Materials and Methods

***In silico* analysis of plasmid transfer genes for Figure S6.** 77 genes putatively associated with plasmid transfer functions in the literature including: antirestriction, pilus formation, incompatibility, stability, and stress response were extracted from 6050 RefSeq plasmid records at NCBI. Genes were clustered with CD-HIT at 98% identity and 90% length difference cutoffs to create a non-redundant database of 8733 gene sequences. Plasmid sequences from conjugation donors and *K. pneumoniae* patient isolate recipient strains as well as several bla_{KPC} or bla_{OXA-48} plasmids from the literature (1, 2) were compared to this database. We extracted putative protein-coding sequences from these plasmids using Prokka ver. 1.12 (3) and compared amino-acid sequences of the encoded proteins to our reference database using TBLASTN ver. 2.2.31+. A gene was considered to be present if it was found at 50% coverage and a minimum E-value of 1e-5. Genes that were found together in 98% clustering were collapsed. The presence/absence of genes and the identity level of best match between plasmid-encoded proteins and our reference set was visualized using R version 3.2.4.

Transfer - Tra	Transfer - Other	Stability/ Toxin-Antitoxin/Replication	Anti-restriction	Stress Response	Entry or Surface Exclusion
traA	*finO*	*kikA*	*ardA*	*mucA*	*eex*
traB	*mobB*	*korA*	*ardB*	*nucB/umuC*	*traS*
traB/traK	*mobC*	*korB*	*ardB/klcA*	*psiA*	*traT*
traB/trbI	*pilT*	*korB/parB*	*ccgC*	*psiB*	
traC	*pilT/vagD*	*nuc*	*ccgD*	*umuC*	
traD	*tir*	*parA*	*klcA*	*umuD*	
traE	*traA/virB3*	*parB*			
traE/traK	*traB/virB4*	*relE*			
traF	*traC/virB5*	*repA*			
traG	*traD/traJ*	*stbA*			
traH	*traD/virB6*	*stbB*			
traI	*traE/virB8*	*stbC*			
traJ	*traF/virB10*	*vagC*			
traK	*traG/virB11*	*vagD*			
traL	*traL/virB1*				

traM	*trbB*
traN	*trbC*
traQ	*trbE*
traR	*trbI*
traU	*trbJ*
traV	*trbL*
traW	*virB4*
traX	*virB8*
traY	*virB9*

Time course of pKpQ-GFP conjugation used to select time of incubation for experiments in this report. Conjugation was measured at hours 1, 2, 4, 8, 12, 16, and 20 between Kpn1760CT_pKpQGFP and Kpn223 in broth culture incubated at 37°C with no agitation. No transconjugants were observed at hours 1 or 2. Kpn223 transconjugants were observed after 4 hours of incubation, and the conjugation frequency was calculated as $2.1 \times 10^{-6} \pm 9.2 \times 10^{-7}$ compared to $6.4 \times 10^{-2} \pm 6.8 \times 10^{-3}$ at 20 h. Conjugation experiments were conducted at hour 4 between Kpn1760CT_pKpQGFP and Kpn731, Kpn808, and *E. coli* XL-1 and no transconjugants were detected (n=3). Based on these data and similar published studies, we selected 20 h as the incubation time for all experiments.

Supplemental Table S1. Conjugation rates of plasmids from KPNIH1760 and transformed isogenic strains to Kpn223 and XL-1

Donor	Recipient	
	Kpn223	XL-1
KPNIH1760	$3.5 \pm 0.9 \times 10^{-2}$[a]	$3.2 \pm 1.3 \times 10^{-4}$
Kpn1760CT_pKpQGFP	$5.6 \pm 0.6 \times 10^{-2}$	$3.8 \pm 0.8 \times 10^{-3}$
Kpn1760CT_pKpQIL	$4.7 \pm 0.7 \times 10^{-2}$	$1.1 \pm 0.3 \times 10^{-3}$

[a]Avg conjugation frequencies (CFU transconjugants / CFU recipients) ± SEM.

