

Supplementary Material

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 \times 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.

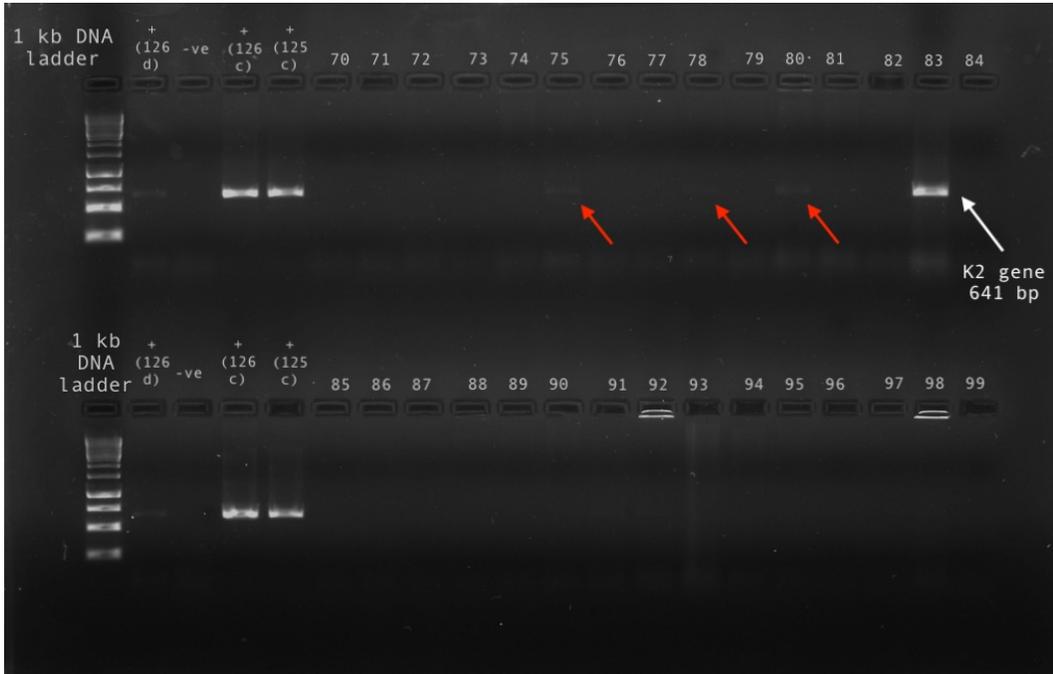
The reaction mixture consisted of 12.5 µl of the GoTaq[®] G2 Green Master Mix, 2X, 1 µl forward primer, 1 µl reverse primer, 1 µl Dimethyl sulfoxide (DMSO) (Sigma-Aldrich, USA), 1.5 µl of the DNA template and finally nuclease-free water was added so that the total volume is 25 µl. DMSO enhances the annealing 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).

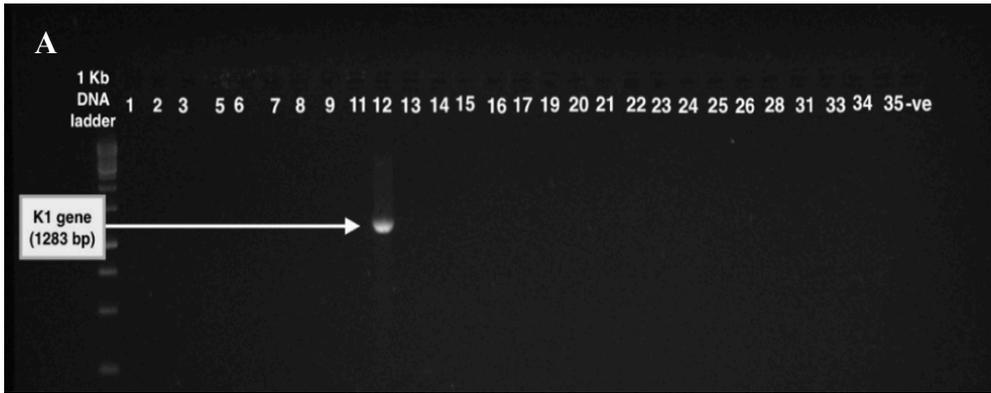
One hundred and twenty-six isolates (Kp 1 – Kp 123, Kp 127 – Kp 129) were screened for five hypervirulent capsular serotypes K1, K2, K20, K54 and K57 using conventional PCR amplification. Kp 124, Kp 125 and Kp126 were used as positive controls in this experiment as they were tested previously. Kp 124 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.

