

Supplementary Materials

1 Supplementary Figures

(A) PCR screening for capsular serotypes in clinical isolates (n=129)

A.1 Colony Polymerase Chain Reaction

Detection and characterization of hypermucovirulent capsular serotypes K1, K2, K20, K54 and K57

DNA was extracted using the boiling method or thermal lysis in preparation for PCR. Firstly, 30 µl of nuclease-free water (Invitrogen, USA) was used to re-suspend three to five colonies picked up from the agar plate. Secondly, the samples were heated for 10 minutes at 100 °C in a heat block (Eppendorf ThermoStat plus, Germany). Thirdly, the samples were centrifuged at 16000 x g for 1 minute and the supernatant was used as a template for PCR whilst the cell pellet was discarded.

The PCR assays were performed using GoTaq® G2 Green Master Mix (Promega Ltd., USA). The cycling conditions for the Go-Taq polymerase are listed in Table 2.2. The detection of the five *K. pneumoniae* hyper-virulent capsular serotypes was performed by the amplification of the capsular genes K1, K2, K20, K54 and K57. The conditions of the PCR cycles were set according to the size of the genes and the melting temperature of the primers.

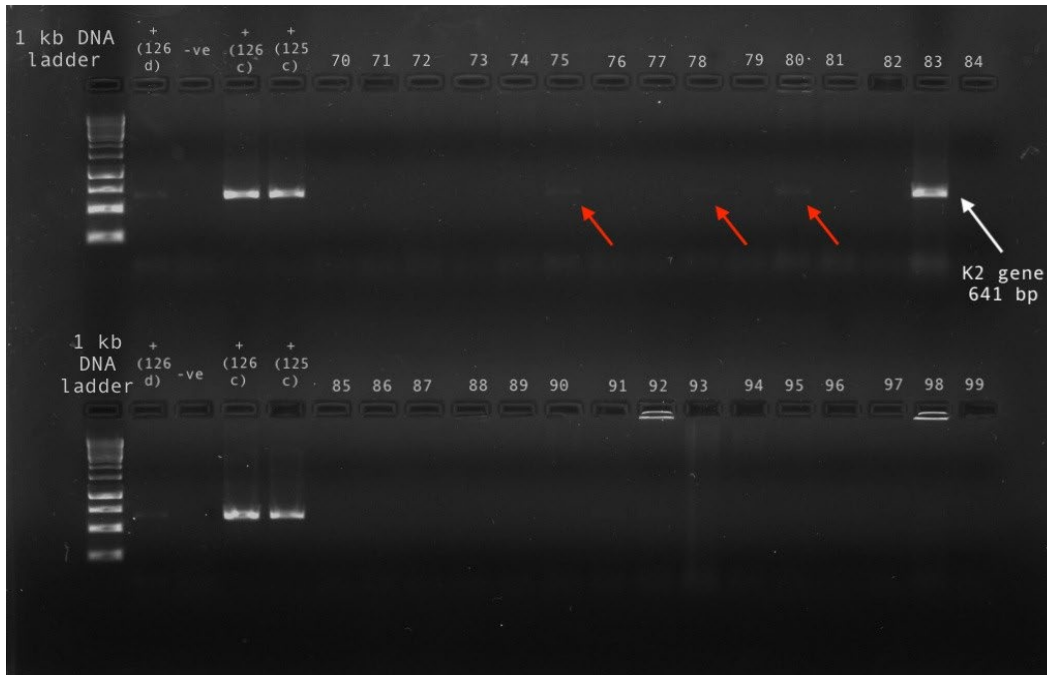
The reaction mixture consisted of 12.5 µl of the GoTaq® G2 Green Master Mix, 2X, 1 µl forward primer, 1 µl reverse primer, 1 µl Dimethyl sulfoxide (DMSO) (Sigma-Aldrich, USA), 1.5 µl of the DNA template and finally nuclease-free water was added so that the total volume is 25 µl. DMSO enhances the annealing of primers to the template and therefore positively impacts the process of amplification. A positive control (when available, depending on the capsular serotype) was used as well as a negative control that consists of the reaction mixture without the DNA template. A thermocycler (Eppendorf master cycler®, MA, Germany) was used to perform the amplification. The annealing temperatures for each gene as they differ based on the melting temperature of the primers as well as the specific primers used for the detection of the 5 different capsular serotypes are listed in Supplementary Table 14.

A.2 Gel Electrophoresis

The gel was prepared using 1.4 g of low electroendosmosis (LE) agarose, 70 ml 1x Tris/Borate/EDTA (TBE) electrophoresis buffer (ThermoFisher Scientific, USA) and 4 µl MIDORI^{Green} Direct (NIPPON-genetics, Europe). GeneRuler 1 kb DNA Ladder (ThermoFisher Scientific, USA) was used to estimate the size of the DNA fragments. Finally, the bands were visualised using iBrightTM CL1500 Imaging System (InvitrogenTM, USA).

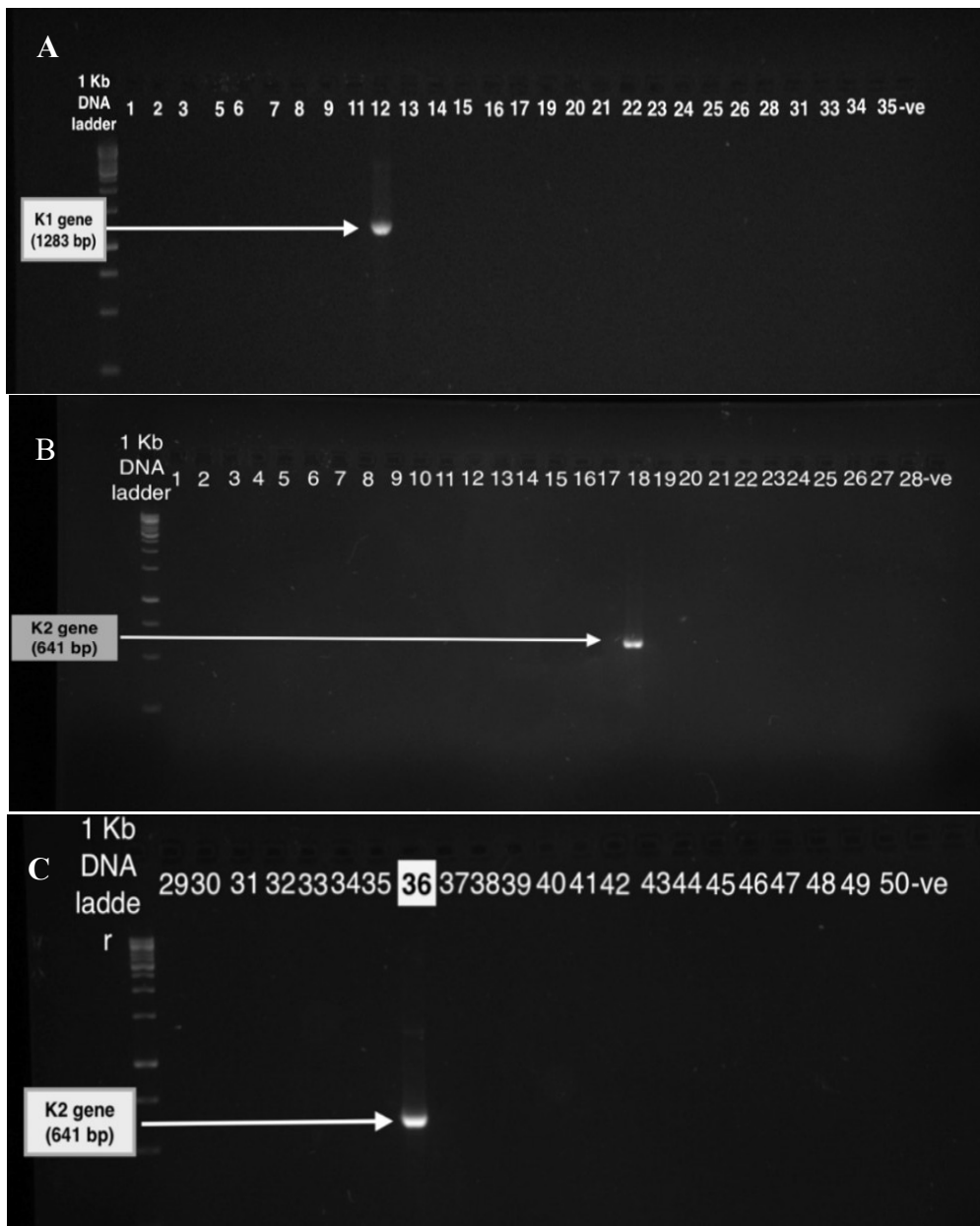
One hundred and twenty-six isolates (Kp 1 – Kp 123, Kp 127 – Kp 129) were screened for five hypervirulent capsular serotypes K1, K2, K20, K54 and K57 using conventional PCR amplification. Kp 124, Kp 125 and Kp126 were used as positive controls in this experiment as they were tested previously. Kp 124 is positive for K1, Kp 125 and Kp 126 are positive for K2 as shown in supplementary figure 1 (A, B and C). Further analysis of their hypervirulent nature including WGS, phylogeny and investigation of *k-loci* was continued in this study.

