Supplementary Methods

Bacterial strain typing. DNA macrorestriction with *XbaI* and subsequent PFGE was performed as previously described [1]. Isolates assigned to 'pulsotype 1' displayed banding patterns that differed from each other by less than three bands [1]. Unique banding patterns were assigned to subgroups 1-1 to 1-13. Multilocus sequence typing (MLST) data were extracted from genome sequences.

Genome sequencing. DNA was extracted from *K. pneumoniae* isolates by using the DNeasy blood and tissue kit (Qiagen). To generate a reference genome sequence, the DNA from isolate 234/12 was sequenced by using a combination of Pacific Biosciences and Illumina technologies. Pacific Biosciences data were generated on the PacBio RS-II platform by applying the DNA Template Prep and Sequencing Kit 2.0 (Pacific Biosciences, Menlo Park, CA, USA), yielding 96,664 sequencing reads (average read length, 5,871 bp) and 85-fold average coverage of the bacterial chromosome. Read assembly was performed applying SMRT Analysis software v2.1.0 (Pacific Biosciences) with implemented Celera assembler, using default parameters and adjusting the expected genome size to 5 Mbp. Illumina sequencing of DNA from isolate 234/12 was performed on a HiScan 2000 machine by applying Nextera XT DNA Sample Preparation Kit and TruSeq SBS Kit v3 - HS sequencing kit (Illumina, San Diego, CA, USA) to yield 74-fold average coverage with 100-bp paired-end reads. Illumina reads were used to detect and correct homopolymer-length sequencing errors at five positions total. Coding sequences were identified and annotated using the RAST web pipeline (http://rast.nmpdr.org/) [2]. In addition, prophage sequences were identified and annotated using PHAST (phage search tool, available at http://phast.wishartlab.com ;[3]).

Illumina re-sequencing of genomic DNA from 46 K. pneumoniae isolates to >27-fold average coverage each was performed on a HiScan 2000 machine (Illumina, San Diego, CA, USA) as described above. Paired-end reads were mapped to the reference genome sequence from isolate 234/12 by using Burrows-Wheeler aligner BWA, version 0.6.2. [4], with default

parameters. Alignment files were processed with Samtools version 0.1.18 (available at http://sourceforge.net/projects/samtools/files/). Single-nucleotide polymorphisms (SNPs) were identified using Varscan version 2.3 using default parameters and custom shell scripts (parameters: coverage ≥8; variant frequency ≥0.75; base quality ≥15). SNPs in repetitive regions identified by using the repeat analysis tool implemented in Kodon software (Applied Maths) were excluded from further analyses. Likewise, SNPs in mobile genetic elements including plasmids, prophages, transposons, or insertion sequence elements (supplementary table 2) were excluded from phylogenetic analyses. Homoplasious SNPs were identified by using the four-gamete test as implemented in DnaSP software, version 5 (available at http://www.ub.edu/dnasp/). Multilocus sequence types were inferred from genome sequences, and relatedness of sequence types was investigated by using eBURST version 3 (http://eburst.mlst.net/).

Phylogenetic analyses. An alignment of core genome SNPs was used to reconstruct the isolates' phylogeny utilizing the PhyML module in Seaview, version 4.2.3. Evolutionary rates and divergence dates were estimated from an alignment of genome sequences dated with the isolates' recovery dates, by using BEAST version 1.7.4 (http://beast.bio.ed.ac.uk). Results were largely independent from tree priors (constant population size, exponential growth, Bayesian skyline). Random permutation of sampling dates among isolates resulted in lower substitution rates, much older dates, and much larger confidence intervals, indicating that our data contained a genuine temporal signal. Usage of a relaxed clock model was justified by the result of a likelihood ratio test performed with PAUP [5 6]. Minimum spanning trees were constructed by using Bionumerics (Applied Maths).

References (Supplementary Methods):

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