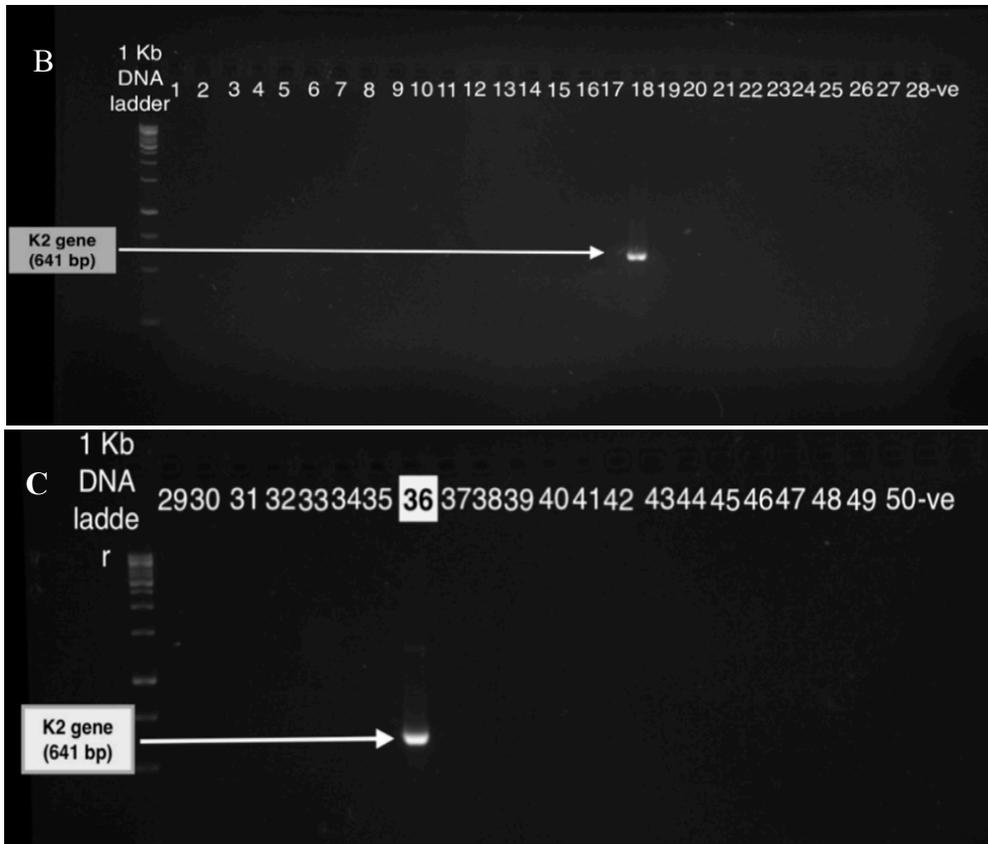
Moreover, Kp 83 was positive for capsular serotype 2 and none of the 129 isolates were positive for K20, K54 and K57. Faint bands in lanes 75, 78 and 80 were observed which might be non-specific bands. Therefore, to double check, PCR and gel electrophoresis were repeated for Kp 75, 78 and 80 with slight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



Supplementary Figure 1. 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 2. 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.

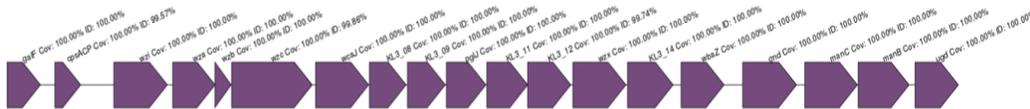
(B) Kaptive

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 (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, 288, 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 3. *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 4. *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, *rmlA*, shown in gray that is very different. The match confidence is just good.

Kp 102



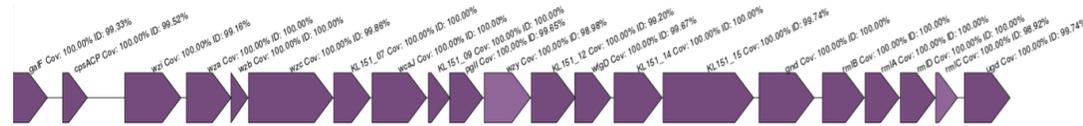
Supplementary Figure 5. *k-loci* gene arrangement in Kp 102. The *k-loci* of the isolate is similar to a known reference, KL51. However, there is a missing part of the genetic sequence represented as a line (____) in the figure above as well as a very different gene, *wcoZ*, represented in gray.