Moreover, Kp 83 was positive for capsular serotype 2 and none of the 129 isolates were positive for K20, K54 and K57. Faint bands in lanes 75, 78 and 80 were observed which might be non-specific bands. Therefore, to double check, PCR and gel electrophoresis were repeated for Kp 75, 78 and 80 with slight modifications including the addition of DMSO which resulted in the disappearing of the bands, therefore, these were excluded from WGS studies.



Supplementary Figure S1. Agarose gel electrophoresis of the K2 hypervirulent isolate Kp 83. Lane 1 is 1 kb DNA ladder. Lane 2 is the positive control Kp 126 (DNA sample isolated in 2019), Lane 3 is the negative control. Lanes 4 and 5 are positive controls Kp 126 and Kp 125 (DNA extracted by boiling method from fresh overnight culture). Lanes 75, 78 and 80 isolates Kp 75, Kp 78 and Kp 80, respectively show very faint bands with an expected size of 641 bp (pointed at with red arrows). Lane 83 is isolate Kp 83, positive for K2 with an expected band size of 641 bp.

The gel electrophoresis images below show PCR products of isolates Kp 124, Kp 125 and Kp 126 for the hypervirulent capsular serotypes. Positive isolates that show dense and significant DNA bands were sent for WGS. Those isolates are Kp 124, Kp 125, Kp 126 and Kp 83.



Supplementary Figure S2. Agarose gel electrophoresis of the K1 and K2 hypervirulent capsular serotypes. Lane 1 is the 1 kb DNA ladder in A, B and C. Lane 36 in A, lane 29 in B and lane 51 in C is the negative control. Lane 12 in A is isolate Kp 124, positive for K1 with an expected band size of 1238 bp. Lane 18 in B is isolate Kp 125, positive for K2 with an expected band size of 641 bp. Lane 36 in C is isolate Kp 126, positive for K2 with an expected band size 641 bp.

(B) Kaptive

Kaptive online tool was used to compare the *k-loci* of the isolates to known *K. pneumoniae* references in its database to analyse the similarities and variabilities between the isolates [1] (Wick et al., 2018). The figures generated by Kaptive represent the arrangement of different *k-loci* in each isolate and the genes that make up these loci as well as percentage of similarity between the isolate to known references. Many of the isolates have similar Kaptive figures therefore only the four most common ones are represented in supplementary figures 27, 28, 29 and 30.

The Kaptive image below shows the *k-loci* genetic arrangement of isolates Kp 102, Kp 106, Kp 110, Kp 112, Kp 113, Kp 114, Kp 115, Kp 116, Kp 118 and Kp 125. The *k-loci* of these isolates are similar to a known reference, KL 51 in the Kaptive database. However, there is a missing part of the genetic sequence represented as a line (____) in the figure below as well as a missing gene, *wcoZ*, represented in gray.

Kaptive figures for all clinical isolates subjected to WGS

Kp 100



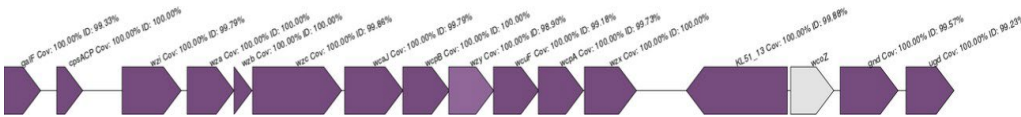
Supplementary Figure S3. *k-loci* gene arrangement in Kp 100. The *k-loci* of the isolate is perfectly similar to a known reference, KL52. The match confidence is very good.

Kp 101



Supplementary Figure S4. *k-loci* gene arrangement in Kp 101. The *k-loci* of the isolate is perfectly similar to a known reference, KL52 except for 1 gene, *rmIA*, shown in gray that is very different. The match confidence is just good.

Kp 102



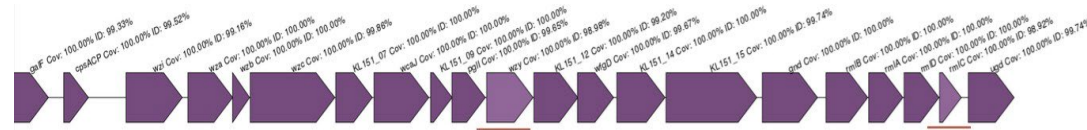
Supplementary Figure S5. *k-loci* gene arrangement in Kp 102. The *k-loci* of the isolate is similar to a known reference, KL51. However, there is a missing part of the genetic sequence represented as a line (—) in the figure above as well as a very different gene, *wcoZ*, represented in gray.

Kp 103



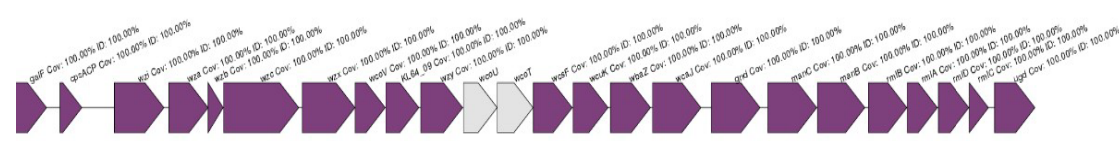
Supplementary Figure S6. *k-loci* gene arrangement in Kp 103. The *k-loci* of the isolate is perfectly similar to a known reference, KL64. The match confidence is very good.