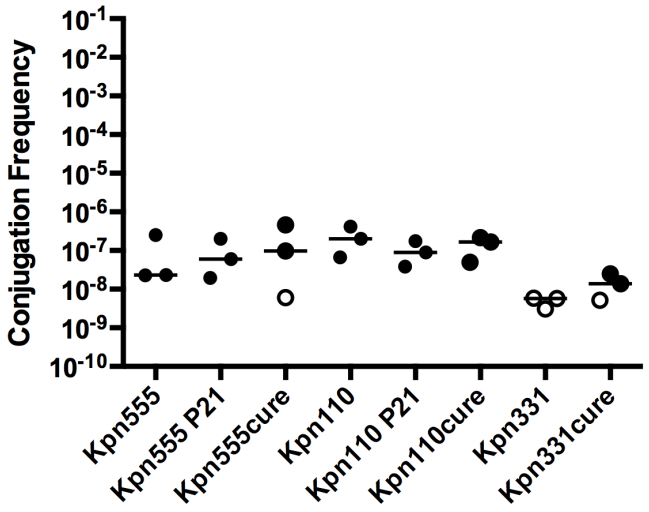
Supplemental Table S2. Plasmid and antibiotic susceptibility profiles of CAV1193 isogenic strains

Strain	CAV1193	C1193_KPCCURE	C1193_GentCURE	C1193_CURE	C1193CT_pKpQGFP
Susceptibility	$Mero^R$, $Gent^R$	$Mero^S$, $Gent^R$	$Mero^R$, $Gent^S$	$Mero^S$, $Gent^S$	$Mero^R$, $Gent^S$
***bla*$_{KPC}$ PCR**	Positive	Negative	Positive	Negative	Positive
Passage #	N/A	22	8	57	57
Plasmid Presence	pKPC_CAV1193		pKPC_CAV1193		
	pCAV1193-3741	pCAV1193-3741		pCAV1193-3741	pCAV1193-3741
	pCAV1193-78	pCAV1193-78			
	pCAV1193-166	pCAV1193-166			
	pCAV1193-258	pCAV1193-258		pCAV1193-258	pCAV1193-258

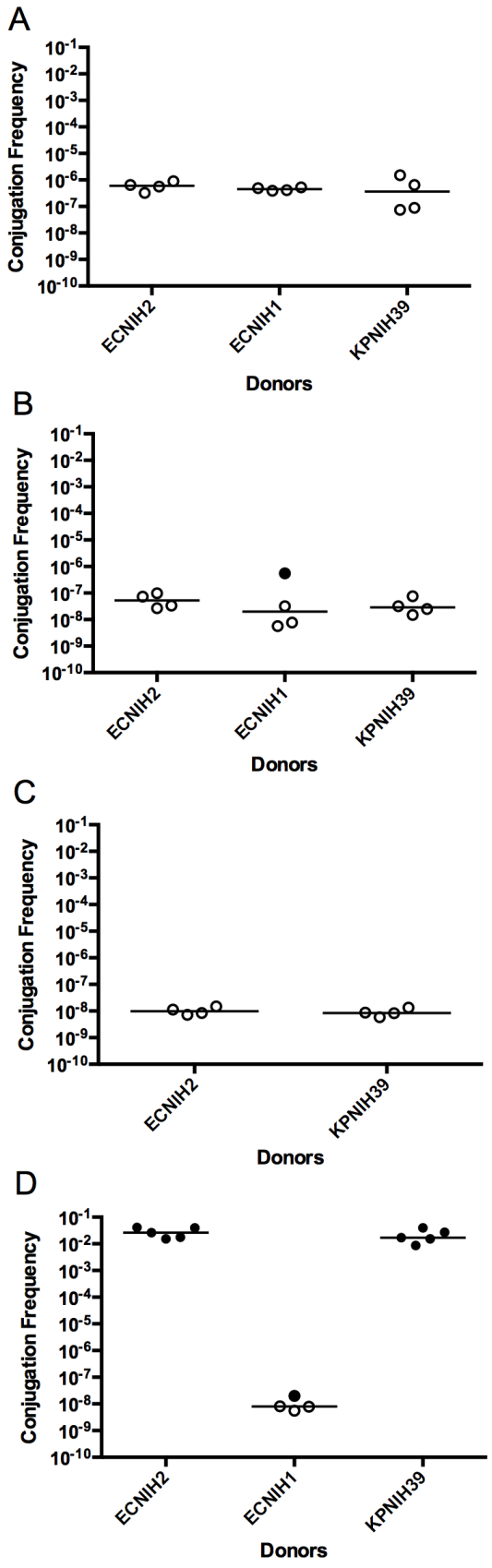
Supplemental Table S3. MIC data of selected transconjugants following *in vitro* mating