Kp 103



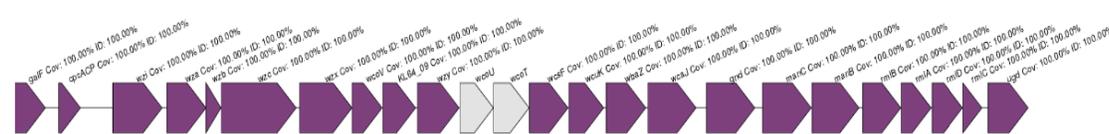
Supplementary Figure 6. *k-loci* gene arrangement in Kp 103. The *k-loci* of the isolate is perfectly similar to a known reference, KL64. The match confidence is very good.

Kp 104



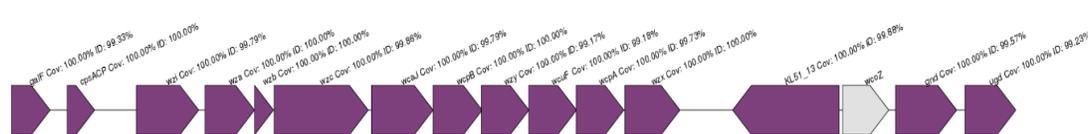
Supplementary Figure 7. *k-loci* gene arrangement in Kp 104. The *k-loci* of the isolate is similar to a known reference, KL64 except for 2 genes *wcoU* & *wcoT*, shown in gray that are very different. The match confidence is just good.

Kp 105



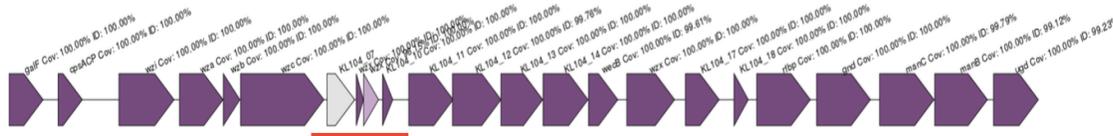
Supplementary Figure 8. *k-loci* gene arrangement in Kp 105. The *k-loci* of the isolate is very similar to a known reference, KL151 except for 2 genes that are a bit different as they are represented in light purple in the figure and underlined in red, *rmIC* and *wzy*.

Kp 106



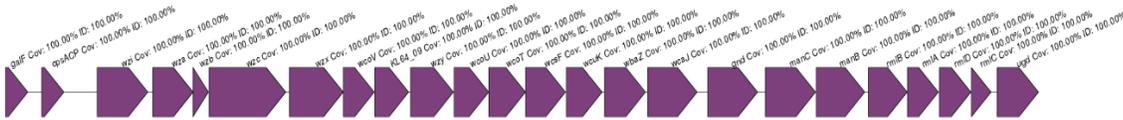
Supplementary Figure 9. *k-loci* gene arrangement in Kp 106. The *k-loci* of the isolate is similar to a known reference, KL51. However, there is a missing part of the genetic sequence represented as a line (____) in the figure above as well as a very different gene, *wcoZ*, represented in gray. The *k-loci* in this isolate is exactly identical to Kp 102.

Kp 107



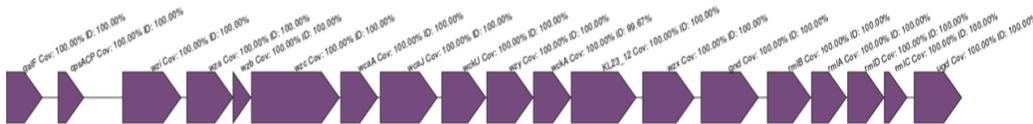
Supplementary Figure 10. *k-loci* gene arrangement in Kp 107. The *k-loci* of the isolate is similar to a known reference, KL104. The match confidence is good but the arrangement of genes in this k-loci is a bit different than other isolates. The light purple means low coverage or identity in gene *wzX* as well as there is a missing gene represented in gray, *KL104_7*.

Kp 108



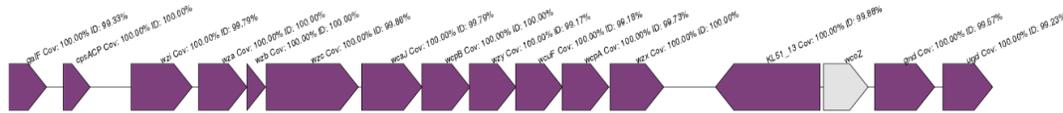
Supplementary Figure 11. *k-loci* gene arrangement in Kp 108. The *k-loci* of the isolate is similar to a known reference, KL64. The match confidence is very good. The *k-loci* in this isolate is exactly identical to Kp 103.