Kp 104



Supplementary Figure S7. *k-loci* gene arrangement in Kp 104. The *k-loci* of the isolate is similar to a known reference, KL64 except for 2 genes *wcoU* & *wcoT*, shown in gray that are very different. The match confidence is just good.

Kp 105



Supplementary Figure S8. *k-loci* gene arrangement in Kp 105. The *k-loci* of the isolate is very similar to a known reference, KL151 except for 2 genes that are a bit different as they are represented in light purple in the figure and underlined in red, *rmIC* and *wzy*.

Kp 106



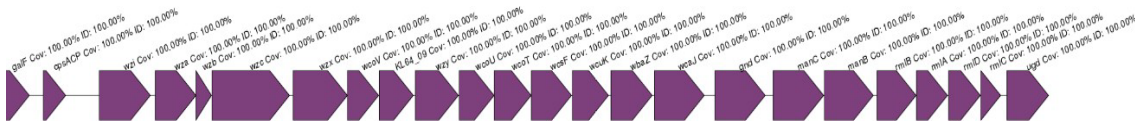
Supplementary Figure S9. *k-loci* gene arrangement in Kp 106. The *k-loci* of the isolate is similar to a known reference, KL51. However, there is a missing part of the genetic sequence represented as a line (—) in the figure above as well as a very different gene, *wcoZ*, represented in gray. The *k-loci* in this isolate is exactly identical to Kp 102.

Kp 107



Supplementary Figure S10. *k-loci* gene arrangement in Kp 107. The *k-loci* of the isolate is similar to a known reference, KL104. The match confidence is good but the arrangement of genes in this *k-loci* is a bit different than other isolates. The light purple means low coverage or identity in gene *wzx* as well as there is a missing gene represented in gray, *KL104_7*.

Kp 108



Supplementary Figure S11. *k-loci* gene arrangement in Kp 108. The *k-loci* of the isolate is similar to a known reference, KL64. The match confidence is very good. The *k-loci* in this isolate is exactly identical to Kp 103.

Kp 109



Supplementary Figure S12. *k-loci* gene arrangement in Kp 109. The *k-loci* of the isolate is similar to a known reference, KL23. The match confidence is very good.

Kp 110



Supplementary Figure S13. *k-loci* gene arrangement in Kp 110. The *k-loci* of the isolate is similar to a known reference, KL51. However, there is a missing part of the genetic sequence represented as a line (—) in the figure above as well as a very different gene, *wcoZ*, represented in gray. The *k-loci* in this isolate is exactly identical to Kp 102 & 106.

Kp 111



Supplementary Figure S14. *k-loci* gene arrangement in Kp 111. The *k-loci* of the isolate is similar to a known reference, KL64. The match confidence is very good. The *k-loci* in this isolate is exactly identical to Kp 108 & 103.

Kp 112



Supplementary Figure S15. *k-loci* gene arrangement in Kp 112. The *k-loci* of the isolate is similar to a known reference, KL51. However, there is a missing part of the genetic sequence represented as a line (—) in the figure above as well as a somewhat different gene, *wcoZ*, represented in light purple. The *k-loci* in this isolate is exactly identical to Kp 102, 106 & 110.

Kp 113



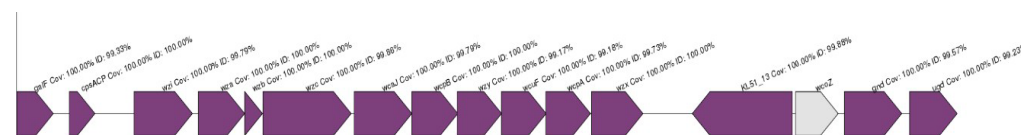
Supplementary Figure S16. *k-loci* gene arrangement in Kp 113. The *k-loci* of the isolate is similar to a known reference, KL51. However, there is a missing part of the genetic sequence represented as a line (—) in the figure above as well as a very different gene, *wcoZ*, represented in gray. The *k-loci* in this isolate is exactly identical to Kp 102, 106, 110 & 112.

Kp 114



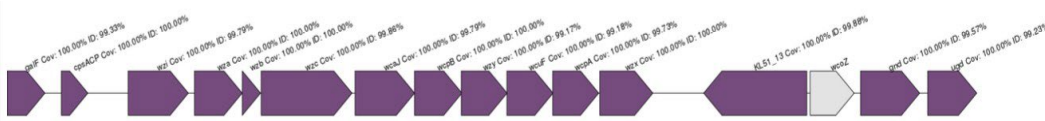
Supplementary Figure S17. *k-loci* gene arrangement in Kp 114. The *k-loci* of the isolate is similar to a known reference, KL51. However, there is a missing part of the genetic sequence represented as a line (—) in the figure above as well as a very different gene, *wcoZ*, represented in gray. The *k-loci* in this isolate is exactly identical to Kp 102, 106, 110, 112 & 113.

Kp 115



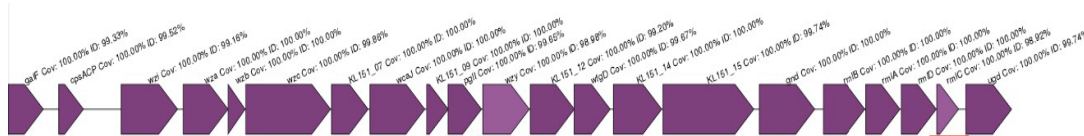
Supplementary Figure S18. *k-loci* gene arrangement in Kp 115. The *k-loci* of the isolate is similar to a known reference, KL51. However, there is a missing part of the genetic sequence represented as a line (—) in the figure above as well as a very different gene, *wcoZ*, represented in gray. The *k-loci* in this isolate is exactly identical to Kp 102, 106, 110, 112, 113 & 114.

Kp 116



Supplementary Figure S19. *k-loci* gene arrangement in Kp 116. The *k-loci* of the isolate is similar to a known reference, KL51. This genetic composition is unique with a very different gene, *wcoZ*, represented in gray. The *k-loci* in this isolate is exactly identical to Kp 102, 106, 110, 112, 113, 114 & 115.

Kp 117



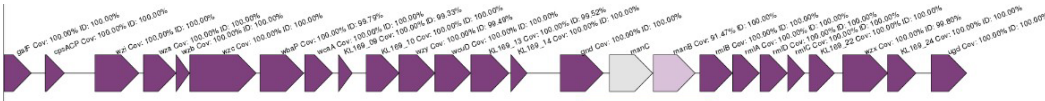
Supplementary Figure S20. *k-loci* gene arrangement in Kp 117. The *k-loci* of the isolate is very similar to a known reference, KL151 except for 2 genes that are a bit different as they are represented in light purple in the figure and underlined in red, *rmIC* and *wzy*. The *k-loci* in this isolate is exactly identical to Kp 105.