	Kpn223	Kpn223 TC[a] pKpQ-GFP	Kpn731	Kpn731 TC pKpQ-GFP	Kpn1760CT_ pKpQ-GFP
Amikacin	≤16	≤16	≤16	≤16	32
Amoxicillin/Clavulanate	≤8	>16	≤8	>16	>16
Ampicillin	>16	>16	>16	>16	>16
Aztreonam	≤4	>8	≤4	>8	>8
Cefazolin	≤2	>4	≤2	>4	>4
Cefepime	≤8	>16	≤8 I	16 R	
Cefotaxime	≤1	64	≤1	16	64
Cefoxitin	16	>16	16	>16	>16
Ceftaroline	≤0.25	>2	≤0.25	>2	>2
Ceftazidime	≤2	64	≤2	128	>128
Ceftriaxone	≤1	>32	≤1	>32	>32
Chloramphenicol		>16		≤8	16
Ciprofloxacin	>2	>2	≤1	≤1	>2
Colistin		≤2		≤2	>4
Doripenem	≤1	>4	≤1	2	>4
Doxycline	>8	>8	>8	>8	
Ertapenem	≤0.5	>4	≤0.5	>4	>4
Gentamicin	>8	>8	≤4	≤4	≤4
Imipenem	≤1	>4	≤1	>4	>4
Levofloxacin	≤2	≤2	≤2	≤2	>4
Meropenem	≤1	>8	≤1	4	>8
Piperacillin	≤16	>64	≤16	>64	>64
Piperacillin/Tazobactam	≤16	>64	≤16	>64	>64
Tigecycline	≤0.5	≤0.5	≤0.5	≤0.5	1
Tobramycin	8	≤4	≤4	≤4	>8
Trimethoprim/sulfamethoxazole	>2	>2	≤2	≤2	>2

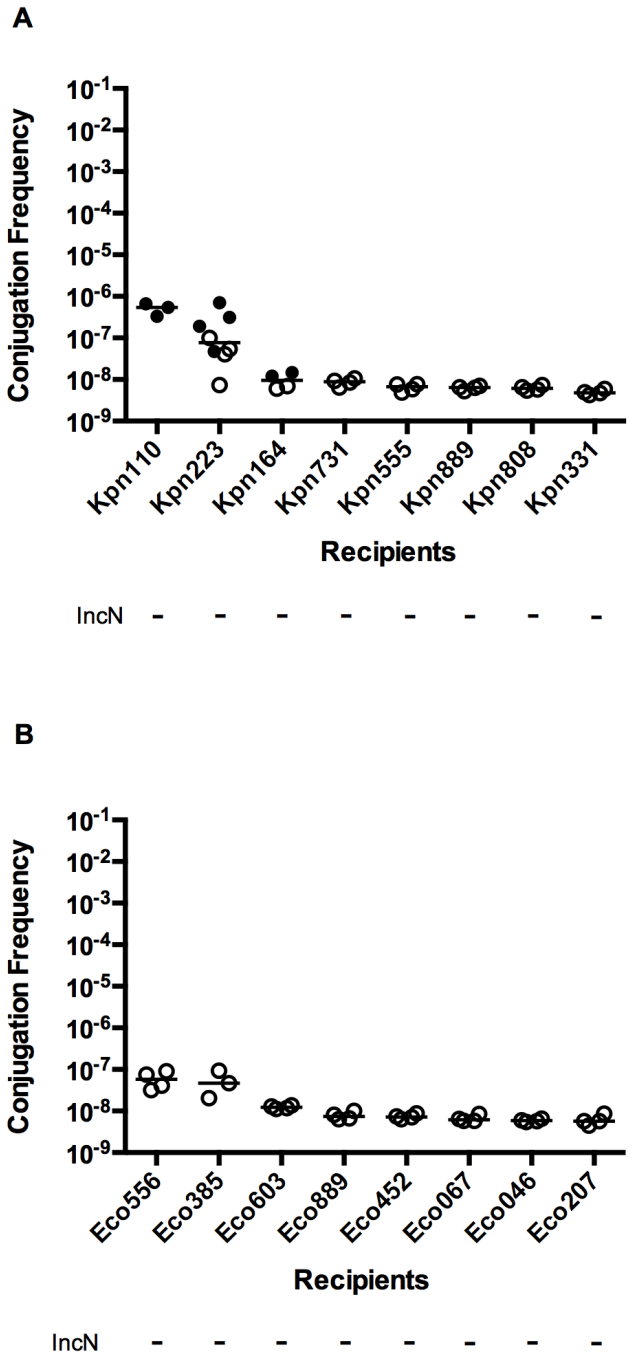
[a]TC = Transconjugant.