Kp 109



Supplementary Figure 12. *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



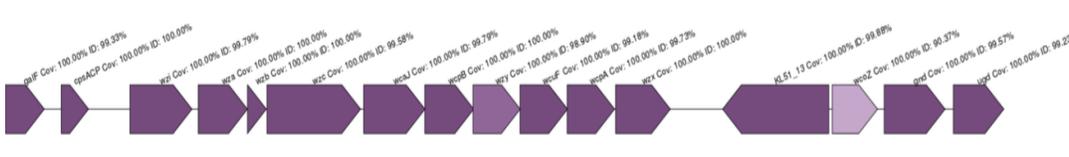
Supplementary Figure 13. *k-loci* gene arrangement in Kp 110. The *k-loci* of the isolate is similar to a known reference, KL51. However, there is a missing part of the genetic sequence represented as a line (____) in the figure above as well as a very different gene, *wcoZ*, represented in gray. The *k-loci* in this isolate is exactly identical to Kp 102 & 106.

Kp 111



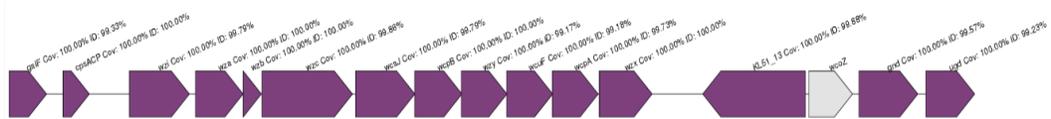
Supplementary Figure 14. *k-loci* gene arrangement in Kp 111. The *k-loci* of the isolate is similar to a known reference, KL64. The match confidence is very good. The *k-loci* in this isolate is exactly identical to Kp 108 & 103.

Kp 112



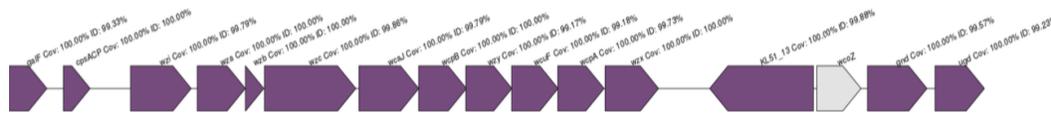
Supplementary Figure 15. *k-loci* gene arrangement in Kp 112. The *k-loci* of the isolate is similar to a known reference, KL51. However, there is a missing part of the genetic sequence represented as a line (____) in the figure above as well as a somewhat different gene, *wcoZ*, represented in light purple. The *k-loci* in this isolate is exactly identical to Kp 102 ,106 & 110.

Kp 113



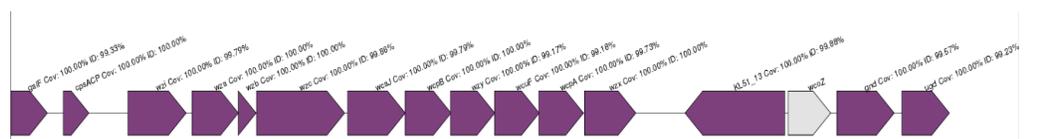
Supplementary Figure 16. *k-loci* gene arrangement in Kp 113. The *k-loci* of the isolate is similar to a known reference, KL51. However, there is a missing part of the genetic sequence represented as a line (____) in the figure above as well as a very different gene, *wcoZ*, represented in gray. The *k-loci* in this isolate is exactly identical to Kp 102, 106, 110 & 112.

Kp 114



Supplementary Figure 17. *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



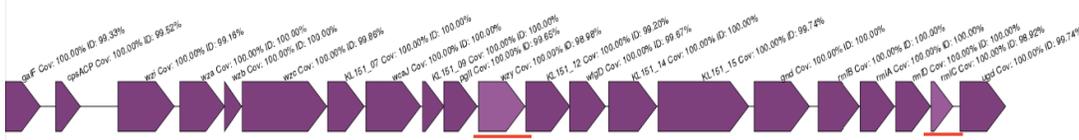
Supplementary Figure 18. *k-loci* gene arrangement in Kp 115. The *k-loci* of the isolate is similar to a known reference, KL51. However, there is a missing part of the genetic sequence represented as a line (____) in the figure above as well as a very different gene, *wcoZ*, represented in gray. The *k-loci* in this isolate is exactly identical to Kp 102, 106, 110, 112, 113 & 114.

Kp 116



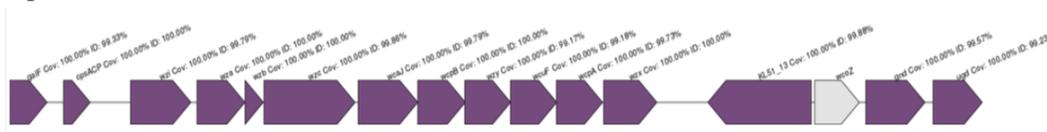
Supplementary Figure 19. *k-loci* gene arrangement in Kp 116. The *k-loci* of the isolate is similar to a known reference, KL51. This genetic composition is unique with a very different gene, *wcoZ*, represented in gray. The *k-loci* in this isolate is exactly identical to Kp 102, 106, 110, 112, 113, 114 & 115.

