/S | ≤0.25/S | 32/S | ≤20/S |
| RJA1253 | 16/R | 4/S  | ≤4/S | ≤4/S | ≤1/S | ≤1/S | ≤1/S | ≤0.5/S | ≤1/S | ≤2/S | ≤1/S | ≤1/S | ≤0.25/S | ≤0.25/S | 32/S | ≤20/S |
| RJA1657 | 16/R | 4/S  | ≤4/S | ≤4/S | ≤1/S | ≤1/S | ≤1/S | ≤0.5/S | ≤1/S | ≤2/S | ≤1/S | ≤1/S | ≤0.25/S | ≤0.25/S | 64/I | ≤20/S |
| RJA565  | 16/R | 4/S  | ≤4/S | ≤4/S | ≤1/S | ≤1/S | ≤1/S | ≤0.5/S | ≤1/S | ≤2/S | ≤1/S | ≤1/S | ≤0.25/S | ≤0.25/S | 32/S | ≤20/S |
| RJA1547 | 16/R | ≤2/S | ≤4/S | ≤4/S | ≤1/S | ≤1/S | ≤1/S | ≤0.5/S | 2/I | 4/S  | ≤1/S | ≤1/S | ≤0.25/S | ≤0.25/S | 64/I | ≤20/S |
| RJA1504 | 16/R | 4/S  | ≤4/S | ≤4/S | ≤1/S | ≤1/S | ≤1/S | ≤0.5/S | ≤1/S | ≤2/S | ≤1/S | ≤1/S | ≤0.25/S | ≤0.25/S | 64/I | ≤20/S |
| RJA1887 | 16/R | ≤2/S | ≤4/S | ≤4/S | ≤1/S | ≤1/S | ≤1/S | ≤0.5/S | ≤1/S | ≤2/S | ≤1/S | ≤1/S | ≤0.25/S | ≤0.25/S | 64/I | ≤20/S |
| RJA726  | 16/R | 4/S  | ≤4/S | ≤4/S | ≤1/S | ≤1/S | ≤1/S | ≤0.5/S | ≤1/S | ≤2/S | ≤1/S | ≤1/S | ≤0.25/S | ≤0.25/S | 128/R | ≤20/S |

\(^a\) The minimal inhibitory concentration was determined by the VITEK2 compact system. AMP, ampicillin; SAM, ampicillin/sulbactam; TZP, piperacillin/tazobactam; KZ, cefazolin; CTT, cefotetan; CAZ, ceftazidime; TX, ceftriaxone; FEP, cefepime; ATM, aztreonam; ETP, ertapenem; IPM, imipenem; AMK, amikacin; CN, gentamycin; TOB, tobramycin; CIP, ciprofloxacin; LEV, levofloxacin; F, nitrofurantoin; SXT, trimethoprim/sulfamethoxazole.

\(^b\) These isolates include the 20 hypermucoviscous *Klebsiella pneumoniae* isolated between September 2014 and March 2016, and RJA726. *K. pneumoniae* RJA726 was isolated from the same patient as RJF293, in April 2014.