Supplemental Figure 1. Effect of recipient native plasmid content on pKpQ-GFP conjugation frequency. Donor is Kpn1760CT_pKpQGFP. Recipients are Kpn555, Kpn110, Kpn331 and their derivatives cured of their IncFII plasmid: Kpn555cure, Kpn110cure, and Kpn331cure. Kpn555 P21 and Kpn110 P21 are controls that were passaged at elevated temperature like cured strains, but retained IncFII. All recipients except for Kpn331 were transformed with the gentamicin resistant plasmid pRU1103 required for selection following conjugation. pRU1103 was a gift from Philip Pool (Addgene plasmid #14467) (4). Conjugations performed for 20 h in broth culture at 37°C. Conjugation frequency given by (CFU transconjugants / CFU recipients). Bar indicates median. Solid circles: conjugation frequency; open circles: no transconjugants observed at the limit of detection. n = 3.


A
Conjugation Frequency
10-1
10-2
10-3
10-4
10-5
10-6
10-7
10-8
10-9
10-10
ECNIH2
ECNIH1
KPNIH39
Donors
B
Conjugation Frequency
10-1
10-2
10-3
10-4
10-5
10-6
10-7
10-8
10-9
10-10
ECNIH2
ECNIH1
KPNIH39
Donors
C
Conjugation Frequency
10-1
10-2
10-3
10-4
10-5
10-6
10-7
10-8
10-9
10-10
ECNIH2
KPNIH39
Donors
D
Conjugation Frequency
10-1
10-2
10-3
10-4
10-5
10-6
10-7
10-8
10-9
10-10
ECNIH2
ECNIH1
KPNIH39
Donors

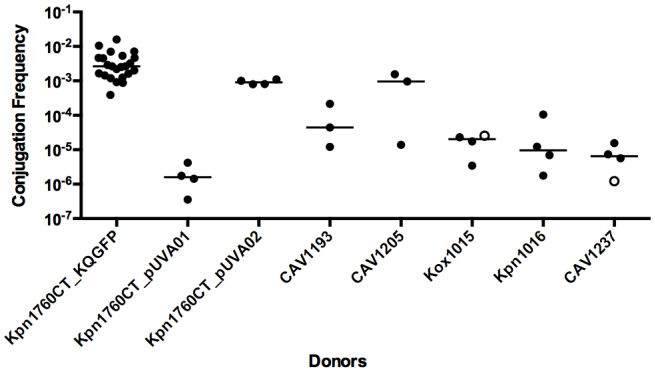
Supplemental Figure 2. Effect of donor species on *bla*$_{KPC}$-plasmid conjugation frequency in broth culture. Donors are listed on the x-axis, and recipients are Eco556 (A), Eco889 (B), ECNIH2CURE (C), and Kpn223 (D). Broth culture conjugations performed for 20 h. Conjugation frequency given by (CFU transconjugants / CFU recipients). Kpn: *K. pneumonia*e and Eco: *E. coli*. Bar indicates median. Solid circles: conjugation frequency; open circles: no transconjugants observed at the limit of detection. n=4.



