Supporting Information

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SI Materials and Methods

Klebsiella pneumoniae **Isolates.** We selected two representative multilocus sequence type 258 (ST258) carbapenem-resistant *K. pneumoniae* isolates for genome sequencing based on the presence of KPC3 and mucoid and nonmucoid phenotypes. Isolates were selected for comparative genome sequencing based on their geographical and temporal distribution, *K. pneumoniae* carbapenemase (KPC) variants, and Tn*4401* isoforms (Dataset S1). *K. pneumoniae* isolates were obtained from healthcare facilities at diverse geographic locations in the United States, Canada, Colombia, and Italy. None of the ST258 isolates contained the gene encoding New Delhi metallo-β-lactamase-1 (Dataset S1). *K. pneumoniae* were cultured overnight in LB broth at 37 °C for subsequent isolation of DNA for genome sequencing (see below). Alternatively, bacteria were cultured overnight in LB and then were subcultured to exponential phase of growth in LB. Bacteria were pelleted by centrifugation to remove the medium and were opsonized with fresh 5% (vol/vol) human autologous serum for 30 min at 37 °C. Bacteria were washed once in PBS, suspended in RPMI/H to the desired concentration, and added to assay tubes with human neutrophils as described below.

Characterization of *K. pneumoniae* Strains. Minimum inhibitory concentrations of *K. pneumoniae* isolates were determined by broth microdilution in cation-adjusted Mueller–Hinton broth using Sensititre GNX2F panels (Thermo Fisher Scientific) according to Clinical and Laboratory Standards Institute methods and interpretations (1, 2). An in-house resistance and plasmid replicon database was developed. The resistance reference genes were identified from the Comprehensive Antibiotic Research Database (http://arpcard.mcmaster.ca) (3), Antibiotic Resistance Genes Database (http://ardb.cbcb.umd.edu/) (4), Lahey Clinic β-lactamase database (www.lahey.org/studies/), and published gene lists (5). The plasmid replicons were identified from plasmid multilocus sequence type databases (http://pubmlst.org/plasmid/), from the PCR-based replicon typing method (6), and from plasmids sequenced in the current study. All unique resistance and plasmid replicon sequences were concatenated and compiled as the reference library. The *cps* regions of two clades were also included in the library. The DNA sequence of the capsular polysaccharide biosynthesis gene cluster *cps 1* from isolate 1787 was generated by de novo assembly of the Illumina HiSeq reads using CLC Genomics Workbench software (v5.5.1, CLC Bio). The *cps 2* DNA sequence was determined from the genome sequence from NJST258_1. The raw reads of each sample then were assembled against the library using CLC Genomics Workbench to identify genes encoding antimicrobial resistance (resistance to β-lactams, aminoglycosides, and quinolones), plasmid replicons, and *cps* gene types.

K. pneumoniae **de Novo DNA Sequencing and Assembly.** *K. pneumoniae* DNA was isolated from overnight cultures using a DNeasy tissue kit (Qiagen) according to the manufacturer's directions. Paired end libraries with an 8-kb span were created for *K. pneumoniae* strains NJST258_1 and NJST258_2 using kits and reagents (Roche) according to the manufacturer's instructions, and each library was sequenced using a two-region gasket and 454 GS FLX Titanium chemistry using a Roche 454/Life Sciences GS FLX sequencer. Two additional fragment libraries were prepared using the same method and were sequenced in an identical fashion. For Illumina sequencing, two libraries were prepared using *TruSeq DNA Sample* prep kits and reagents (Illumina) following the low sample (LS) protocol provided by the manufacturer. The libraries were pooled in equal amounts and were clustered in an individual lane of a paired-end flowcell, and 100 bp were sequenced from both ends using an Illumina HisEq 2000 sequencer. De novo assembly was accomplished by using a combination of GS De Novo Assembler (Roche/454 Life Sciences), Mira (7), and Velvet (8). The best assemblies from each method were combined using Sequencher. These contigs were ordered by genome position and orientation using optical whole-genome mapping (OpGen) to create a complete scaffold with 27 or 37 gaps. Gap closures were accomplished using a combination of traditional Sanger sequencing or long-range PCR amplification of the gaps followed by sequencing and assembly using a Roche 454/Life Sciences GS FLX sequencer and GS De Novo Assembler. Genome annotation was performed by Integrated Genomics, as described previously (9). The genome and plasmid DNA sequences of NJST258_1 and NJST258_2 are deposited in GenBank under National Center for Biotechnology Information (NCBI) accession numbers CP006923 (NJST258_1 chromosome), CP006927 (pNJST258N1), CP006926 (pNJST258N2), CP006925 (pNJST258N3), CP006928 (pNJST258N4), and CP006924 (pNJST258N5), and NCBI accession numbers CP006918 (NJST258_2 chromosome), CP006922 (pNJST258C1), CP006919 (pNJST258C2), and CP006921 (pNJST258C3).