Table S6. Putative virulence factor genes detected in the genome of hypervirulence *K. pneumoniae* of RJF293 (ST374 and K2 serotype) a

| Locus tag     | Length (aa) | Identities (%) | Ha-value | Hit description                                                                 |
|---------------|-------------|----------------|----------|---------------------------------------------------------------------------------|
| Chromosome    |             |                |          |                                                                                 |
| RJF2_RS01505  | 752         | 72.2           | 0.695    | fepA, ferrienterobactin outer membrane transporter                               |
| RJF2_RS04305  | 305         | 77.6           | 0.774    | lpxC, UDP-3-O-R-3-hydroxyacyl-N-acetylglucosamine deacetylase                   |
| RJF2_RS04615  | 140         | 68.1           | 0.657    | gspG, general secretion pathway protein G                                       |
| RJF2_RS04810  | 341         | 65.1           | 0.645    | lpxD, UDP-3-O-3-hydroxyacyl-N-acetylglucosamine N-acyltransferase               |
| RJF2_RS04820  | 262         | 67.6           | 0.676    | lpxA, UDP-N-acetylglucosamine acyltransferase                                   |
| RJF2_RS04905  | 271         | 69             | 0.69     | ilpA, immunogenic lipoprotein A                                                 |
| RJF2_RS05030  | 192         | 91             | 0.91     | gmhA, phosphoheptose isomerase                                                  |
| RJF2_RS05325  | 195         | 95.9           | 0.959    | yagZ/ecpA, *E. coli* common pilus structural subunit EcpA                         |
| RJF2_RS05330  | 222         | 91.4           | 0.914    | yagY/ecpB, *E. coli* common pilus chaperone EcpB                                 |
| RJF2_RS05335  | 841         | 93.3           | 0.933    | yagX/ecpC, *E. coli* common pilus usher EcpC                                    |
| RJF2_RS05340  | 547         | 94.1           | 0.941    | yagW/ecpD, polymerized tip adhesin of ECP fibers                                |
| RJF2_RS05345  | 236         | 86.9           | 0.869    | yagV/ecpE, *E. coli* common pilus chaperone EcpE                                |
| RJF2_RS06870  | 742         | 81.4           | 0.823    | fepA, ferrienterobactin outer membrane transporter                              |
| RJF2_RS06875  | 402         | 66.4           | 0.659    | fes, enterobactin/ferric enterobactin esterase                                  |
| RJF2_RS06885  | 1293        | 78.1           | 0.78     | entF, enterobactin synthase multienzyme complex component, ATP-dependent        |
| RJF2_RS06890  | 264         | 88.6           | 0.883    | fepC, ferrienterobactin ABC transporter ATPase                                  |
| RJF2_RS06895  | 330         | 83             | 0.83     | fepG, iron-enterobactin ABC transporter permease                               |
| RJF2_RS06900  | 335         | 86.2           | 0.857    | fepD, ferrienterobactin ABC transporter permease                                |
| RJF2_RS06905  | 413         | 84.5           | 0.847    | entS, enterobactin exporter, iron-regulated                                     |
| RJF2_RS06910  | 319         | 84.3           | 0.79     | fepB, ferrienterobactin ABC transporter periplasmic binding protein             |
| RJF2_RS06915  | 391         | 75.4           | 0.754    | entC, isochorismate synthase 1                                                 |
| RJF2_RS06920  | 535         | 81.1           | 0.802    | entE, 2,3-dihydroxybenzoate-AMP ligase component of enterobactin synthase multienzyme complex |
| RJF2_RS06925  | 283         | 87.3           | 0.876    | entB, isochorismatase                                                          |
