| Strain | Size (bp) | ORFs | RNAs | Contigs | Accession No. |
|---------------|-----------|------|------|---------|---------------|
| 48-IT | 5,682,001 | 5373 | 81 | 112 | LKAB0000000 |
| CIV4-IT | 5,580,815 | 5310 | 129 | 115 | MRBE00000000 |
| КН-43-СО | 5,724,434 | 5472 | 125 | 169 | MQUU00000000 |
| KL-49-CO | 5,680,218 | 5394 | 125 | 140 | MQNA0000000 |
| H150820806-UK | 5,586,185 | 5302 | 81 | 179 | MQUT0000000 |
| H154440769-UK | 5,541,345 | 5246 | 79 | 188 | MQUZ0000000 |
| H151300628-UK | 5,498,686 | 5207 | 85 | 128 | MQUW0000000 |
| H151400610-UK | 5,485,228 | 5226 | 79 | 211 | MQUX0000000 |
| H151400611-UK | 5,636,878 | 5533 | 85 | 546 | MQUY0000000 |
| H151440672-UK | 5,489,845 | 5223 | 82 | 154 | MQUV00000000 |
| H155360912-UK | 5,439,556 | 5136 | 85 | 130 | MQVB0000000 |
| H151440671-UK | 5,358,482 | 5043 | 86 | 126 | MQVA0000000 |

Table S1. Characteristics and features of the ST307 genomes

| Contig ^a | Positions ^a | Features | HS11286 | NJST258_2 | KPNIH1 | 48IT |
|---------------------|-------------------------------|---|---------|-----------|----------|-------------|
| | | | ST11 | ST258 | ST258 | ST307 |
| | | | (wzi74) | (wzi154) | (wzi154) | (wzi173) |
| 1 | 756959-821560 | ICE, T4SS ^b , yersiniabactin | + | _ | - | + |
| 1 | 121459-129485 | Cellobiose PTS ^c | + | - | - | + |
| 2 | 178022-200498 | T6SS ^b | - | - | - | + |
| 2 | 398541-434010 | Type 1 Fimbria, RND multidrug efflux | - | + | + | + |
| 3 | 57266-78773 | RND efflux system, acrivinflavin resistance | + | - | + | + |
| 3 | 175062-197386 | Phage φ2 | + | - | + | + |
| 4 | 300588-377780 | Phage φ1 | - | - | - | + |
| 4 | 30798-42976 | Capsule 1 | - | - | - | + |
| 6 | 315697-323095 | 4-hydroxyproline metabolism | - | - | - | + |
| 10 | 8054-44477 | Antirestriction KlcA, YfiZ-YpJF antitoxin-toxin | - | - | - | + |
| 12 | 1-13623 | T1SS ^b | + | - | + | + |
| 12 | 134263-142016 | Sorbitol PTS ^c | - | - | - | + |
| 18 | 69121-82910 | π -fimbrial cluster, urophatogenic | - | - | - | + |
| 18 | 30161-44180 | Capsule 2 Enterobacter | - | - | - | + |
| 28 | 13267-20969 | Ethanolamine utilization | - | - | - | + |

Table S2. Major chromosomal differences identified by genome comparison among ST307 48-IT and HS11286, NSJ258_2 and KPNIH1 reference genomes

^a: Contig positions refers to the 48-IT genome sequence Acc. No. LKAB00000000

^b: T4SS:Type IV Secretion System, T6SS: Type VI Secretion System, T1SS: Type I Secretion System

^c: Sugar transport process via the phosphoenolpyruvate phosphotransferase system (PTS)