Kp 117



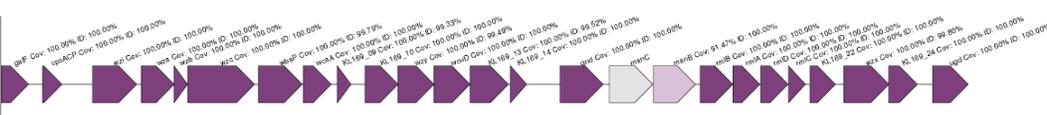
Supplementary Figure 20. *k-loci* gene arrangement in Kp 117. The *k-loci* of the isolate is very similar to a known reference, KL151 except for 2 genes that are a bit different as they are represented in light purple in the figure and underlined in red, *rmlC* and *wzy*. The *k-loci* in this isolate is exactly identical to Kp 105.

Kp 118



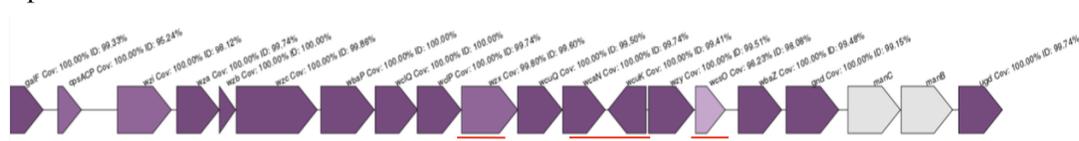
Supplementary Figure 21. *k-loci* gene arrangement in Kp 118. The *k-loci* of the isolate is similar to a known reference, KL51. However, there is a missing part of the genetic sequence represented as a line (____) in the figure above as well as a very different gene, *wcoZ*, represented in gray. The *k-loci* in this isolate is exactly identical to Kp 102, 106, 110, 112, 113, 114, & 115.

Kp 119



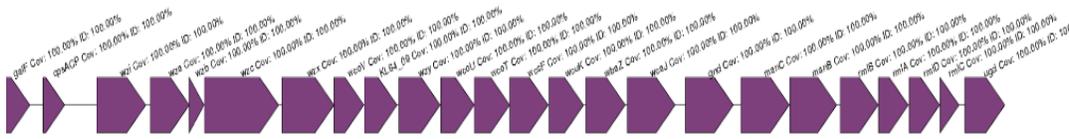
Supplementary Figure 22. *k-loci* gene arrangement in Kp 119. The *k-loci* of the isolate is similar to a known reference, KL169. The match confidence is not very good as the genetic sequence of this isolate was broken into 7 pieces hence, the assembly was poor. The underlined genes are inserted in a 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



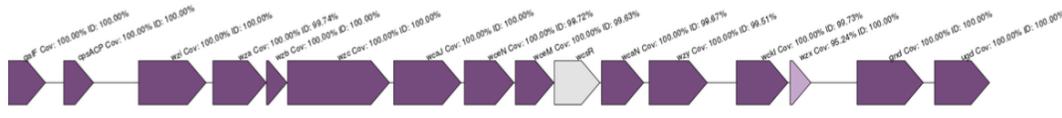
Supplementary Figure 23. *k-loci* gene arrangement in Kp 120. The *k-loci* of the isolate is similar to a known reference, KL64. The match confidence is not very good. The *k-loci* in this isolate is somewhat similar to Kp 103, 108 & 111. However, the gene *wzx* is not very similar as it is in light 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



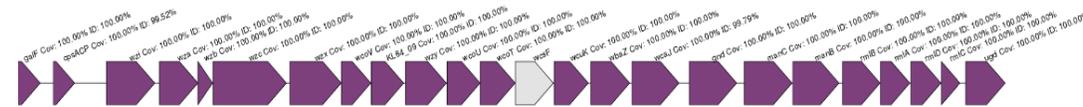
Supplementary Figure 24. *k-loci* gene arrangement in Kp 121. The *k-loci* of the isolate is similar to a known reference, KL64. The match confidence is very good. The *k-loci* in this isolate is exactly identical to Kp 103, 108 & 111.

Kp 122



Supplementary Figure 25. *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, *wscR* and a gene with low coverage in lavender, *wscX*. This isolate has a *k-loci* that is very different than other isolates.

Kp 123



Supplementary Figure 26. *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 *wscF* 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.