| RJF2_RS06930  | 251         | 88.7           | 0.876    | entA, 2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase                         |
| Gene        | Length | Identity | E-Value | Description                                                                 |
|-------------|--------|----------|---------|----------------------------------------------------------------------------|
| RJF2_RS08655 | 582    | 66.4     | 0.66    | msbA, lipid transporter ATP-binding/permease                              |
| RJF2_RS08675 | 248    | 66.5     | 0.673   | kdsB, 3-deoxy-manno-octulosonate cytidyltransferase                       |
| RJF2_RS08865 | 356    | 85.4     | 0.854   | ompA, outer membrane protein A                                           |
| RJF2_RS09005 | 338    | 99.7     | 0.997   | kfuA, iron ABC transporter substrate-binding protein                      |
| RJF2_RS09010 | 524    | 99.6     | 0.996   | kfuB, iron ABC transporter permease                                      |
| RJF2_RS09015 | 342    | 100      | 1       | kfuC, Fe(3+) ions import ATP-binding protein                              |
| RJF2_RS09560 | 729    | 98.6     | 0.986   | iutA, Ferric aerobactin receptor precursor                                |
| RJF2_RS13305 | 226    | 70.9     | 0.699   | mgtC, Mg2+ transport protein                                              |
| RJF2_RS14205 | 193    | 66.5     | 0.658   | sodB, superoxide dismutase                                                |
| RJF2_RS15420 | 300    | 74.4     | 0.717   | galU, glucosephosphate uridylyltransferase                                |
| RJF2_RS15580 | 284    | 83       | 0.827   | kdsA, 2-dehydro-3-deoxyphosphooctonate aldolase                          |
| RJF2_RS16675 | 434    | 98.4     | 0.984   | ybtS, salicylate synthase irp9                                            |
| RJF2_RS16680 | 426    | 98.4     | 0.984   | ybtX, putative signal transducer                                          |
| RJF2_RS16685 | 600    | 99.8     | 0.998   | ybtQ, inner membrane ABC-transporter YbtQ                                 |
| RJF2_RS16690 | 570    | 99.6     | 0.996   | ybtP, lipoprotein inner membrane ABC-transporter                          |
| RJF2_RS16695 | 319    | 99.4     | 0.994   | ybtA, transcriptional regulator YbtA                                     |
| RJF2_RS16700 | 2035   | 99.5     | 0.995   | irp2, yersiniabactin biosynthetic protein                                 |
| RJF2_RS16705 | 3163   | 99.5     | 0.995   | irp1, yersiniabactin biosynthetic protein                                 |
| RJF2_RS16710 | 366    | 99.5     | 0.995   | ybtU, yersiniabactin biosynthetic protein YbtU                             |
| RJF2_RS16715 | 267    | 98.9     | 0.989   | ybtT, yersiniabactin biosynthetic protein YbtT                             |
| RJF2_RS16720 | 525    | 99.4     | 0.994   | ybtE, yersiniabactin siderophore biosynthetic protein                     |
| RJF2_RS17085 | 334    | 97.9     | 0.979   | uge, uridine diphosphate galacturonate 4-epimerase (LPS gene cluster)     |
| RJF2_RS17105 | 456    | 73.2     | 0.732   | manB, phosphomannomutase                                                  |
| RJF2_RS19275 | 191    | 64.4     | 0.644   | algU, alginate biosynthesis protein AlgZ/FimS                             |
| RJF2_RS19855 | 171    | 73.1     | 0.731   | luxS, S-ribosylhomocysteinase [AI-2 (VF0406)]                             |
| RJF2_RS20220 | 343    | 65.2     | 0.644   | chuS, heme oxygenase ChuS                                                 |
| RJF2_RS21105 | 234    | 100      | 1       | mrkH, c-di-GMP-Dependent Transcriptional Activator                        |
| GenBank ID   | Coverage | Identity | Similarity | Description                                      |