| Target | Oligo name | Sequence | Amplicon size (bp) | Annealing | |
|-------------------------|-------------|-------------------------|--------------------|------------------|--|
| | _ | | | temperature (°C) | |
| π -fimbrial cluster | Usher_C18Fw | GATCATAGCAACGGTTCGGA | 628 | 57 | |
| | Usher_C18Rv | TCAAATGACAGCGCTCCCA | | | |
| Capsule 2 | CA_C18Fw | TACCGTTATTCTTGCATCTC | 499 | 55 | |
| Enterobacter | CA_C18Rv | CGCGAGATCAAACATACCGG | | | |
| Phage ф48 | C4_Fw | GAAATGGCTTGCTCATGTG | 679 | 52 | |
| | C4_Rv | CGGCATTTTCTCACCTGTC | | | |
| Glycogen | glico-fw | TGATAGGCAATGTTGTG | 373 | 50 | |
| synthesis cluster | glico-Rv | CCTGGGTTTCCGGCGATCCGC | | | |
| on pKPN-307 | | | | | |
| Urea transport | urtC-fw | CGCCCGCCCACGGCACA | 217 | 60 | |
| system on pKPN- | urtC-rv | CTCGGCCGACTGATGGTTGCC | | | |
| 307 | | | | | |
| FIB replicon on | FIB-KN fw | GCTGCTCCATCGCCTTACGC | 631 | 57 | |
| pKPN-307 | FIB-KN Rv | GAAATCAACAGGGAAGAGCAAGG | | | |
| FIB replicon on | FIB-KQ fw | GTATCTGGTCTGTTAAGTCG | 258 | 57 | |
| pKpQIL | FIB-KQ rv | TCTGCTGGTTATTGGGTGAG | | | |

Table S3. Primers used in this study for PCR amplification of specific ST307 features

| Strain | Fimbriae ^a | | | | | | mrk alleles | ybt alleles | fyuA, irp1, irp2 |
|-----------------|-----------------------|------------------------------|-------------|-------------|-------------|-------------------------|---|---|------------------------------|
| | 1040037 | 160037 | 20008 | 220009 | 610080 | 90118-26 | | | alleles |
| | 1040041 | 160044 | 20010 | 220012 | 610083 | 90133-37 | | | |
| 48-IT | Contig 14 | Contig 2 | Contig 3 | Contig 3 | Contig 7 | Contig 2 | mrkA_12, mrkB-2, mrkD-8, mrkF- 4, mrkH-2, mrkI-4, mrkJ-2 | ybtA-38, ybtE-5, ybtP-25, ybtQ-6, ybtS-41, ybtU-39, ybtX-11 | fyuA-11, irp1-37 |
| CIV4-IT | Scaffold 17 | Scaffold 14 | Scaffold 7 | Scaffold 7 | Scaffold 0 | Scaffold 10 | mrkB-2, mrkD-8, mrkF-4, mrkH-2, mrkI-4, mrkJ-2 | ybtA-38, ybtE-5, ybtP-25, ybtQ-6, ybtS-41, ybtT-5, ybtU-39, ybtX-11 | fyuA-11, irp1-37 |
| KH-43-CO | Scaffold 17 | Scaffold 13 | Scaffold 21 | Scaffold 29 | Scaffold 0 | Scaffold 8 | mrkA_12, mrkB-2, mrkD-8, mrkF- 4, mrkH-2, mrkI-4, mrkJ-2 | neg | neg |
| KL-49-CO | Scaffold 12 | Scaffold 18 | Scaffold 0 | Scaffold 0 | Scaffold 1 | Scaffold 7 | mrkA_12, mrkB-2, mrkD-8, mrkF- 4, mrkH-2, mrkI-4, mrkJ-2 | neg | neg |
| H154440769-UK | Scaffold 11 | Scaffold 0 | Scaffold 4 | Scaffold 4 | Scaffold 6 | Scaffold 0 | mrkA_12, mrkB-2, mrkD-8, mrkF- 4, mrkH-2, mrkI-4, mrkJ-2 | neg | neg |
| H150820806-UK | Scaffold 13 | Scaffold 0 | Scaffold 2 | Scaffold 2 | Scaffold 5 | Scaffold 0 | mrkA_12, mrkB-2, mrkD-8, mrkF- 4, mrkH-2, mrkI-4, mrkJ-2 | neg | neg |
| H151300628-UK | Scaffold 17 | Scaffold 1 | Scaffold 0 | Scaffold 0 | Scaffold 6 | Scaffold 1 | mrkA_12, mrkB-2, mrkD-8, mrkF- 4, mrkH-2, mrkI-4, mrkJ-2 | ybtA-3, ybtE-9, ybtP-3, ybtQ- 4, ybtS-16, ybtT-10, ybtU-3, ybtX-12 | fyuA-39, irp1-54, irp2-61 |
| H151400610-UK | Scaffold 16 | Scaffold 15 | Scaffold 46 | Scaffold 20 | Scaffold 22 | Scaffold 3 | mrkA_12, mrkB-2, mrkD-8, mrkF- 4, mrkH-2, mrkI-4, mrkJ-2 | ybtA-3, ybtE-9, ybtP-3, ybtQ- 4, ybtS-16, ybtT-10, ybtU-3, ybtX-12 | fyuA-39, irp1-54, irp2-61 |
| H151400611-UK | Scaffold 36 | Scaffolds 323,447,2 23 | Scaffold 99 | Scaffold 9 | Scaffold 87 | Scaffold 179- 212-28 | mrkB-2, mrkD-8, mrkF-4, mrkH-2, mrkI-4, mrkJ-2 | ybtP-3, ybtQ-4, ybtS-16, ybtT- 10, ybtU-3, ybtX-12 | fyuA-39 |
| H151440672-UK | Scaffold 4 | Scaffold 17 | Scaffold 1 | Scaffold 2 | Scaffold 20 | Scaffold 5 | mrkA_12, mrkB-2, mrkD-8, mrkF- 4, mrkH-2, mrkI-4, mrkJ-2 | ybtA-3, ybtE-9, ybtP-3, ybtQ- 4, ybtS-16, ybtT-10, ybtU-3, ybtX-12 | fyuA-39, irp1-54, irp2-61 |
| H15536091201-IT | Scaffold 11 | Scaffold 2 | Scaffold 4 | Scaffold 4 | Scaffold 1 | Scaffold 2 | mrkA_12, mrkB-2, mrkD-8, mrkF- 4, mrkH-2, mrkI-4, mrkJ-2 | ybtA-3, ybtE-9, ybtP-3, ybtQ- 4, ybtS-16, ybtT-10, ybtU-3, ybtX-12 | fyuA-39, irp1-54, irp2-61 |
| H151440671-UK | Scaffold 11 | Scaffold 13 | Scaffold 2 | Scaffold 2 | Scaffold 12 | Scaffold 3 | mrkA_12, mrkB-2, mrkD-8, mrkF- 4, mrkH-2, mrkI-4, mrkJ-2 | ybtA-3, ybtE-9, ybtP-3, ybtQ- 4, ybtS-16, ybtT-10, ybtU-3, ybtX-12 | fyuA-39, irp1-54, irp2-61 |