Kp 118



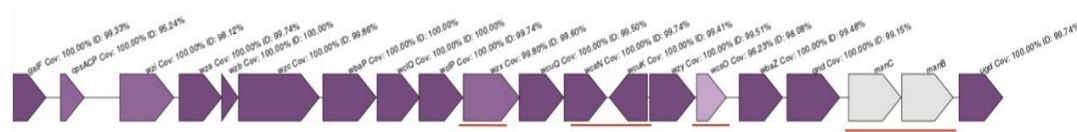
Supplementary Figure S21. *k-loci* gene arrangement in Kp 118. The *k-loci* of the isolate is similar to a known reference, KL51. However, there is a missing part of the genetic sequence represented as a line (—) in the figure above as well as a very different gene, *wcoZ*, represented in gray. The *k-loci* in this isolate is exactly identical to Kp 102, 106, 110, 112, 113, 114, & 115.

Kp 119



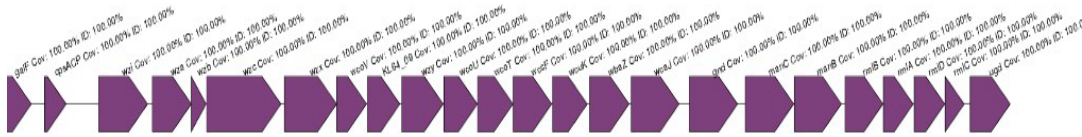
Supplementary Figure S22. *k-loci* gene arrangement in Kp 119. The *k-loci* of the isolate is similar to a known reference, KL169. The match confidence is not very good as the genetic sequence of this isolate was broken into 7 pieces hence, the assembly was poor. The underlined genes are inserted in a different position than the rest of the isolates, they are usually in the end. The gene that is absent or very different represented in gray is *manC* and the light purple which is a little different than the reference is *manB*.

Kp 120



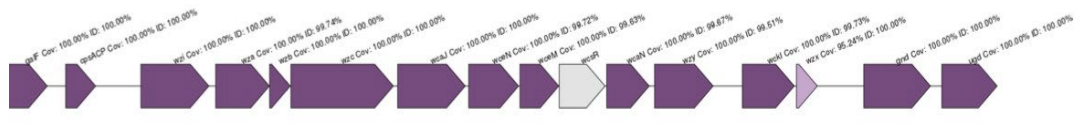
Supplementary Figure S23. *k-loci* gene arrangement in Kp 120. The *k-loci* of the isolate is similar to a known reference, KL64. The match confidence is not very good. The *k-loci* in this isolate is somewhat similar to Kp 103, 108 & 111. However, the gene *wzx* is not very similar as it is in light purple, gene *wcsD* is quite different as it is represented in lavender as well as the *manB* & *manC* genes are absent. Also, the gene *wcuZ* is inserted in reverse.

Kp 121



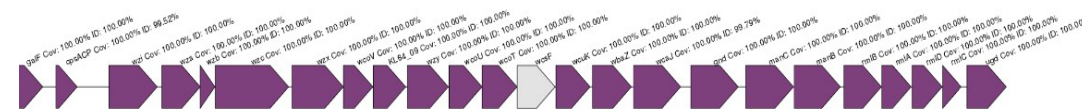
Supplementary Figure S24. *k-loci* gene arrangement in Kp 121. The *k-loci* of the isolate is similar to a known reference, KL64. The match confidence is very good. The *k-loci* in this isolate is exactly identical to Kp 103, 108 & 111.

Kp 122



Supplementary Figure S25. *k-loci* gene arrangement in Kp 122. The *k-loci* of the isolate is similar to a known reference, KL15. The match confidence is not very good. There are a few gaps in the loci as well as a missing gene, *wcsR* and a gene with low coverage in lavender, *wzx*. This isolate has a *k-loci* that is very different than other isolates.

Kp 123

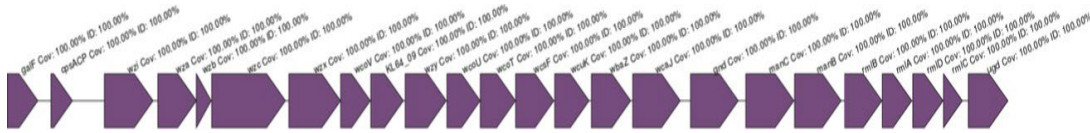


Supplementary Figure S26. *k-loci* gene arrangement in Kp 123. The *k-loci* of the isolate is similar to a known reference, KL64. The match confidence is good except for *wcsF* which is represented in gray, it might be absent or completely different than the reference. The *k-loci* in this isolate is exactly identical to Kp 103, 108 & 111.

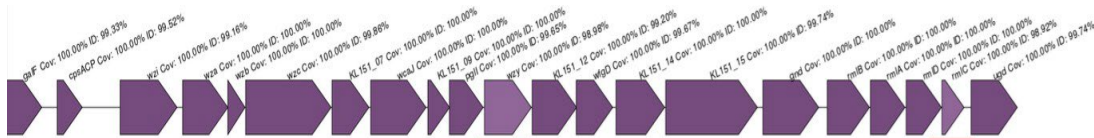


Supplementary Figure S27. *k-loci* gene arrangement of isolates Kp 102, Kp 106, Kp 110, Kp 112, Kp 113, Kp 114, Kp 115, Kp 116, Kp 118 and Kp 125.

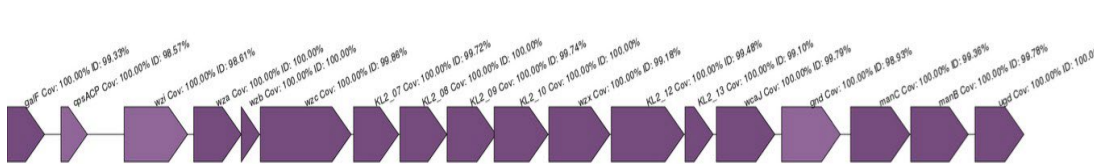
The Kaptive image below shows the *k-loci* genetic arrangement of isolates Kp 103, Kp 104, Kp 108, Kp 111, Kp 120, Kp 121 and Kp 123. The *k-loci* of these isolates are similar to a known reference, KL 64 in the Kaptive database. The match confidence is very good.



Supplementary Figure S28. *k-loci* gene arrangement of isolates Kp 103, Kp 104, Kp 108, Kp 111, Kp 120, Kp 121 and Kp 123.