|-------------|----------|----------|------------|--------------------------------------------------|
| RJF2_RS21110 | 194      | 99.5     | 0.995      | mrkI, transcriptional regulator                  |
| RJF2_RS21115 | 238      | 93.2     | 0.929      | mrkJ, regulator of Type III fimbriae             |
| RJF2_RS21120 | 211      | 100      | 1          | mrkF, type 3 fimbriae anchor protein             |
| RJF2_RS21125 | 331      | 100      | 1          | mrkD, type 3 fimbriae adhesin                    |
| RJF2_RS21130 | 828      | 100      | 1          | mrkC, type 3 fimbriae usher protein              |
| RJF2_RS21135 | 233      | 100      | 1          | mrkB, type 3 fimbriae assembly chaperone protein |
| RJF2_RS21140 | 202      | 99.5     | 0.995      | mrkA, type 3 fimbriae major subunit              |
| RJF2_RS21165 | 201      | 83.5     | 0.831      | fimB, type 1 fimbriae regulatory protein fimB    |
| RJF2_RS21170 | 202      | 82.8     | 0.787      | fimE, type 1 fimbriae regulatory protein fimE    |
| RJF2_RS21180 | 182      | 81.3     | 0.813      | fimA, type-1 fimbrial protein, A chain precursor |
| RJF2_RS21335 | 207      | 100      | 1          | kvgA, DNA-binding transcriptional activator     |
| RJF2_RS21340 | 1214     | 99.6     | 0.996      | kvgS, hybrid sensor histidine kinase             |
| RJF2_RS22025 | 477      | 70.2     | 0.686      | rfaE, ADP-heptose synthase                       |
| RJF2_RS24595 | 310      | 78.2     | 0.777      | rfaD, ADP-L-glycero-D-mannoheptose-6-epimerase   |
| **Plasmid** |          |          |            |                                                  |
| RJF2_RS26130 | 210      | 100      | 1          | rmpA, regulator of mucoid phenotype              |
| RJF2_RS26145 | 726      | 100      | 0.997      | iroN, iron outer membrane receptor              |
| RJF2_RS26150 | 409      | 100      | 1          | iroD, salmochelin siderophore ferric enterochelin esterase |
| RJF2_RS26155 | 1214     | 99.9     | 0.999      | iroC, salmochelin siderophore ATP-binding cassette |
| RJF2_RS26160 | 371      | 100      | 1          | iroB, salmochelin siderophore glycosyltransferase |
| RJF2_RS26550 | 574      | 100      | 1          | iucA, aerobactin siderophore biosynthesis protein |
| RJF2_RS26555 | 315      | 100      | 1          | iucB, aerobactin siderophore biosynthesis protein |
| RJF2_RS26560 | 577      | 100      | 1          | iucC, aerobactin siderophore biosynthesis protein |
| RJF2_RS26565 | 425      | 100      | 1          | iucD, aerobactin siderophore biosynthesis protein |
| RJF2_RS26570 | 733      | 89.6     | 0.895      | iutA, ferric aerobactin receptor precursor       |
| RJF2_RS26605 | 114      | 94.2     | 0.851      | rmpA2, regulator of mucoid phenotype             |
Detection the putative virulence factors with the protein sequence similarities by using BLASTp-based \( H_a \)-value

To examine the degree of sequence similarities at an amino acid level between each query protein and the VRprofile-collected virulence factors, the NCBI BLASTp-derived \( H_a \)-value was employed.\(^5\) For each query, the \( H_a \)-value was calculated as follows:

\[
H_a = i \times \frac{lm}{lq}
\]

where \( i \) was the level of BLASTp identities of the region with the highest Bit score expressed as a frequency of between 0 and 1, \( lm \) the length of the highest scoring matching sequence (including gaps) and \( lq \) the query length. If there were no matching sequences with a BLASTp \( E \) value < 0.01, the \( H_a \)-value assigned to that query sequence was defined as zero. Therefore \( H_a \)-value belonged to the set, \( H_a \in [0,1] \). Here, a strict \( H_a \)-value cut-off \( \geq 0.64 \) was used to determine the significant sequence similarities; for example, the identities is 80% and the ratio of matching length is 80%.