Table S4. Fimbriae and virulence genes identified in the ST307 genomes

^a All usher loci included between the two numbers were identified in the respective scaffolds of assembled genomes (i.e. Usher_1040037, Usher_1040038, Usher_1040039, Usher_1040040 and Usher_1040041 are indicated as 1040037-1040041 in Table). Usher loci numbers refer to nomenclature adopted at the BIGSdb-Kp database (<u>http://bigsdb.pasteur.fr/</u>)





The comparison was obtained uploading the ST307 annotated genomes at the RAST server (<u>http://rast.nmpdr.org/seedviewer.cgi</u>) by Blast P analysis of CDS, visualized by the Seed Viewer version 2.0. The major differences among the strains of the ST307 clone consisted in phages (ϕ 48), prophages (ϕ 1 and ϕ 2), plasmids (pKPN3 variants and pKpQIL presence or absence) and ICE typeA and type B content.



Figure S2. Comparative analysis of ST307 genomes vs Klebsiella pneumoniae complete reference genomes

The comparison was obtained uploading 3 prototypic ST307 genomes and the *K. pneumoniae* NJST258_2 (NZ_CP006918), KPNIH1 (NZ_CP008827) and HS11286 (CP003200), reference genomes at the RAST server (<u>http://rast.nmpdr.org/seedviewer.cgi</u>) by Blast P analysis of CDS, visualized by the Seed Viewer version 2.0. The major differences identified in this comparison are listed in Table S2.





ST307 genomes were ordered and aligned using the MAUVE comparison tool against the *K. pneumoniae* NJST258_2 (NZ_CP006918), and HS11286 (CP003200) reference genomes.



Figure S4. Prophages identified in the ST307 genomes and $\varphi48$

Arrows indicate phage genes and their direction of transcription.Colours indicate clusters encoding replication control (green), capside assembly (red), tail assembly (blue). Partitioning and lysozyme encoding genes are indicated by violet and yellow arrows, respectively





Gene clusters of the two capsular clusters Cp1 (panel A) and Cp2 (panel B) identified in ST307. The intact Cp1 and IS*Kpn7* deleted Δ Cp1 are drawn in comparison to the capsular loci of ST258 and ST101 strains. Arrows indicate genes and their direction of transcription. Yellow arrows indicate genes that are common, white arrows those that are different among ST307, ST258 and ST101 clusters. The second capsular cluster Cp2 is indicated by green arrows