Supplementary Information for

Kpi, a chaperone-usher pili system associated with the worldwide-disseminated high-risk clone *Klebsiella pneumoniae* ST-15.

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www.pnas.org/cgi/doi/10.1073/pnas.1921393117

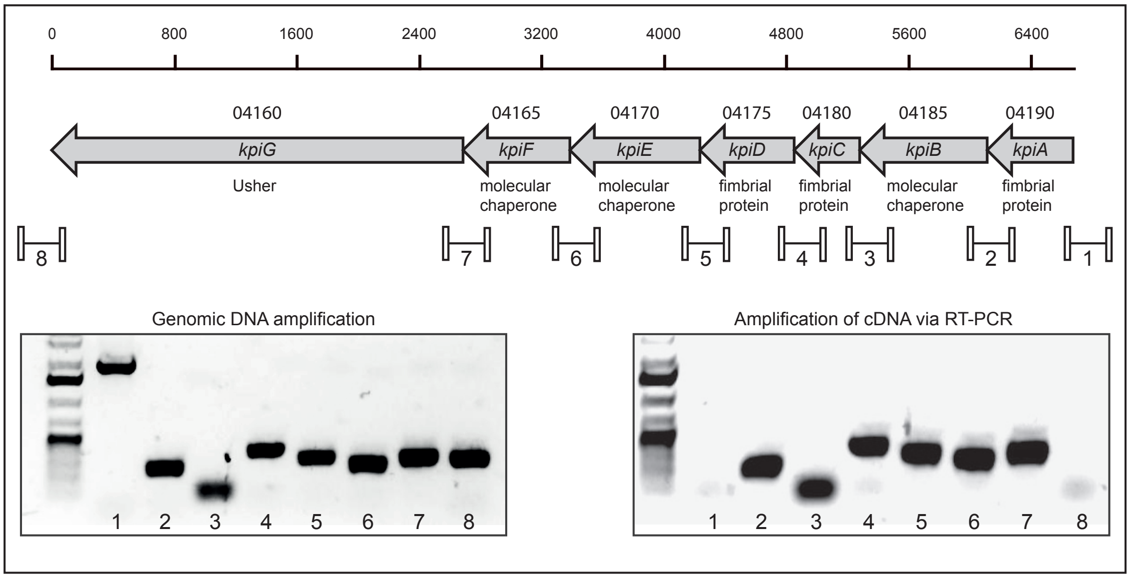
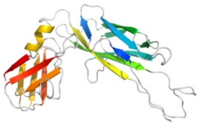
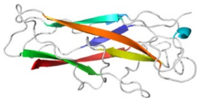
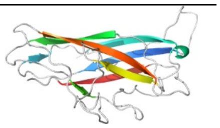
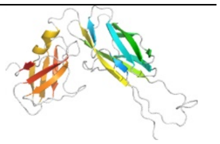
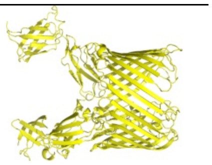


Fig. S1. Genetic organization of the *kpiABCDEFG* operon. Arrows indicate the location and direction of gene transcription. The numbers above the arrows represent the genes located within this operon. The connected open rectangles below the arrows indicate the regions amplified in PCR and RT-PCR assays using genomic DNA and DNA-free total RNA, respectively. Agarose gel electrophoresis revealed the results of genomic DNA amplification (left) and cDNA amplification by RT-PCR (right). The intergenic regions between genes within the operon are shown in lanes 2, 3, 4 5, 6 and 7. Intergenic regions between the upstream gene and *kpiA* gene and between the downstream gene and *kpiG* are shown in lines 1 and 8, respectively

Table S1. Protein structure modeling of the CUP system *kpiABCDEFG* in *K. pneumoniae* using RaptorX**.**