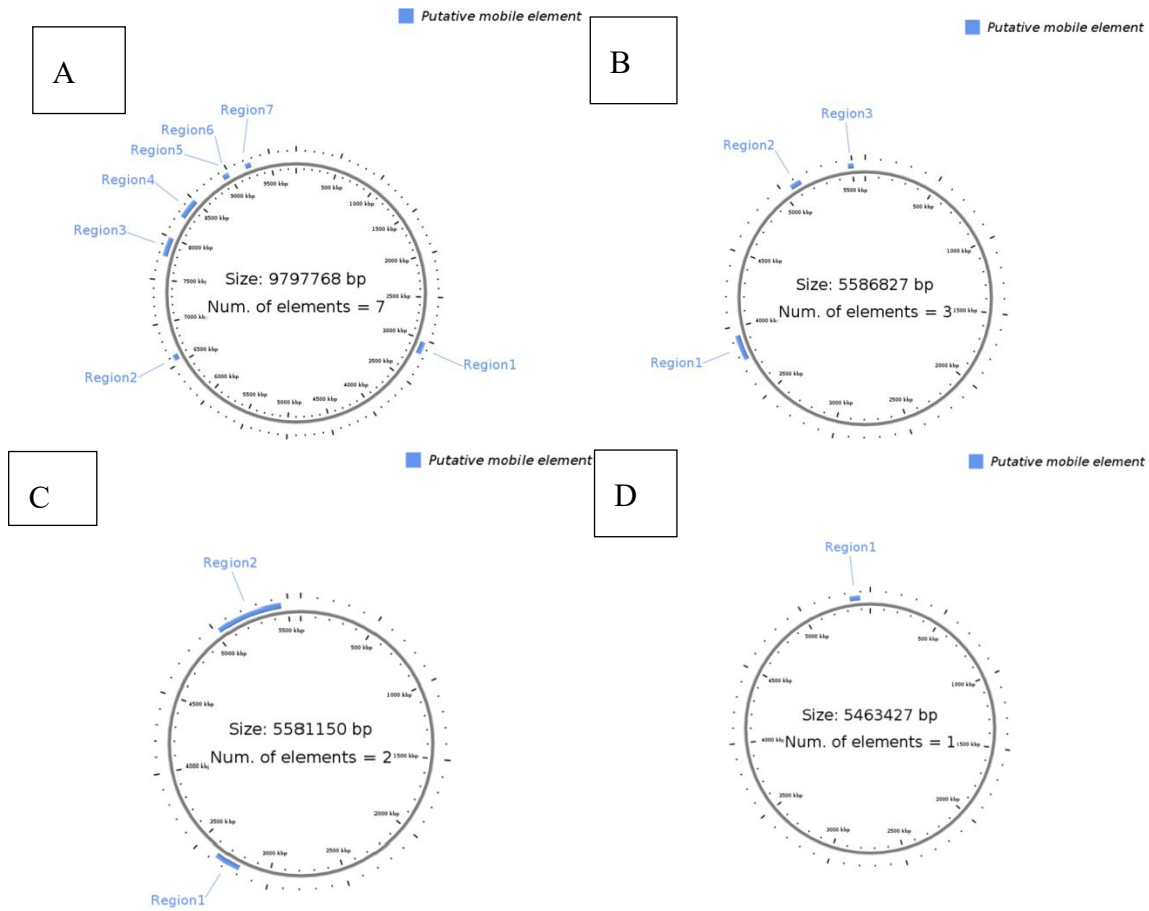


Supplementary Figure S29. *k-loci* gene arrangement of isolates Kp 105 and Kp 117. The *k-loci* of the isolates are very similar to a known reference, KL151 except for 2 genes that are a bit different as they are represented in light purple in the figure and underlined in red, *rmIC* and *wzy*.



Supplementary Figure S30. *k-loci* gene arrangement of isolates Kp 83, Kp 126 and Kp 129. The Kaptive image below shows the *k-loci* genetic arrangement of isolates Kp 83, Kp 126 and Kp 129. The *k-loci* of these isolate are similar to a known reference, KL2. The match confidence is very high.

(C) Plasmid analysis



Supplementary Figure S33. Putative integrative and conjugative elements (ICEs) in (A) KP83 K2/ST-14, (B) KP124 K1/ST-23, (C) KP125 K2/ST231, and (D) KP126 K2/ST-881. The ICEs region in the genome is indicated in blue boxes, the size in bps and the number of elements are indicated inside each circular genome.

Supplementary Materials

1 Supplementary Tables

(A) List of clinical isolates

Supplementary Table S1. List of *K. pneumoniae* isolates (n=129).

| Isolate | Year of isolation | Specimen/ Source | Place of collection |
|---------|-------------------|-------------------|---------------------|
| Kp 1 | 2018 | Blood culture | CPHL |
| Kp 2 | 2018 | Urine | CPHL |
| Kp 3 | 2018 | Urine | CPHL |
| Kp 4 | 2018 | Sputum | CPHL |
| Kp 5 | 2018 | Sputum | CPHL |
| Kp 6 | 2018 | Urine | CPHL |
| Kp 7 | 2018 | Sputum | CPHL |
| Kp 8 | 2018 | Wound | CPHL |
| Kp 9 | 2018 | Blood culture | CPHL |
| Kp 10 | 2018 | Tracheal aspirate | CPHL |
| Kp 11 | 2020 | Tracheal aspirate | CPHL |
| Kp 12 | 2020 | Blood culture | CPHL |
| Kp 13 | 2020 | Tracheal aspirate | CPHL |
| Kp 14 | 2020 | Urine | CPHL |
| Kp 15 | 2020 | Urine | CPHL |
| Kp 16 | 2020 | Urine | CPHL |
| Kp 17 | 2020 | Urine | CPHL |
| Kp 18 | 2020 | Urine | CPHL |
| Kp 19 | 2020 | Urine | CPHL |
| Kp 20 | 2020 | Tracheal aspirate | CPHL |
| Kp 21 | 2016 | Blood culture | CPHL |
| Kp 22 | 2016 | Blood culture | CPHL |
| Kp 23 | 2016 | Sputum | CPHL |
| Kp 24 | 2016 | Sputum | CPHL |

| | | | |
|-------|------|-------------------|------|
| Kp 25 | 2016 | Blood culture | CPHL |
| Kp 26 | 2016 | Wound | CPHL |
| Kp 27 | 2016 | Wound | CPHL |
| Kp 28 | 2016 | Urine | CPHL |
| Kp 29 | 2016 | Blood culture | CPHL |
| Kp 30 | 2016 | Urine | CPHL |
| Kp 31 | 2016 | Wound | CPHL |
| Kp 32 | 2020 | Blood culture | CPHL |
| Kp 33 | 2020 | Tracheal aspirate | CPHL |
| Kp 34 | 2020 | Tracheal aspirate | CPHL |
| Kp 35 | 2020 | Blood culture | CPHL |
| Kp 36 | 2020 | Blood culture | CPHL |
| Kp 37 | 2020 | Tracheal aspirate | CPHL |
| Kp 38 | 2020 | Tracheal aspirate | CPHL |
| Kp 39 | 2020 | Urine | CPHL |
| Kp 40 | 2020 | Tracheal aspirate | CPHL |
| Kp 41 | 2020 | Sputum | CPHL |
| Kp 42 | 2015 | Tracheal aspirate | CPHL |
| Kp 43 | 2015 | Tracheal aspirate | CPHL |
| Kp 44 | 2015 | Urine | CPHL |
| Kp 45 | 2015 | Urine | CPHL |
| Kp 46 | 2015 | Tracheal aspirate | CPHL |
| Kp 47 | 2015 | Tracheal aspirate | CPHL |
| Kp 48 | 2015 | Pus | CPHL |
| Kp 49 | 2015 | Urine | CPHL |
| Kp 50 | 2019 | Sputum | CPHL |
| Kp 51 | 2019 | Blood culture | CPHL |
| Kp 52 | 2019 | Urine | CPHL |
| Kp 53 | 2019 | Urine | CPHL |
| Kp 54 | 2019 | Tracheal aspirate | CPHL |
| Kp 55 | 2019 | Sputum | CPHL |