Table S7. List of the putative virulence factors detected in the 106 *K. pneumoniae* complete genome sequences

| #  | Gene     | Product                                                                 |
|----|----------|-------------------------------------------------------------------------|
| 1  | allA     | Ureidoglycolate hydrolase                                               |
| 2  | allB     | Allantoinase                                                            |
| 3  | allC     | Allantoate amidohydrolase                                               |
| 4  | allD     | Ureidoglycolate dehydrogenase                                           |
| 5  | allR     | Regulator of the allantoin metabolism gene cluster                      |
| 6  | allS     | Transcriptional activator of the allantoin metabolism gene cluster      |
| 7  | arcC     | Carbamate kinase                                                        |
| 8  | fdrA     | NAD(P)-binding acyl-CoA synthetase                                       |
| 9  | gcl      | Glyoxylate carboligase                                                   |
| 10 | glxK     | Glycerate kinase                                                        |
| 11 | glxR     | Tartronic semialdehyde reductase                                         |
| 12 | hyi      | Hydroxypyruvate isomerase                                                |
| 13 | KP1_1364 | Probable metabolite transport protein                                    |
| 14 | KP1_1371 | Putative glyoxylate utilization gene                                     |
| 15 | ybbW     | Allantoin permease                                                      |
| 16 | ybbY     | Purine permease                                                         |
| 17 | ylbE     | Putative cytoplasmic protein                                             |
| 18 | ylbF     | Anaerobic allantoin catabolic oxamate carbamoyltransferase               |
| 19 | rmpA     | Regulator of mucoid phenotype                                            |
| 20 | rmpA2    | Regulator of mucoid phenotype                                            |
| 21 | kvgA     | DNA-binding transcriptional activator                                    |
| 22 | kvgS     | Hybrid sensor histidine kinase                                           |
| 23 | iroB     | Salmochelin siderophore glycosyltransferase                             |
| 24 | iroC     | Salmochelin siderophore ATP-binding cassette                            |
| 25 | iroD     | Salmochelin siderophore ferric enterochelin esterase                    |
| 26 | iroN     | Iron outer membrane receptor                                            |
| 27 | irp1     | Yersiniabactin biosynthetic protein                                      |
| 28 | irp2     | Yersiniabactin biosynthetic protein                                      |
| 29 | iucA     | Aerobactin siderophore biosynthesis protein                             |
| 30 | iucB     | Aerobactin siderophore biosynthesis protein                             |
| 31 | iucC     | Aerobactin siderophore biosynthesis protein                             |
| 32 | iucD     | Aerobactin siderophore biosynthesis protein                             |
| 33 | iutA14   | Ferric aerobactin receptor precursor                                     |
| 34 | kfuA     | Iron ABC transporter substrate-binding protein                           |
| 35 | kfuB     | Iron ABC transporter permease                                            |
| 36 | kfuC     | Fe(3+) ions import ATP-binding protein                                  |
| 37 | mrkA     | Type 3 fimbriae major subunit                                            |
| 38 | mrkB     | Type 3 fimbriae assembly chaperone protein                              |
| 39 | mrkC     | Type 3 fimbriae usher protein                                            |
| Gene | Function |
|------|----------|
| mrkD | Type 3 fimbriae adhesin |
| mrkF | Type 3 fimbriae anchor protein |
| mrkH | c-Di-GMP-Dependent Transcriptional Activator |
| mrkI | Transcriptional regulator |
| mrkJ | Regulator of Type III fimbriae |
| algU | Alginate biosynthesis protein AlgZ/FimS |
| rfaE | ADP-heptose synthase |
| rfaD | ADP-L-glycero-D-mannoheptose-6-epimerase |
| ybtE | Yersiniabactin siderophore biosynthetic protein |