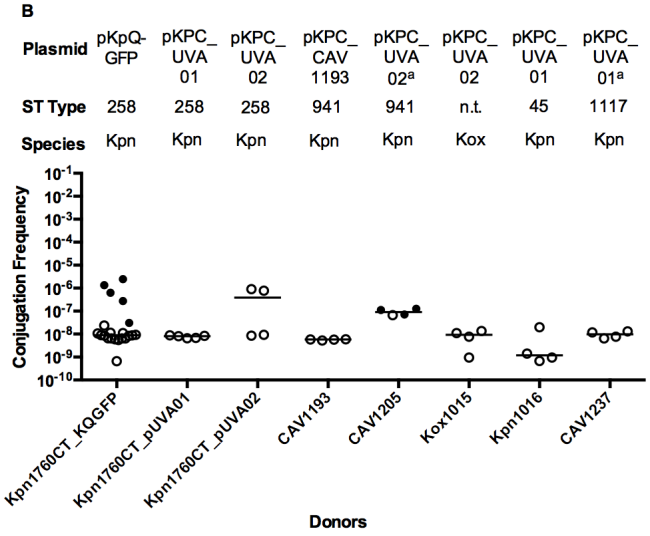
Supplemental Figure 3. Effect of recipient strain and species on p47e-GFP *in vitro* conjugation rates in broth culture. Donor is Kpn1760CT_p47eGFP; *K. pneumoniae* (A) or *E. coli* (B) recipients are listed on the x-axis. Broth culture matings performed for 20 h. Conjugation frequency given by (CFU transconjugants / CFU recipients). Replicon typing performed as

described. Bar indicates median. Solid circles: conjugation frequency; open circles: no transconjugants observed at the limit of detection. n≥3.

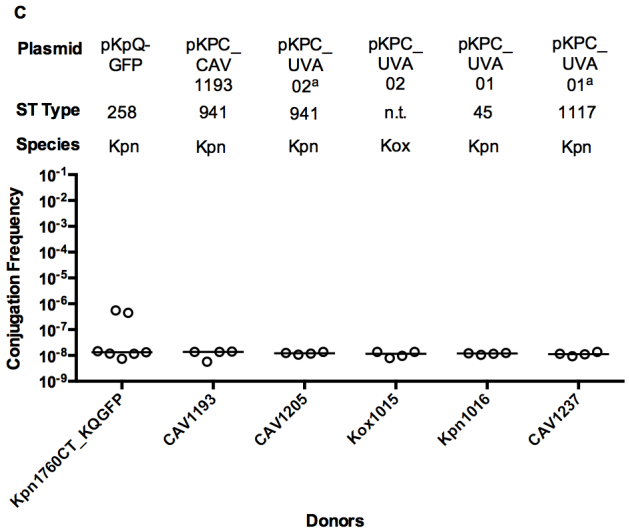
A


Plasmid
pKpQ-GFP
pKPC_UVA01
pKPC_UVA02
pKPC_CAV1193
pKPC_UVA02a
pKPC_UVA02
pKPC_UVA01
pKPC_UVA01a
ST Type
258
258
258
941
941
n.t.
45
1117
Species
Kpn
Kpn
Kpn
Kpn
Kpn
Kox
Kpn
Kpn
Conjugation Frequency
10-1
10-2
10-3
10-4
10-5
10-6
10-7
Kpn1760CT_KQGFP
Kpn1760CT_pUVA01
Kpn1760CT_pUVA02
CAV1193
CAV1205
Kox1015
Kpn1016
CAV1237
Donors