| | | | |
|-------|------|-------------------|------|
| Kp 56 | 2019 | Tracheal aspirate | CPHL |
| Kp 57 | 2019 | Tracheal aspirate | CPHL |
| Kp 58 | 2019 | Urine | CPHL |
| Kp 59 | 2019 | Tracheal aspirate | CPHL |
| Kp 60 | 2017 | Wound | CPHL |
| Kp 61 | 2017 | Tracheal aspirate | CPHL |
| Kp 62 | 2017 | Blood culture | CPHL |
| Kp 63 | 2017 | Blood culture | CPHL |
| Kp 64 | 2017 | Blood culture | CPHL |
| Kp 65 | 2017 | Blood culture | CPHL |
| Kp 66 | 2017 | Sputum | CPHL |
| Kp 67 | 2017 | Wound | CPHL |
| Kp 68 | 2017 | Sputum | CPHL |
| Kp 69 | 2017 | Sputum | CPHL |
| Kp 70 | 2019 | Urine | CPHL |
| Kp 71 | 2019 | Tracheal aspirate | CPHL |
| Kp 72 | 2019 | Sputum | CPHL |
| Kp 73 | 2019 | Blood culture | CPHL |
| Kp 74 | 2019 | Blood culture | CPHL |
| Kp 75 | 2019 | Blood culture | CPHL |
| Kp 76 | 2019 | Blood culture | CPHL |
| Kp 77 | 2019 | Blood culture | CPHL |
| Kp 78 | 2019 | Blood culture | CPHL |
| Kp 79 | 2019 | Blood culture | CPHL |
| Kp 80 | 2017 | Urine | CPHL |
| Kp 81 | 2017 | Tracheal aspirate | CPHL |
| Kp 82 | 2017 | Urine | CPHL |
| Kp 83 | 2017 | Urine | CPHL |
| Kp 84 | 2017 | Sputum | CPHL |
| Kp 85 | 2017 | Urine | CPHL |
| Kp 86 | 2017 | Urine | CPHL |
| Kp 87 | 2017 | Urine | CPHL |

Supplementary Materials

| | | | |
|--------|------|-------------------|------|
| Kp 88 | 2017 | Urine | CPHL |
| Kp 89 | 2017 | Urine | CPHL |
| Kp 90 | 2018 | Blood culture | CPHL |
| Kp 91 | 2018 | Urine | CPHL |
| Kp 92 | 2018 | Blood culture | CPHL |
| Kp 93 | 2018 | Urine | CPHL |
| Kp 94 | 2018 | Urine | CPHL |
| Kp 95 | 2018 | Tracheal aspirate | CPHL |
| Kp 96 | 2018 | Urine | CPHL |
| Kp 97 | 2018 | Blood culture | CPHL |
| Kp 98 | 2018 | Blood culture | CPHL |
| Kp 99 | 2018 | Blood culture | CPHL |
| Kp 100 | 2019 | Urine | SQUH |
| Kp 101 | 2019 | Urine | SQUH |
| Kp 102 | 2019 | Urine | SQUH |
| Kp 103 | 2019 | Wound | SQUH |
| Kp 104 | 2019 | Wound | SQUH |
| Kp 105 | 2019 | Blood culture | SQUH |
| Kp 106 | 2019 | Tracheal aspirate | SQUH |
| Kp 107 | 2019 | Peritoneal fluid | SQUH |
| Kp 108 | 2019 | Tracheal aspirate | SQUH |
| Kp 109 | 2019 | Urine | SQUH |
| Kp 110 | 2019 | Wound | SQUH |
| Kp 111 | 2019 | Urine | SQUH |
| Kp 112 | 2019 | Urine | SQUH |
| Kp 113 | 2019 | Urine | SQUH |
| Kp 114 | 2019 | Tracheal aspirate | SQUH |
| Kp 115 | 2019 | Tracheal aspirate | SQUH |
| Kp 116 | 2019 | Wound | SQUH |
| Kp 117 | 2019 | Tracheal aspirate | SQUH |
| Kp 118 | 2019 | Catheter urine | SQUH |

| | | | |
|--------|------|-------------------|------|
| Kp 119 | 2019 | Urine | SQUH |
| Kp 120 | 2019 | Urine | SQUH |
| Kp 121 | 2019 | Sputum | SQUH |
| Kp 122 | 2019 | Tracheal aspirate | SQUH |
| Kp 123 | 2019 | Urine | SQUH |
| Kp 124 | 2019 | Pus | SQUH |
| Kp 125 | 2019 | Urine | SQUH |
| Kp 126 | 2019 | Urine | SQUH |
| Kp 127 | 2021 | Blood culture | SQUH |
| Kp 128 | 2021 | Blood culture | SQUH |
| Kp 129 | 2021 | Blood culture | SQUH |

(B) *Galleria Mellonella* virulence assays**Supplementary Table S2. Virulence assay observation table (Replicates 1, 2 and 3).**

| | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
|-------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Control 1 | All alive | All alive | All alive | All dead | All dead |
| | All alive | 4 alive, 1 dead | 4 alive, 1 dead | 3 alive, 2 dead | 3 alive, 2 dead |
| | All alive | All alive | All alive | All alive | All alive |
| Control 2 | All alive | All alive | All alive | All dead | All dead |
| | All alive | All alive | All alive | All alive | All alive |
| Control 3 | 2 alive, 3 dead | 2 alive, 3 dead | 2 alive, 3 dead | 2 alive, 3 dead | 2 alive, 3 dead |
| | 1 dead, 4 alive | 1 dead, 4 alive | 1 dead, 4 alive | 2 dead, 3 alive | 2 dead, 3 alive |
| Isolate 12 | 6 dead, 4 alive | 7 dead, 3 alive | 7 dead, 3 alive | 7 dead, 3 alive | 7 dead, 3 alive |
| | All dead | All dead | All dead | All dead | All dead |
| Isolate 18 | 5 alive, 5 dead | 3 alive, 7 dead | 3 alive, 7 dead | 2 alive, 8 dead | 2 alive, 8 dead |
| | 4 alive, 6 dead | 2 alive, 8 dead | 1 alive, 9 dead | All dead | All dead |
| | 2 alive, 8 dead | 2 alive, 8 dead | 2 alive, 8 dead | 2 alive, 8 dead | 2 alive, 8 dead |
| | 4 alive, 6 dead | 3 alive, 7 dead | 3 alive, 7 dead | 2 alive, 8 dead | 1 alive, 9 dead |
| Isolate 36 | 8 alive, 2 dead | 8 alive, 2 dead | 7 alive, 3 dead | 5 alive, 5 dead | 3 alive, 7 dead |
| | 4 alive, 6 dead | 3 alive, 6 dead | 2 alive, 8 dead | 1 alive, 9 dead | 1 alive, 9 dead |
| | 5 alive, 5 dead | 5 alive, 5 dead | 4 alive, 6 dead | 4 alive, 6 dead | 3 alive, 7 dead |
| Isolate 84 | 6 alive, 4 dead | 4 alive, 6 dead | 4 alive, 6 dead | 2 alive, 8 dead | All dead |
| | 2 alive, 8 dead | 1 alive, 9 dead | 1 alive, 9 dead | 1 alive, 9 dead | 1 alive, 9 dead |
| | All dead | All dead | All dead | All dead | All dead |