| ybtT | Yersiniabactin biosynthetic protein YbtT |
| ybtU | Yersiniabactin biosynthetic protein YbtU |
| ybtA | Transcriptional regulator YbtA |
| ybtP | lipoprotein inner membrane ABC-transporter |
| ybtQ | Inner membrane ABC-transporter YbtQ |
| ybtX | Putative signal transducer |
| ybtS | Salicylate synthase Irp9 |
| mgtB | Mg2+ transport protein |
| mgtC | Mg2+ transport protein |
| fimB | Type 1 fimbriae Regulatory protein fimB |
| fimE | Type 1 fimbriae Regulatory protein fimE |
| fimA | Type-1 fimbrial protein, A chain precursor |
| fimI | Fimbrin-like protein fimI precursor |
| fimC | Chaperone protein fimC precursor |
| fimD | Outer membrane usher protein fimD precursor |
| fimF | FimF protein precursor |
| fimG | FimG protein precursor |
| fimH | FimH protein precursor |
| chuS | Heme oxygenase ChuS |
| fepA | Ferrienterobactin outer membrane transporter |
| fepB | Ferrienterobactin ABC transporter periplasmic binding protein |
| fepC | Ferrienterobactin ABC transporter ATPase |
| fepD | Ferrienterobactin ABC transporter permease |
| fepG | Iron-enterobactin ABC transporter permease |
| entF | Enterobactin synthase multienzyme complex component, ATP-dependent |
| entC | Isochorismate synthase 1 |
| entE | 2,3-Dihydroxybenzoate-AMP ligase component of enterobactin synthase multienzyme complex |
| entB | isochorismatase |
| entA | 2,3-Dihydro-2,3-dihydroxybenzoate dehydrogenase |
| ompA | Outer membrane protein A |
| sodB | Superoxide dismutase |
| gspG | General secretion pathway protein G |
| manB | Phosphomannomutase |
| Gene | Function |
|------|----------|
| fcl  | GDP-fucose synthetase [O-antigen (VF0392)] |
| gmd  | GDP-mannose 4,6-dehydratase [O-antigen (VF0392)] |
| yagY | E. coli common pilus chaperone EcpB |
| gmhA | Phosphoheptose isomerase |
| yagZ | E. coli common pilus structural subunit EcpA |
| yagX | E. coli common pilus usher EcpC |
| yagW | Polymerized tip adhesin of ECP fibers |
| kdsB | 3-Deoxy-manno-octulosonate cytidylyltransferase |
| msbA | Lipid transporter ATP-binding/permease |
| galU | Glucosephosphate uridylyltransferase |
| lpdD | UDP-3-O-(3-hydroxymyristoyl) glucosamine N-acyltransferase; Lipopolysaccharides |
| lpxA | UDP-N-acetylg glucosamine acyltransferase; Lipopolysaccharides |
| lpxC | UDP-3-O-(R-3-hydroxymyristoyl)-N-acetylg glucosamine deacetylase; Lipopolysaccharides |
| kdsA | 2-Dehydro-3-deoxyphosphooctonate aldolase |
| luxS | S-ribosylhomocysteinase |
| fes  | Enterobactin/ferric enterobactin esterase |
| entS | Enterobactin exporter, iron-regulated |
| lipA | Immunogenic lipoprotein A |
| uge  | Uridine diphosphate galacturonate 4-epimerase (LPS synthesis gene cluster) |
| cibB | Putative peptide/polyketide synthase (colibactin synthesis gene cluster cibB-Q) |
| cibN | Putative non-ribosomal peptide synthetase (colibactin synthesis gene cluster cibB-Q) |
| cibQ | Iron acquisition yersiniabactin synthesis enzyme (colibactin synthesis gene cluster cibB-Q) |
| mceC | Microcin E492 modification (microcin E492 synthesis gene cluster) |
| mceD | Apo and ferric salmochelin esterase (microcin E492 synthesis gene cluster) |
| mceJ | Microcin E492 modification with salmochelin (microcin E492 synthesis gene cluster) |
| mceI | Microcin E492 modification with salmochelin (microcin E492 synthesis gene cluster) |
| mceH | Second component of microcin E492 exporter (microcin E492 synthesis gene cluster) |
| mceG | ABC protein of microcin E492 exporter (microcin E492 synthesis gene cluster) |
| mceF | Similar to McmM of CAAX amino terminal protease family (microcin E492 synthesis gene cluster) |
Figure S1. Detection of the ICE or prophage excision by PCR. The excision of ICE or prophage can result in an attB site on the chromosome. PCR assay with primers outside the attL and attR sites (P1 and P4 in the diagram) can be employed to amplify the attB site upon the ICE or prophage excision. No amplicon can be obtained with this pair of primers if the ICE or prophage remains integrated in the chromosome, as the size of ICE or prophage is outside the range of DNA polymerase capability.
Figure S2. Positive hypermucoviscosity phenotype of *K. pneumoniae* RJF293 by string test. The K1 and ST23 hvKP strain NTUH-K2044 was used for positive control while the ST11 cKP strain HS11286 for negative control.