Operon Components	Ranking	Score[#]	% Sequence Identity[##]	PDB code	Description	Model
KpiA (Fimbriae)[###]	---	---	---	No structure	---	---
KpiB (Chaperone)	1	150/243	24	5ghu	Fimbrial chaperone YadV from *E. coli*	
	2	160/243	24	4djm	Fimbrial chaperone DraB from *E. coli*	
	3	161/243	23	2co6	Type 1 chaperone from *Salmonella enterica*	
KpiC (Fimbriae)	1	128/163	16	5lp9	Type 1 pilus subunit FimA from *Shigella flexneri*	
	2	119/163	20	6erj	Type 1 pilus subunit FimA from *Salmonella paratyphi*	
	3	104/163	14	2jty	Type 1 pilus main subunit from *E. coli*	
KpiD (Fimbriae)	1	115/179	15	5lp9	Type 1 pilus subunit FimA from *Shigella flexneri*	
	2	104/179	15	6erj	Type 1 pilus subunit FimA from *Salmonella paratyphi*	
	3	110/179	14	5afo	Type 1 fimbriae adhesin LpfD from *E. coli*	
KpiE (Chaperone)	1	157/264	26	3rfz	Chaperone protein FimC from *E. coli*	
	2	145/264	25	4djm	Fimbrial chaperone DraB from *E. coli*	
	3	150/264	24	2co6	Type 1 chaperone from *Salmonella enterica*	
KpiF (Chaperone)	1	155/242	25	5ghu	Fimbrial chaperone YadV from *E. coli*	
	2	144/242	27	4djm	Fimbrial chaperone DraB from *E. coli*	
	3	144/242	27	2co6	Type 1 chaperone from *Salmonella enterica*	
KpiG (Usher)	1	668/864	34	3rfz	Type 1, Outer membrane usher protein from *E. coli*	

[#] Score (alignment length *vs* sequence length); [##] number of identical residues multiplied by 100; [###]KpiA was not structurally related to any crystalized protein.

Table S2. Set of usher proteins for the alignment and phylogenetic classification of KpiG.

Fimbrial clade	Usher	Usher Accession (NCBI Entrez)	Organism
γ1	AtfC	CAB01714.1	*Proteus mirabilis* HI4320
γ1	AufC	AAN82650.1	*Escherichia coli* CFT073
γ1	BcfC	AAL18987.1	*Salmonella enterica* Serotype Typhimurium LT2
γ1	ECs0594	BAB34017.1	Escherichia coli 0157:H7 str. Sakai
γ1	FimD	CAD56973.1	*Citrobacter freundii* 3009
γ1	FimD	AAC77273.1	*Escherichia coli* K12
γ1	FimD	AAL19500.1	*Salmonella enterica* Serotype Typhimurium LT2
γ1	FmlC	ABE07195.1	*Escherichia coli* UTI89
γ1	FocD	AAN79699.1	*Escherichia coli* CFT073
γ1	SfaF	CAC16953.2	*Escherichia coli* 536
γ1	SfmD	AAC73634.1	*Escherichia coli* K12
γ1	YcbS	AAC74026.1	*Escherichia coli* K12
γ1	YdeT	AAC74578.1	*Escherichia coli* K12
γ1#	ECs4022	BAB37445.1	*Escherichia coli* 0157:H7 str. Sakai
γ1#	ECs4667	BAB38090.1	*Escherichia coli* 0157:H7 str. Sakai
γ1#	LpfC	AAY18078.1	*Escherichia coli* 789
γ1#	LpfC	AAL18163.1	*Escherichia coli* EH41
γ1#	LpfC	AAL22498.1	*Salmonella enterica* Serotype Typhimurium LT2
γ1#	PhfD	CAE13290.1	*Photorhabdus luminescens* subsp. laumondii TTO1
γ1#	plu2157	CAE14450.1	*Photorhabdus luminescens* subsp. laumondii TTO1
γ1#	StgC	AAS99231.1	Escherichia coli X7122
γ1#	SthB	AAL23408.1	*Salmonella enterica* Serotype Typhimurium LT2
γ1#	StiC	AAL19139.1	*Salmonella enterica* Serotype Typhimurium LT2
γ1#	YraJ	AAC76178.1	*Escherichia coli* K12
γ2	CswD (CS12)	AAK09050.1	*Escherichia coli* 350C1
γ2	ECP_3814	ABG71785.1	*Escherichia coli* 536
γ2	FasD (987P)	AAB02687.1	*Escherichia coli* 987
γ2	FotD (CS18)	AAO73849.1	*Escherichia coli* ARG-2
γ2	plu0268	CAE12563.1	*Photorhabdus luminescens* subsp. laumondii TTO1
γ2	plu0784	CAE13079.1	*Photorhabdus luminescens* subsp. laumondii TTO1
γ2	plu0791	CAE13086.1	*Photorhabdus luminescens* subsp. laumondii TTO1
γ3	AfaC-3	CAA54117.1	*Escherichia coli* A30
γ3	AfaC-7	AAD44025.2	*Escherichia coli* 262 KH 89
γ3	AfaC-8	AAD44022.2	*Escherichia coli* 239 KH 89
γ3	Agg3C	AAM88296.1	*Escherichia coli* 55989
γ3	AggC	AAA57452.1	*Escherichia coli* 17-2
γ3	Caf1A	CAB55265.1	*Yersinia pestis* CO92
γ3	CS3-2	P15484	*Escherichia coli* PB176
γ3	CssD (CS6)	AAB51364.1	*Escherichia coli* E8775
γ3	DraC	AAK16477.1	*Escherichia coli* IH11128
γ3	HdaC	BAF33889.1	*Escherichia coli* O86:H-