(C) Human serum resistance assays**Supplementary Table S3. Serum resistance assay observation table.**

| Time | Isolate | Replicate A | Replicate B | Replicate C |
|---------------|----------------|-------------|-------------|-------------|
| 0 hour | Control | 115 | 63 | 55 |
| 1 hour | | 0 | 0 | 0 |
| 2 hour | | 0 | 0 | 0 |
| 3 hour | | 0 | 0 | 0 |
| 0 hour | Kp 124 | 10 | 16 | 43 |
| 1 hour | | 19 | 11 | 23 |
| 2 hour | | 18 | 8 | 20 |
| 3 hour | | 10 | 16 | 43 |
| 0 hour | Kp 125 | 181 | 136 | 68 |
| 1 hour | | 118 | 101 | 59 |
| 2 hour | | 200 | 222 | 129 |
| 3 hour | | 238 | 251 | 155 |
| 0 hour | Kp 83 | 63 | 27 | 20 |
| 1 hour | | 48 | 18 | 5 |
| 2 hour | | 12 | 6 | 3 |
| 3 hour | | 41 | 90 | 39 |

| | | | | |
|---------------|---------------|-----|-----|-----|
| 0 hour | Kp 126 | 160 | 133 | 236 |
| 1 hour | | 70 | 166 | 323 |
| 2 hour | | 118 | 82 | 174 |
| 3 hour | | 249 | 266 | 344 |

(D) Phylogenetic tree serial numbers of the study isolates

Supplementary Table S4. Clinical isolate serial numbers of phylogenetic tree in Figures 4 and 5.

| Isolate number | Serial number |
|-----------------------|----------------------|
| Kp 100 | 30121wA1_4 |
| Kp 101 | 30121wA2_21 |
| Kp 102 | 30121wB1_5 |
| Kp 103 | 30121wB2_22 |
| Kp 104 | 30121wA3_41 |
| Kp 105 | 30121wB3_42 |
| Kp 106 | 30121wC1_6 |
| Kp 107 | 30121wC_25 |
| Kp 108 | 30121wC_43 |
| Kp 109 | 30121wD2_27 |
| Kp 110 | 30121wD1_7 |
| Kp 111 | 30121wD3_44 |
| Kp 112 | 30121wE2_28 |
| Kp 113 | 30121wE1_10 |
| Kp 114 | 30121wE3_45 |
| Kp 115 | 30121wF1_11 |
| Kp 116 | 30121wF2_30 |
| Kp 117 | 30121wF3_46 |
| Kp 118 | 30121wG1_15 |
| Kp 119 | 30121wG2_37 |
| Kp 120 | 30121wG3_49 |
| Kp 121 | 30121wH1_16 |
| Kp 122 | 30121wH2_40 |
| Kp 123 | 30121wH3_50 |
| Kp 124 | 34548_12 |
| Kp 125 | 34549_18 |
| Kp 126 | 34550_36 |
| Kp 127 | 34551_KP1 |
| Kp 128 | 34552_KP2 |
| Kp 129 | 34554_KP4 |
| Kp 83 | 34553_KP3 |

(E) PCR assays**Supplementary Table S5. Colony PCR cycling conditions.**

| Stage | Temperature (°C) | Time | Cycles |
|-----------------|------------------|--------------|--------|
| Initialization | 95 | 2 min | 1 |
| Denaturation | 95 | 30 s | 30 |
| Annealing | variable | 30 s | 30 |
| Extension | 72 | 1 min per kb | 30 |
| Final extension | 72 | 10 min | 1 |
| Hold | 4 | | |

Supplementary Table S6. List of PCR primer names, nucleotide sequences and annealing temperatures used in this study [2] (Xu, et al., 2017) .

| Primer | Nucleotide sequence (5' -3') | Target | Expected size (bp) | Annealing temperature |
|----------|------------------------------|--------|--------------------|-----------------------|
| magAF | GGTGCTCTTTACATCATTGC | K1 | 1283 | 58 |
| magAR | GCAATGGCCATTTGCGTTAG | | | |
| wzy-K2F | GACCCGATATTCATACTTGACAGAG | K2 | 641 | 59 |
| wzy-K2R | CCTGAAGTAAAATCGTAAATAGATGGC | | | |
| wzy-K20F | CGGTGCTACAGTGCATCATT | K20 | 741 | 53 |
| wzy-K20R | GTTATACGATGCTCAGTCGC | | | |
| wzx-K54F | CATTAGCTCAGTGGTTGGCT | K54 | 881 | 52 |
| wzx-K54R | GCTTGAAAACACCATAGCAG | | | |
| wzy-K57F | CTCAGGGCTAGAAGTGTCAT | K577 | 1037 | 58 |
| wzx-K57R | CACTAACCCAGAAAGTCGAG | | | |

Supplementary Table S7. Whole Genome Sequencing assembly data.