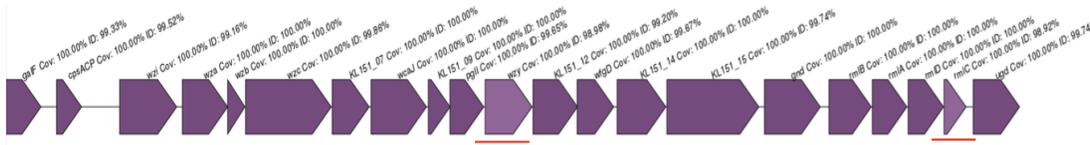


Supplementary Figure 27. *k-loci* gene arrangement of isolates Kp 102, Kp 106, Kp 110, Kp 112, Kp 113, Kp 114, Kp 115, Kp 116, Kp 118 and Kp 125.

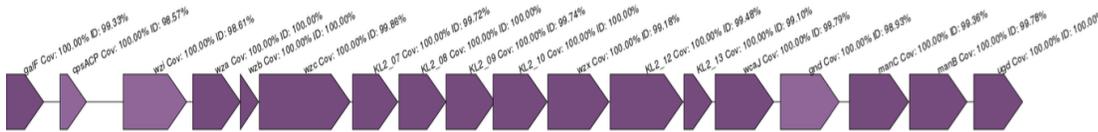
The Kaptive image below shows the *k-loci* genetic arrangement of isolates Kp 103, Kp 104, Kp 108, Kp 111, Kp 120, Kp 121 and Kp 123. The *k-loci* of these isolates are similar to a known reference, KL 64 in the Kaptive database. The match confidence is very good.



Supplementary Figure 28. *k-loci* gene arrangement of isolates Kp 103, Kp 104, Kp 108, Kp 111, Kp 120, Kp 121 and Kp 123.

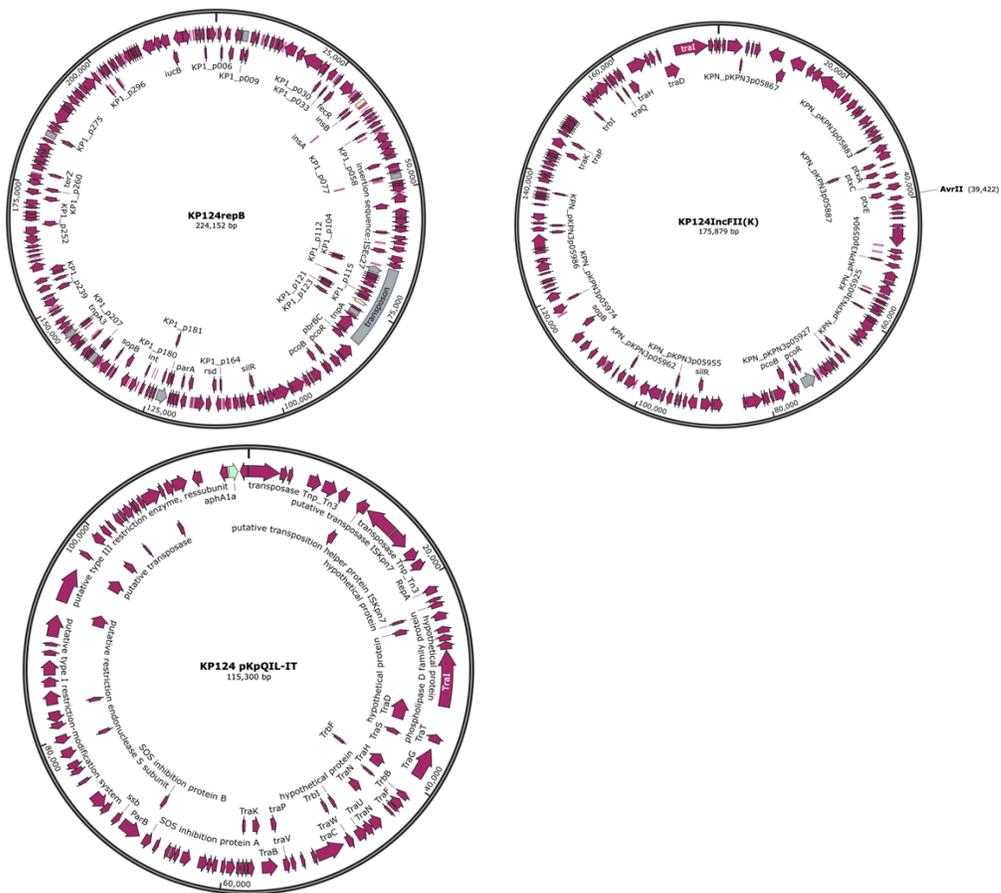


Supplementary Figure 29. *k-loci* gene arrangement of isolates Kp 105 and Kp 117. The *k-loci* of the isolates are very similar to a known reference, KL151 except for 2 genes that are a bit different as they are represented in light purple in the figure and underlined in red, *rmIC* and *wzy*.