γ3	MyfC	CAA79953.1	*Yersinia enterocolitica*
γ3	PsaC	CAL19958.1	*Yersinia pestis* CO92
γ3	PSPPH_A0064	AAZ38018.1	*Pseudomonas syringae* pv. phaseolicola 1448A
γ3	SafC	AAL19258.1	*Salmonella enterica* Serotype Typhimurium LT2
γ3	SefC	AAV80035.1	*Salmonella enterica* Serotype Paratyphi A ATCC 9150

γ1#, γ1-Fimbriae related

Table S3. Bacterial strains and plasmids used in the present study.

Strains and plasmids	Relevant characteristics	Reference or Source
***K. pneumoniae* strains**		
MGH 78578	ATCC 700721 strain showing a highly adherent phenotype	ATCC
ATCC 10031	Susceptible strain with no-adherent phenotype	ATCC
Kp09107	Clinical isolate with a no-adherent phenotype	This study
Kp727	Clinical isolate with a no-adherent phenotype	This study
Kp924	Clinical isolate with a no-adherent phenotype	This study
Kp1278	Clinical isolate with a no-adherent phenotype	This study
Kp3380	Clinical isolate with a highly adherent phenotype.	This study
Kp3380Δ*kpiD*	*KpiD*-defective Kp3380 mutant strain	This study
Kp3380Δ*kpiD_C*	Kp3380Δ*KpiD* strain harboring pUC_*kpiD*	This study
E. coli		
TG1	Host strain used for cloning procedures	Invitrogen
Plasmids		
pIJ773	Template vector for apramycin gene amplification	(1)
pACBSR-hyg	Plasmid (hygromycin) containing an arabinose-inducible λ-Red recombinase	(1)

pFLP-hyg	Plasmid (hygromycin) containing a heat-shock inducible FLP recombinase and a p15A replicon	(1)
pWH1266	Cloning vector (tetracycline)	ATCC
pUCP24	Cloning vector (gentamicin)	(2)
pUCP24/T	Modified pUCP24 expression vector (tetracycline promoter)	This study
pUC_*kpiD*	pUCP24/T containing the *kpiD* gene	This study

Table S4. Oligonucleotides used in the present work.