K. pneumoniae **Comparative Genome Sequencing and Analysis.** Total DNA was isolated from overnight cultures of 85 *K. pneumoniae* clinical isolates using a DNeasy tissue kit (Qiagen) according to the manufacturer's directions. Samples were sheared individually on a Covaris S2 Focused-ultrasonicator (Covaris, Inc.) using the glass microtube format, stored in a 96-well plate, and processed using the LS protocol (Illumina). Samples were uniquely indexed to represent eight pools and were size selected on a Pippin Prep electrophoresis platform (Sage Science, Inc.). Each pool was clustered in an individual lane of a paired-end flowcell, and 50 bp were sequenced from both ends on an Illumina HisEq 2000. Raw reads were trimmed and filtered for adaptor and poor-quality sequence. Reads were mapped to the genome of *K. pneumoniae* strain NJST258_1 using Bowtie 2 (10), and SNPs and small indels were called using GATK Unified Genotyper (11). Metadata were deposited in the NCBI sequence read archive (SRA) (www.ncbi.nlm.nih.gov/sra) under the accession number SRP036874.

Bioinformatics. *K. pneumoniae* strains NJST258_1 and NJST258_2 were compared with eight other closed *K. pneumoniae* genomes currently in NCBI using the BLAST Ring Image Generator program (12). Phylogenetic analysis was performed using concatenated SNP sequences; SNPs located within a mobile genetic element were excluded. Concatenated SNP sequences were aligned using the MAFFT plugin for Geneious Pro (13). Phylogenetic trees were generated using the neighbor-joining method with the Tamura–Nei distance model (14), using isolate 1813 as an outgroup in some analyses, and were resampled 1,000 times with the bootstrap method in Geneious Pro.

Human Neutrophil Assays. Neutrophils were isolated from venous blood of healthy volunteers using dextran sedimentation coupled with Ficoll-Hypaque gradient density centrifugation as described previously (15). These studies were performed in accordance with a protocol approved by the Institutional Review Board for human subjects, National Institute of Allergy and Infectious Diseases, National Institutes of Health. All blood donors provided written informed consent before participation in the studies.

To measure neutrophil bactericidal activity, polymorphonuclear neutrophils (PMNs) in RPMI/H were combined with serum opsonized or unopsonized bacteria in 1.5-mL Eppendorf tubes (10^6 PMN + 10^6 cfus *K. pneumoniae* in 600 μL final volume; cfu:PMN ratio = 1:1) and were incubated at 37 °C for 1 h with gentle rotation. Saponin (0.1% final concentration) was added to each tube, the contents were mixed, and the tubes were chilled on ice for 15 min before diluting and plating on LB. Cfus were enumerated the following day. Percent survival was determined with the following equation: $\mathrm{cfu}_{+\mathrm{PMN}\ 60\ \mathrm{min}}/\mathrm{cfu}_{\mathrm{w/o\ PMN}\ 60\ \mathrm{min}} * 100$. Data were analyzed using a one-way ANOVA and Tukey's posttest (GraphPad Prism 6.0b for Mac OS X, GraphPad Software).