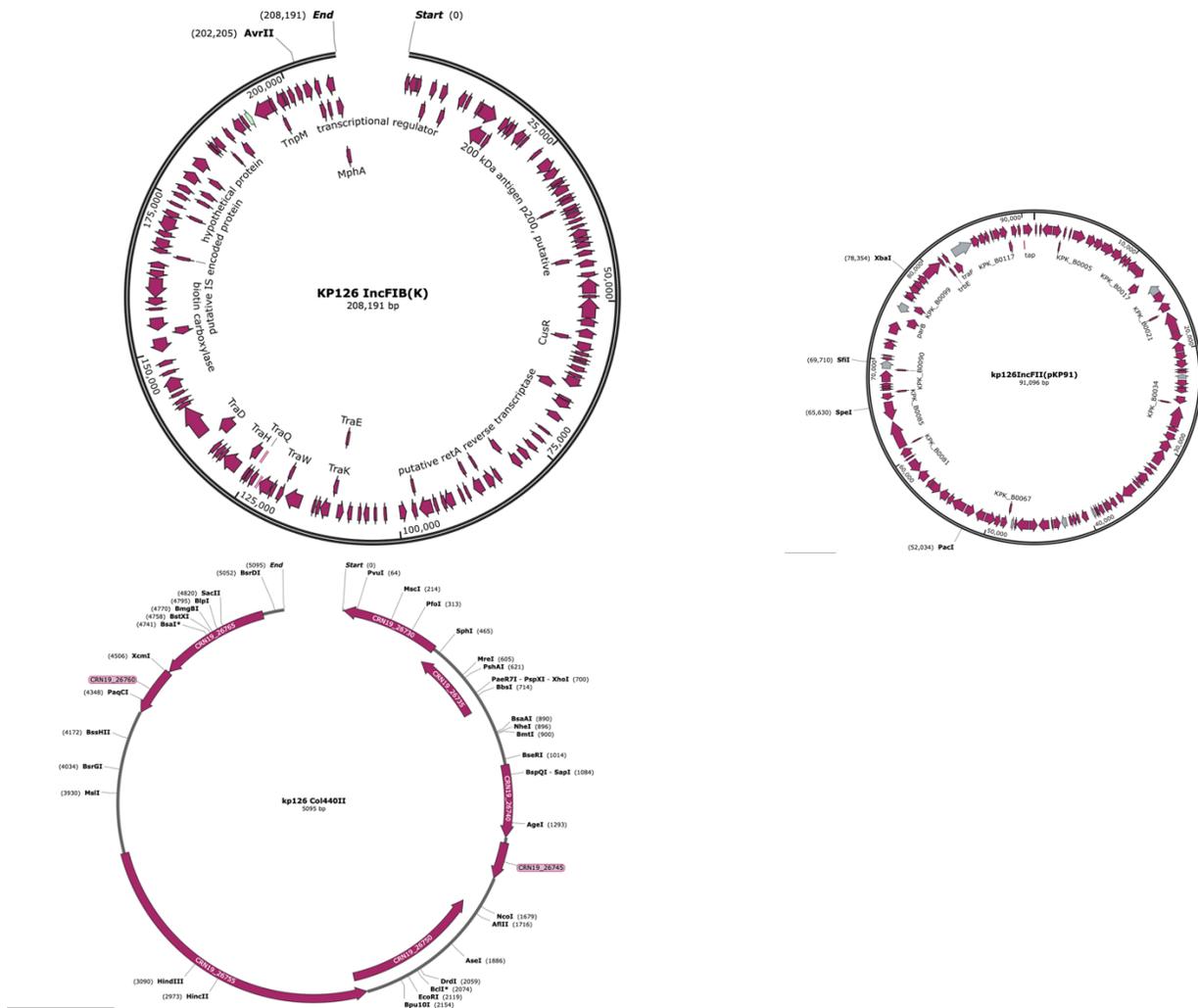


Supplementary Figure 30. *k-loci* gene arrangement of isolates Kp 83, Kp 126 and Kp 129. The Kaptive image below shows the *k-loci* genetic arrangement of isolates Kp 83, Kp 126 and Kp 129. The *k-loci* of these isolate are similar to a known reference, KL2. The match confidence is very high.