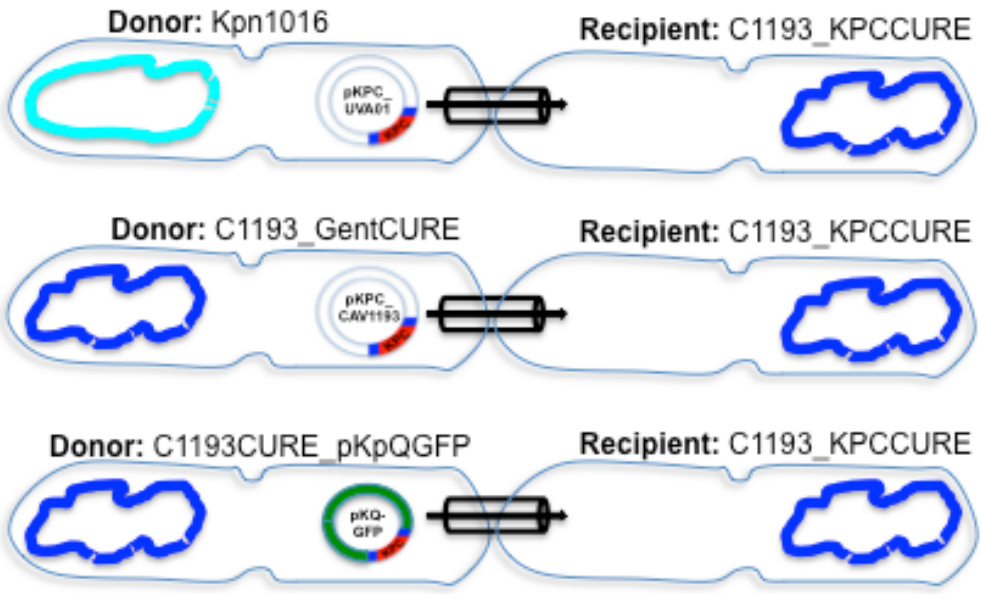
B


Plasmid
pKpQ-GFP
pKPC_UVA01
pKPC_UVA02
pKPC_CAV1193
pKPC_UVA02a
pKPC_UVA02
pKPC_UVA01
pKPC_UVA01a
ST Type
258
258
258
941
941
n.t.
45
1117
Species
Kpn
Kpn
Kpn
Kpn
Kpn
Kox
Kpn
Kpn
Conjugation Frequency
10-1
10-2
10-3
10-4
10-5
10-6
10-7
10-8
10-9
10-10
Kpn1760CT_KQGFP
Kpn1760CT_pUVA01
Kpn1760CT_pUVA02
CAV1193
CAV1205
Kox1015
Kpn1016
CAV1237
Donors

C


Plasmid
pKpQ-GFP
pKPC_CAV1193
pKPC_UVA02a
pKPC_UVA02
pKPC_UVA01
pKPC_UVA01a
ST Type
258
941
941
n.t.
45
1117
Species
Kpn
Kpn
Kpn
Kox
Kpn
Kpn
Conjugation Frequency
10-1
10-2
10-3
10-4
10-5
10-6
10-7
10-8
10-9
Kpn1760CT_KQGFP
CAV1193
CAV1205
Kox1015
Kpn1016
CAV1237
Donors

Supplemental Figure 4. Conjugation ability of Enterobacteriaceae isolates containing pKPC_UVA01, pKPC_CAV1193, or pKPC_UVA02 to Enterobacteriaceae recipients. Donors are indicated on the x-axis; recipients are XL-1 (A), Kpn555 (B), and Eco385 (C). Broth culture conjugations performed for 20 h. Conjugation frequency given by (CFU transconjugants / CFU recipients). Kpn: *K. pneumonia*e and Eco: *E. coli*. Bar indicates median. Solid circles: conjugation frequency; open circles: no transconjugants observed at the limit of detection. n≥3. [a]As determined by whole genome sequencing on the Illumina HiSeq platform and long-range PCR (5).



Supplemental Figure 5. Diagram of CAV1193 isogenic strains and Kpn1016 used in the self-transfer experiment. Kpn: *K. pneumoniae*.