| Sample id | # conti gs (>= 0 bp) | # conti gs (>= 1000 bp) | Total length (>= 0 bp) | Total length (>= 1000 bp) | # conti gs | Larges t contig | Total length | GC (%) | N50 | N75 | L50 | L75 | # N's per 100 kbp |
|-------------|----------------------------------|-------------------------------------|---------------------------------|------------------------------------|------------------|-----------------------|-----------------|-----------|------------|------------|-----|-----|----------------------------|
| 34551_KP1 | 193 | 81 | 60536 95 | 60202 04 | 98 | 39241 1 | 60319 50 | 56.63 | 19529 1 | 81844 | 11 | 24 | 0 |
| 34552_KP2 | 135 | 29 | 56712 09 | 56325 39 | 48 | 10790 67 | 56459 56 | 57.1 | 32730 4 | 23808 4 | 5 | 10 | 0 |
| 34553_KP3 | 448 | 258 | 97977 68 | 97174 39 | 315 | 42344 1 | 97588 78 | 49.74 | 16206 4 | 73567 | 19 | 42 | 0 |
| 34554_KP4 | 108 | 35 | 54976 72 | 54788 88 | 37 | 57212 4 | 54801 02 | 57.2 | 36215 3 | 24713 6 | 6 | 11 | 0 |
| 34548_12 | 192 | 130 | 55868 27 | 55642 99 | 140 | 26413 1 | 55720 70 | 57.27 | 92449 | 42287 | 22 | 45 | 0 |
| 34549_18 | 110 | 58 | 55811 50 | 55620 44 | 67 | 13581 08 | 55694 68 | 57.12 | 35572 1 | 19230 8 | 4 | 9 | 0 |
| 34550_36 | 95 | 30 | 54634 27 | 54463 76 | 32 | 15228 22 | 54477 56 | 57.22 | 36879 5 | 28979 0 | 4 | 8 | 0 |
| 30121wA1_4 | 94 | 50 | 57561 97 | 57427 85 | 53 | 78266 8 | 57447 72 | 56.74 | 41930 3 | 22541 0 | 5 | 10 | 0 |
| 30121wA2_21 | 156 | 57 | 55657 47 | 55382 70 | 64 | 60780 6 | 55436 31 | 57.21 | 20167 8 | 13308 8 | 8 | 17 | 0 |
| 30121wA3_41 | 156 | 82 | 58859 62 | 58602 74 | 92 | 48714 2 | 58671 69 | 56.57 | 14265 7 | 87033 | 12 | 25 | 0 |
| 30121wB1_5 | 147 | 67 | 55752 95 | 55490 57 | 78 | 56714 2 | 55567 97 | 57.08 | 33382 9 | 14445 6 | 7 | 13 | 0 |
| 30121wB2_22 | 137 | 76 | 55017 37 | 54834 79 | 79 | 59215 0 | 54857 92 | 57.19 | 19780 7 | 94980 | 10 | 21 | 0 |
| 30121wB3_42 | 114 | 45 | 55266 67 | 55022 86 | 57 | 78325 3 | 55111 83 | 57.13 | 31482 3 | 13988 6 | 6 | 13 | 0 |
| 30121wC1_6 | 153 | 62 | 55961 62 | 55625 22 | 70 | 70356 5 | 55687 62 | 57.1 | 30150 9 | 13317 7 | 7 | 13 | 0 |
| 30121wC2_25 | 91 | 35 | 54141 63 | 53976 67 | 38 | 62752 4 | 53998 18 | 57.28 | 34981 7 | 17029 7 | 6 | 11 | 0 |
| 30121wC3_43 | 150 | 71 | 55462 16 | 55210 58 | 78 | 54696 1 | 55258 83 | 57.08 | 23026 4 | 11568 1 | 9 | 17 | 0 |
| 30121wD1_7 | 139 | 61 | 55763 90 | 55481 83 | 69 | 69617 9 | 55540 43 | 57.11 | 26695 9 | 14445 6 | 7 | 14 | 0 |
| 30121wD2_27 | 152 | 51 | 55274 92 | 54964 81 | 65 | 73350 9 | 55055 76 | 57.26 | 29212 1 | 21489 6 | 6 | 12 | 0 |
| 30121wD3_44 | 159 | 73 | 56588 42 | 56322 15 | 83 | 78083 0 | 56386 61 | 57.04 | 21413 4 | 95942 | 10 | 20 | 0 |
| 30121wE1_10 | 174 | 79 | 55512 62 | 55163 12 | 96 | 59334 0 | 55295 27 | 57.05 | 30655 1 | 14642 6 | 7 | 14 | 0 |
| 30121wE2_28 | 119 | 64 | 55639 47 | 55411 31 | 78 | 93616 7 | 55517 12 | 57.13 | 33860 2 | 14445 6 | 6 | 12 | 0 |
| 30121wE3_45 | 133 | 64 | 55176 23 | 54929 11 | 72 | 60101 4 | 54992 12 | 57.13 | 35555 4 | 14445 6 | 7 | 12 | 0 |
| 30121wF1_11 | 169 | 74 | 55760 75 | 55369 19 | 94 | 63014 6 | 55508 74 | 57.1 | 26695 9 | 13144 0 | 8 | 15 | 0 |
| 30121wF2_30 | 114 | 57 | 55820 20 | 55637 00 | 63 | 94279 2 | 55681 29 | 57.1 | 38774 9 | 14445 6 | 4 | 10 | 0 |
| 30121wF3_46 | 229 | 44 | 55763 34 | 55040 95 | 58 | 13924 36 | 55139 22 | 57.13 | 36828 6 | 16364 6 | 5 | 10 | 0 |
| 30121wG1_15 | 420 | 85 | 57564 01 | 55874 25 | 215 | 70457 6 | 56743 11 | 56.95 | 29238 6 | 13315 7 | 7 | 14 | 0 |
| 30121wG2_37 | 122 | 40 | 54353 09 | 54112 76 | 48 | 86340 0 | 54165 97 | 57.31 | 37250 0 | 19066 0 | 5 | 10 | 0 |
| 30121wG3_49 | 167 | 69 | 56988 03 | 56675 45 | 78 | 70875 7 | 56730 17 | 56.94 | 21490 9 | 16945 7 | 10 | 17 | 0 |

| | | | | | | | | | | | | | |
|-----------------|-----|----|-------------|-------------|----|------------|-------------|-------|------------|------------|----|----|---|
| 30121wH1_ 16 | 202 | 71 | 55341 35 | 54822 61 | 90 | 59215 0 | 54953 41 | 57.16 | 18327 1 | 11568 1 | 10 | 19 | 0 |
| 30121wH2_ 40 | 206 | 49 | 55301 42 | 54736 89 | 65 | 65739 8 | 54846 26 | 57.15 | 29479 7 | 19589 1 | 6 | 12 | 0 |
| 30121wH3_ 50 | 175 | 77 | 58943 57 | 58621 19 | 91 | 79630 5 | 58712 36 | 56.56 | 21413 4 | 95942 | 10 | 21 | 0 |

Supplementary Table S8. Virulence associated genes in *K. pneumoniae* isolates.

| Gene products | Strains | | | |
|-------------------------------|---------|--------|-------|-------|
| | KP83 | KP124 | KP125 | KP126 |
| Capsule-related | | | | |
| capsular synthesis | | | wzz | |
| wzi | 0 | 0 | 0 | 0 |
| wza | 0 | 0 | 0 | 0 |
| wzb | 0 | 0 | 0 | 0 |
| wzc | 1 | 1 | 0 | 0 |
| wzy | 0 | 0 | 1 | 1 |
| wbaP | 0 | 0 | 0 | 0 |
| Capsule biosynthesis | | | | |
| RcsB | 1 | 1 | 1 | 1 |
| RmpA | 0 | 0 | 0 | 0 |
| RmpA2 | 0 | 0 | 0 | 0 |
| RmpC | 0 | 0 | 0 | 0 |
| RmpD | 0 | 0 | 0 | 0 |
| KvrA | 0 | 0 | 0 | 0 |
| RfaH | 1 | 1 | 1 | 1 |
| KvrB | 0 | 0 | 0 | |
| Inhibit capsule production | | | | |
| FNR | 1 | 1 | 1 | 1 |
| Fur | 1 | 0 | 0 | 1 |
| CRP | 1 | 1 | 1 | 1 |
| carbohydrates metabolism | | | | |
| capsule translocation | | | | |
| GDP-D-mannose synthesis | | | | |
| Other sugar-modifying enzymes | | | | |
| | | | | |
| lipopolysaccharide (LPS) | | | | |
| O-antigen | 1 | 1 | 1 | 1 |
| Adhesion | 1 | 1 | 1 | 1 |
| Type 3 fimbriae | | | | |
| fim | 1 | 1 | 1 | 1 |
| mrk | 0 | 1 | 1 | 1 |
| OMPs | | 9 OMPs | 10 | |

| | | | | |
|--------------------------|---|---|---|---|
| PalA | 1 | 1 | 1 | 1 |
| LppA | 1 | 1 | 1 | 1 |
| OmpK35 | 1 | 1 | 1 | 1 |
| OmpK36 | 1 | 1 | 1 | 1 |
| KpnO | | | | |
| T6SS | | | | |
| PLD1 | 1 | 1 | 1 | 1 |
| Tle1 | 1 | 1 | 1 | 0 |
| Iron acquisition | | | | |
| siderophores including: | | | | |
| Enterobactin entB | 1 | 1 | 1 | 1 |
| yersiniabactin irp2, ybt | 0 | 0 | 0 | 0 |
| salmochelin* iroB | 0 | 0 | 0 | 0 |
| Aerobactin iucA | 1 | 1 | 1 | 0 |

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