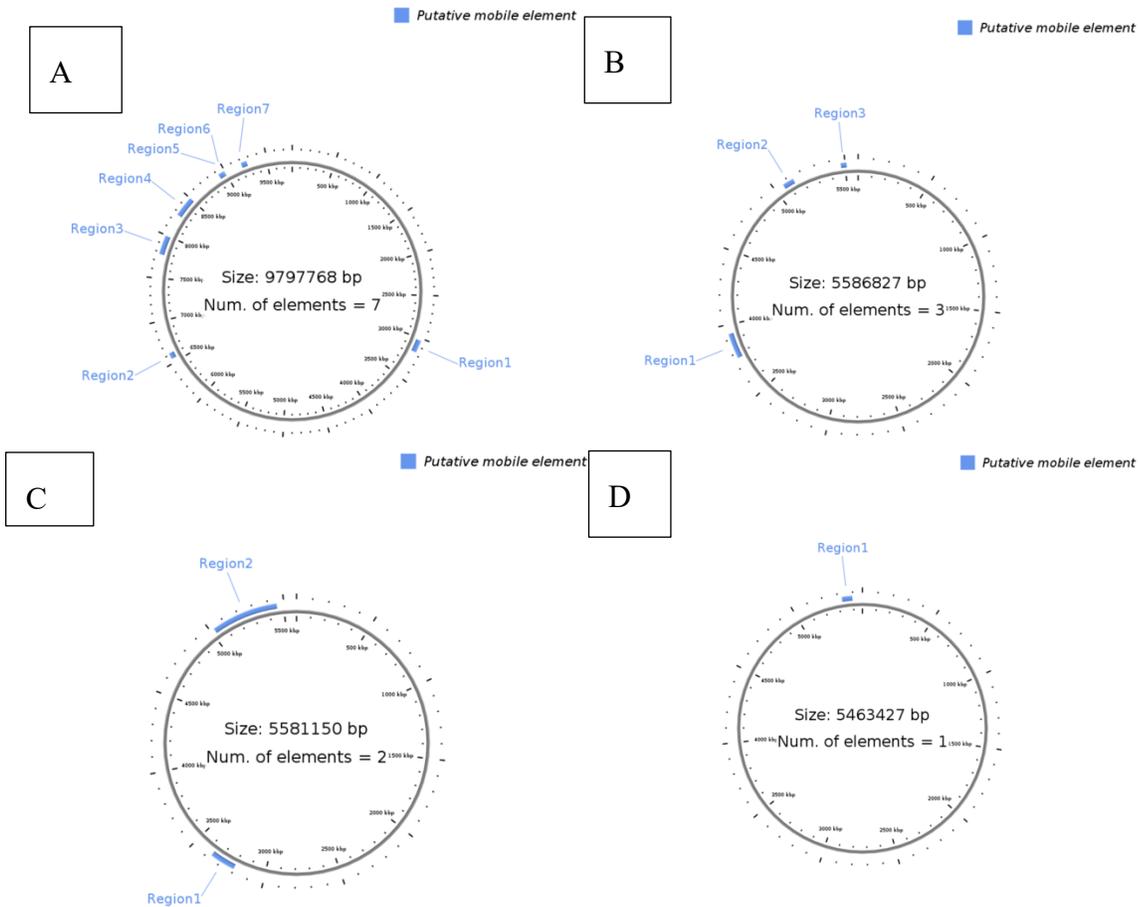
(C) Plasmid analysis



Supplementary figure 31. Acquired plasmids carrying antibiotic resistance genes in KP124 K1/ST-23. The Open reading frames (ORFs) are indicated in purple arrows, the name and the size of each plasmid is indicated in bps is indicated inside each circular genome.



Supplementary figure 32. Acquired plasmids carrying antibiotic resistance genes in KP126 K2/ST-881. The Open reading frames(ORFs) are indicated in purple arrows, the name and the size of each plasmid is indicated in bps is indicated inside each circular genome.



Supplementary figure 33. 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 Material

1 Supplementary tables

(A) List of clinical isolates

Supplementary Table 1. 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

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 2. 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				
Control 2	All alive	All alive	All alive	All dead	All dead
	All alive				
Control 3	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			
	All dead				
	5 alive, 5 dead	3 alive, 7 dead	3 alive, 7 dead	2 alive, 8 dead	2 alive, 8 dead
Isolate 18	4 alive, 6 dead	2 alive, 8 dead	1 alive, 9 dead	All dead	All 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			
	All dead				

(C) Human serum resistance assays**Supplementary Table 3. 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	Kp 124	0	0	0
0 hour		10	16	43
1 hour		19	11	23
2 hour	Kp 125	18	8	20
3 hour		10	16	43
0 hour		181	136	68
1 hour	Kp 83	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 4. Clinical isolate serial numbers of phylogenetic tree in figure 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

Supplementary Table 5. 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 6. List of PCR primer names, nucleotide sequences and annealing temperatures used in this study obtained from (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			