Figure S3. Gel image of PFGE for hypermucoviscous *K. pneumoniae* clinical isolates. (A) PFGE analysis of the 20 hypermucoviscous *K. pneumoniae* isolates collected from September 2014 to March 2016. Four reference isolates, *Salmonella* serotype Braenderup H9812, *K. pneumoniae* NTUH-K2044, HS11286 and MGH78578, were used as controls. Genomic DNA was digested with XbaI and subjected to pulsed-field gel electrophoresis (PFGE). (B) The four isolates (RJF293, RJF294, RJA898 and RJA726) collected from the same patient showed same PFGE patterns.
**Figure S4.** Gel image of S1-PFGE for *K. pneumoniae* RJF293. Genomic DNA was digested using S1-nuclease and subjected to pulsed-field gel electrophoresis. The band marked in a red rectangle is corresponding to the plasmid pRJF293.
**Figure S5.** The *in silico* subtractive hybridization of the *K. pneumoniae* RJF293 genome against genomes of other seven completely sequenced hvKP isolates by using mGenomeSubtractor. The seven subject hvKP chromosomes include (supplementary Table S4): 1084, CG43, ED23, Kp52.145, KPPR1, NTUH-K2044 and RJF999. (A) Histogram of BLASTn-based $H$-values for all RJF293 genes against all seven subject chromosome sequences (color-coded). (B) Chromosome map of RJF293 with gene black/white-shade-coded based on the number of comparator *K. pneumoniae* genomes identified as harboring a nucleotide sequence-conserved homolog. Genes shown in absolute black ('7') are conserved across all seven *K. pneumoniae* comparator genomes, with genes shown in decreasing shades of black being conserved in lower numbers of comparator genomes, while at the other extreme those shown in white ('0') are unique to RJF293. Non-coding regions are shown as gaps. The genomic island-like variable regions (also see Table 2) are marked by red rectangles.
Figure S6. Alignment maps of the five representative *K. pneumoniae* CPS gene clusters. The *cps* gene cluster was located between *galF* and *ugd* as reported by the previous study. The conserved protein-coding genes are presented in grey. Variable regions are differentiated by colors. GT, glycosyl transferas
Figure S7. Alignments between the O1 LPS gene cluster in *K. pneumoniae* NTUH-K2044, RJF293 and Friedlander 204.
Figure S8 Excision of PHAGE_KpnRJF293 from the RJF293 chromosome. The attB site sequence was determined to be TTGAACAT. The chromatogram shows the sequencing result of attB site and its flanking region after prophage excision. The exact attB site determined by DNA sequencing was highlighted with yellow background. The sites attL and attR (named according to the integrase gene orientation) were shown in bold and underlined. The sequences flanking attB were 100% identical to the sequences outside attR and attL sites, indicated by the grey parallelogram.
Figure S9. Sequence alignments between four ICEKp1 family ICEs. ICEKpnRJF293, ICEKp1, and ICEKpn1084 are located respectively on the chromosomes of the hvKP strain RJF293, NTUH-K2044, and 1084. ICEKpnHS11286-1 is carried by the chromosome of the cKP strain HS11286. All of the four ICEs harbored the integrase gene, yersiniabactin biosynthesis gene cluster, and Type-F type IV secretion system gene cluster. The regions in the 3'-end of ICEs are variable among these strains.
Figure S10. Site-specifically excision of ICEKpnRJF293 from the 3'-end of the tRNA<sup>Asn</sup> gene on the RJF293 chromosome. The attB site sequence was determined to be CCAGTCAGAGGAGCCAAATT. The chromatogram shows the sequencing result of attB site and its flanking region after ICE excision. The exact attB site determined by DNA sequencing was highlighted with yellow background. The sites attL and attR (named according to the integrase gene orientation) were shown in bold and underlined. The sequences flanking attB were 100% identical to the sequences outside attL and attR sites, indicated by the grey parallelogram.
Figure S11. Diagram of the plasmid pRJF293 and the sequence alignments to four other completely sequenced *K. pneumoniae* virulence plasmids: pK2044 (224,152 bp in size), pLVPK (219,385 bp), pKCTC2242 (202,852 bp) and p52.145II (121,703 bp).
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