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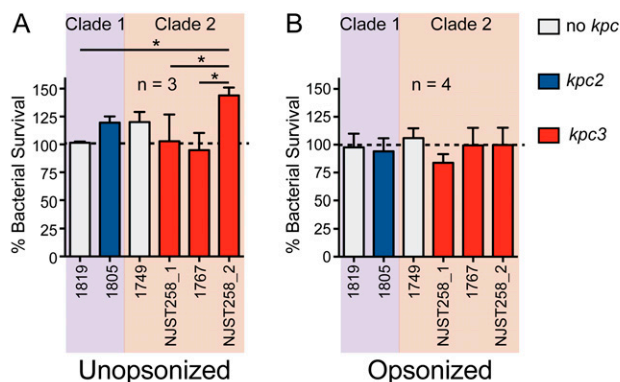

Fig. S1. Interaction of *K. pneumoniae* with human neutrophils. *(A* and *B)* Survival of the indicated *K. pneumoniae* isolates following phagocytic interaction with human neutrophils as described in *SI Materials and Methods*. Assays in *A* were performed with unopsonized bacteria; assays in *B* were performed with bacteria opsonized in 5% (vol/vol) human serum as indicated. Data are the mean ± SD of three or four experiments (PMN donors) as indicated. Data were analyzed using a one-way ANOVA and Tukey's posttest. $*P < 0.05$ for the indicated comparisons.

Table S1. ST258 genes involved in antibiotic or heavy metal resistance

Gene/operon	Predicted function	NJST258_1	NJST258_2
Chromosome			
gyrA	Quinolone resistance	Ser83Ile, Asp87Gly	Ser83Ile, Asp87Asn
gyrB	Quinolone resistance	WT	WT
parC	Quinolone resistance	Ser80Ile	Ser80Ile
parE	Quinolone resistance	WT	WT
rpoB	Rifampin resistance	WT	WT
folp	Sulfonamide resistance	WT	WT
ompA	Outer membrane protein	WT	WT
ompK26	Outer membrane protein	WT	WT
ompK35	Outer membrane protein	STOP89	STOP89
ompK36	Outer membrane protein	WT	WT
ompK37	Outer membrane protein	WT	WT
lamB	Outer membrane protein	+	+
phoE	Outer membrane protein	+	+
arcRAB	Multidrug efflux pump	+	+
tolC	Multidrug efflux pump	+	+
oqxAB	Multidrug efflux pump	+	+
rob	Transcription factor	+	+
marARC	Transcription factor	*marA*-, *marR* trunc, *marC*+	+
soxRS	Transcription factor	*soxR*-, *soxS* trunc	+
romAR	Transcription factor	+	—
hfg	RNA-binding protein	+	+
scrYAB	Sucrose-specific porin	+	+
rpoS	RNA polymerase sigma factor	+	+
pagP	Lipid A acylation protein	+	+
phoBR	TCS	+	+
phoPQ	TCS	+	+
pmrAB	TCS	+	+
pmrD	Signal transduction protein	+	+
pmrF	Lipid A biosynthesis protein	+	+
rcsCBD	TCS	+	+
bla$_{SHV\text{-}11}$	β-Lactam resistance	—	+
Plasmid			
bla$_{KPC\text{-}3}$	β-Lactam resistance	+	+
bla$_{TEM\text{-}1}$	β-Lactam resistance	+	trunc
bla$_{OXA\text{-}9}$	β-Lactam resistance	+	—
aacA4	Aminoglycoside resistance	+	+
aadA1	Aminoglycoside resistance	+	trunc
strAB	Aminoglycoside resistance	+	—
dfrA14	Trimethoprim resistance	+	—
sul2	Sulfonamide resistance	+	—
ars operon	Arsenic resistance	+	—
cop operon	Copper resistance	+	—
sil operon	Silver resistance	+	—
nic operon	Nickel resistance	+	—

Where a specific mutation is listed, the amino acid changed caused antibiotic resistance. TCS, two-component gene regulatory system; trunc, truncated gene; WT, wild-type allele (and thus the isolate was susceptible to the antibiotic); +, gene is present; —, gene is absent.

Other Supporting Information Files

Dataset S1 (XLSX)
Dataset S2 (XLSX)
Dataset S3 (XLSX)