Primer /Probe name	sequence	Use in the present study
KO_*kpiD*_fow	ctggttggaggctcctgtcagatttcagtggatcggcc cagtattacattttcgcctgtgattccggggatccgtcgacc	Construction of Kp3380Δ*kpiD*
KO_*kpiD*_rev	aaccacagtataaatctgcccgcagcttgtgcgaataca ttcatttttatcgccaattcatgtaggctggagctgcttc	Construction of Kp3380Δ*kpiD*
ConfKO_ *kpiD* _fow	ggaattgaaagtgtgacccc	Testing of *kpiD* gene deletion
ConfKO_ *kpiD* _rev	ctttatcgtcgcctgtgtatc	Testing of *kpiD* gene deletion
BamHI_pTET_Fw	cccggatcctactcttccttttcaatattatt	Cloning the tetracycline promoter into pUCP24 plasmid
SmaI_pTET_Rv	ggcccgggccgcaagaggcccgg	Cloning the tetracycline promoter into pUCP24 plasmid
*kpiD*_cloning_Fw	atgcattacaatgaacggacccgtt	Cloning of *kpiD* gene into the pUCP24/T plasmid for complementation.
SacI_ *kpiD*_cloning_Rv	cccgagctctcattcataaataaactgaagcgtc	Cloning of the *kpiD* gene into the pUCP24/T plasmid for complementation.
pUCp24_check_Fw	gcctgcctttcaggctgcgcaactgtt	Testing of the plasmid construction pUCP24_ *kpiD* for complementation.

pUCp24_check_Rv	tggcacgacaggtttcccgactgga	Testing of the plasmid pUCP24_ *kpiD* for complementation.
Kp_C5G826_Fow	cagggcccggtatccagt	Reverse transcription.
Kp_C5G825_Rev	catgctgacaccagaaaggc	Reverse transcription.
Kp_C5G825_Fow	ccactgacgctacgccgg	Reverse transcription.
Kp_C5G824_Rev	ctctggcatcatcactgctc	Reverse transcription.
Kp_C5G824_Fow	ttacattacgctcgccgatcttaaggtggc	Reverse transcription.
Kp_C5G823_Rev	atgataccaacacgcaggcgagcgcca	Reverse transcription.
Kp_C5G823_Fow	caaagatgtcggtgtcggg	Reverse transcription.
Kp_C5G822_Rev	tgcctgacaacataaagccg	Reverse transcription.
Kp_C5G822_Fow	taatatggataagctaattgtcggtactg	Reverse transcription.
Kp_C5G821_Rev	aatataacgcgtgtctgactgagct	Reverse transcription.
Kp_C5G821_Fow	ggtaagagcatggatgtgaaaggga	Reverse transcription.
Kp_C5G820_Rev	attaccagtcgacccatatgatggc	Reverse transcription.
Kp_C5G820_Fow	aaaaaacgcgctttcaaaaatgatac	Reverse transcription.

Kp_C5G819_Rev	gaaatcccggatcaaaataatcacc	Reverse transcription.
Kp_C5G819_Fow	gtcagtgccgtgtcagttttgacat	Reverse transcription.
Kp_C5G818_Rev	ctgcagttttctgagaaacgcttcc	Reverse transcription.
Kp04160 Fow 53	gcagttgccacgttactgaa	qRT-PCR
Kp04160 Rev 53	atcacccacgatgctgcta	qRT-PCR
Kp820 Fow 141	gctcagtcagacacgcgtta	qRT-PCR
Kp820 Rev 141	acctggccgctgttattct	qRT-PCR
Kp04175 Fow 92	atttttcagccacgctggt	qRT-PCR
Kp04175 Rev 92	gtaatactgggccgatccac	qRT-PCR
Kp822-823 Fow 152	ctaccgtggcgatgttacct	qRT-PCR
Kp822-823 Rev 152	gacatctttggcgttgtcg	qRT-PCR
Kp824 Fow 122	gtcggcgtaacaagttcagtc	qRT-PCR
Kp824 Rev 122	ccttcgatatcccgtgagac	qRT-PCR
Kp825 Fow 102	ccgttggctttatgattaagga	qRT-PCR
Kp825 Rev 102	tcacatccaaagccacattc	qRT-PCR
PreChapFimC Fow 2	tgagcctgaaaaccatacctg	qRT-PCR
PreChapFimC Rev 2	ttgcatagccagcgtcaa	qRT-PCR

References

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2. Pérez A, et al. (2012) Effect of Transcriptional Activators SoxS, RobA, and RamA on Expression of Multidrug Efflux Pump AcrAB-TolC in *Enterobacter cloacae*. *Antimicrob Agents Chemother* 56(12):6256–6266.