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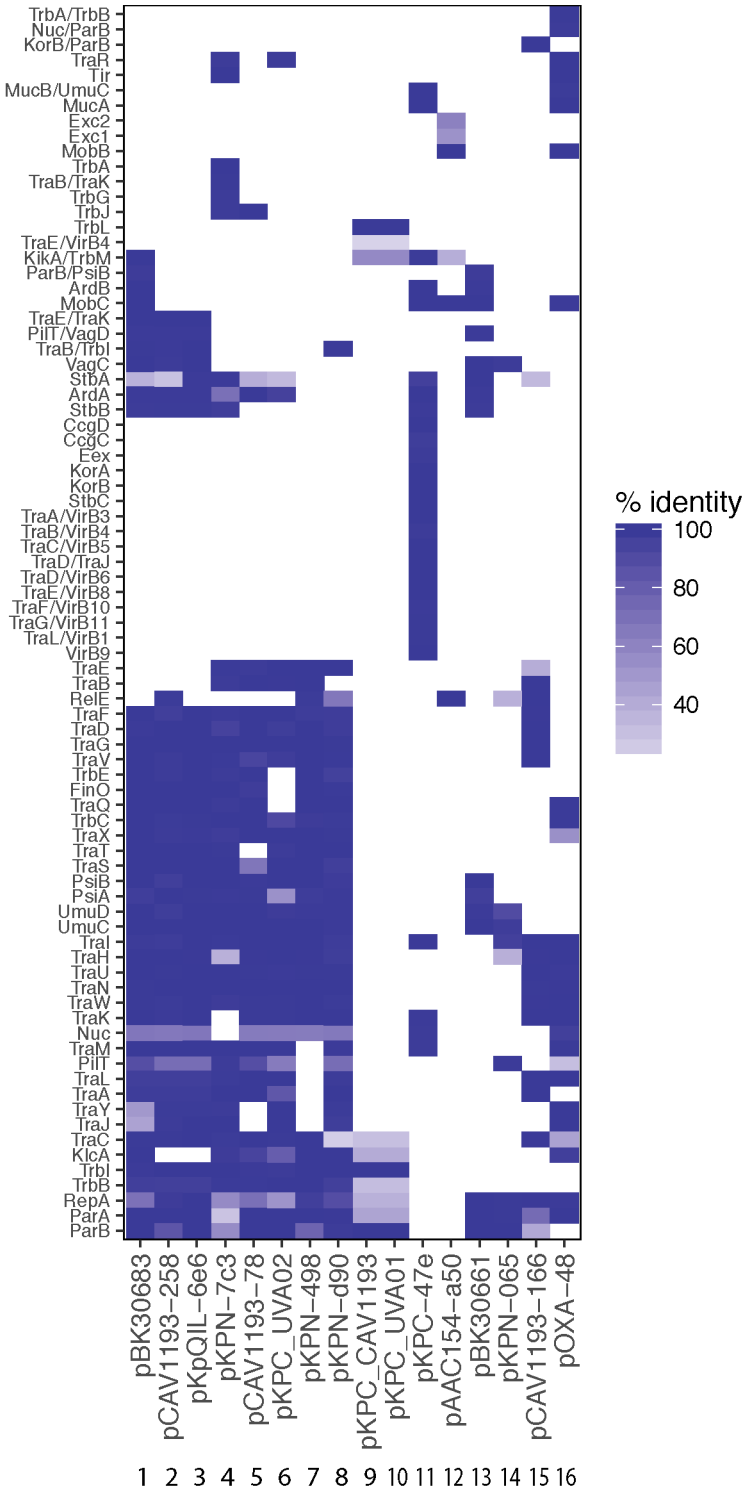

Supplemental Figure 6. Heat map of genes putatively associated with plasmid transfer and other plasmid-related functions. Numbers 1, 3, 6, 9-11, 13, 16: carbapenemase containing plasmids. *denotes referenced plasmids from (1, 2, 6). Number 14: plasmid of Kpn223, a strain used as a conjugation recipient that efficiently accepted pKpQ-GFP. Number 4, 8: plasmids of Kpn555, a strain greatly attenuated as a recipient for pKpQ-GFP via conjugation. Numbers 2, 5, 7, 12, 15: additional plasmids identified in conjugation donors. Note: CAV1193-3741 is a small 3.7 kb plasmid and is not predicted to contain any proteins from our list. Plasmid-Strain associations are listed in Table 1. Kpn: *K. pneumoniae*. Plasmid sequences are arranged on the x-axis labeled by plasmid name. Genes are arranged on the y-axis. For every gene that was found on the plasmid (>50% coverage and E-value < 1e-5), we plotted the identity level for the best alignment between plasmid-encoded gene and database representative. Before plotting, genes and plasmids were clustered using hierarchical clustering in R (hclust) using Ward's method. Transposon insertion in pKPC_CAV1193 in the TrbI gene compared to pKPC_UVA01 is not seen as a disrupted protein in this heatmap, as the criteria for protein match were still met despite the disruption of the sequence (7).

References:

1. **Chen L, Chavda KD, Melano RG, Hong T, Rojtman AD, Jacobs MR, Bonomo RA, Kreiswirth BN.** 2014. Molecular survey of the dissemination of two *bla*KPC-harboring IncFIA plasmids in New Jersey and New York hospitals. Antimicrobial agents and chemotherapy **58:**2289-2294.
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5. **Mathers AJ, Stoesser N, Sheppard AE, Pankhurst L, Giess A, Yeh AJ, Didelot X, Turner SD, Sebra R, Kasarskis A, Peto T, Crook D, Sifri CD.** 2015. Klebsiella pneumoniae Carbapenemase (KPC)-Producing *K. pneumoniae* at a Single Institution: Insights into Endemicity from Whole-Genome Sequencing. Antimicrobial agents and chemotherapy **59:**1656-1663.
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