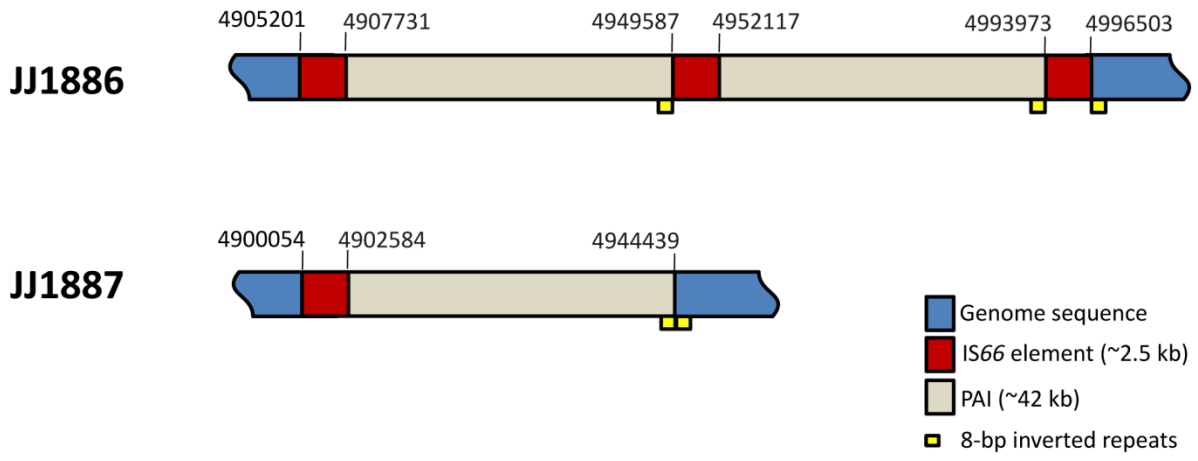

Figure S1. Schematic representation of pathogenicity-associated island (PAI) duplication in chromosome of JJ1886.

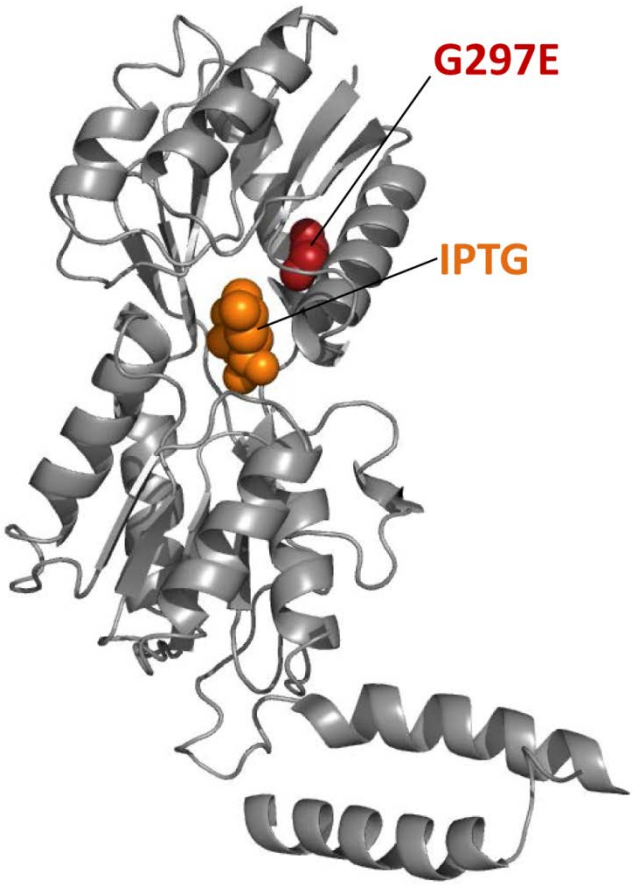

Figure S2. Distribution of structural mutations in the tertiary structure of *E. coli* LacI. The ribbon representation of the *E. coli* K12 LacI monomer as in a crystal structure 1LBH (PDB) (1). The position of G297 is shown as a red spheres. An inhibitor molecule of the LacI (isopropyl β-D-1-thiogalactopyranoside, IPTG) occupying the substrate-binding pocket is shown in orange.

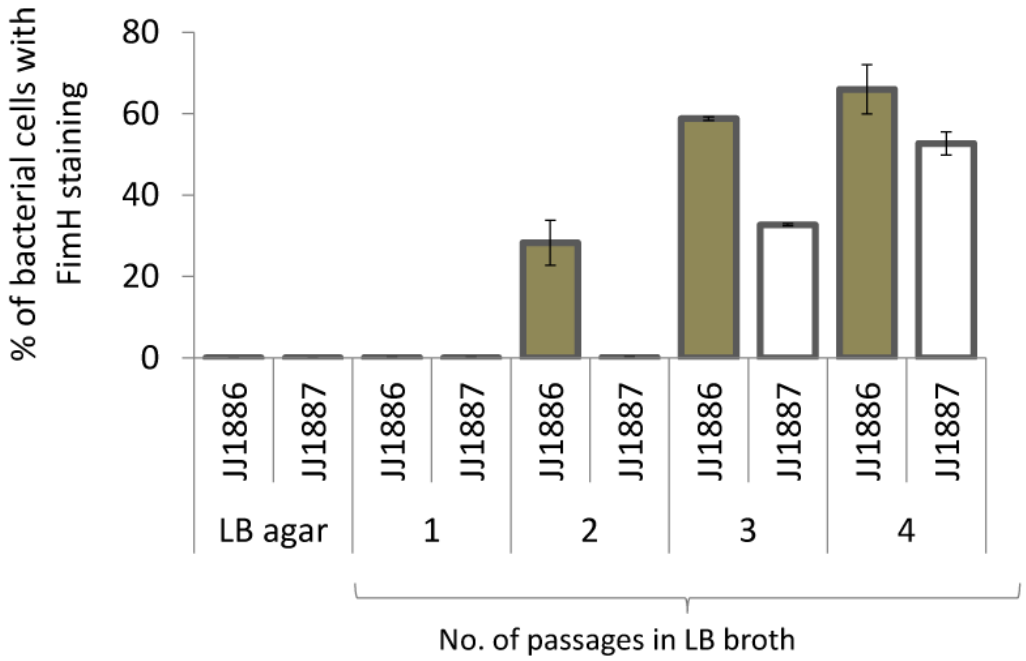

Figure S3. Flow cytometry analysis of type-1 fimbriae expression. JJ1886 and JJ1887 cells were fixed, then stained with an anti-FimH monoclonal antibody (mAb21), followed by a secondary Alexa Fluor488-conjugated anti-mouse antibody. For each sample, the fluorescence of 100, 000 events was measured using FACScan (Becton Dickinson). Data shown are mean ± SD for two biological replicates from one representative experiment.

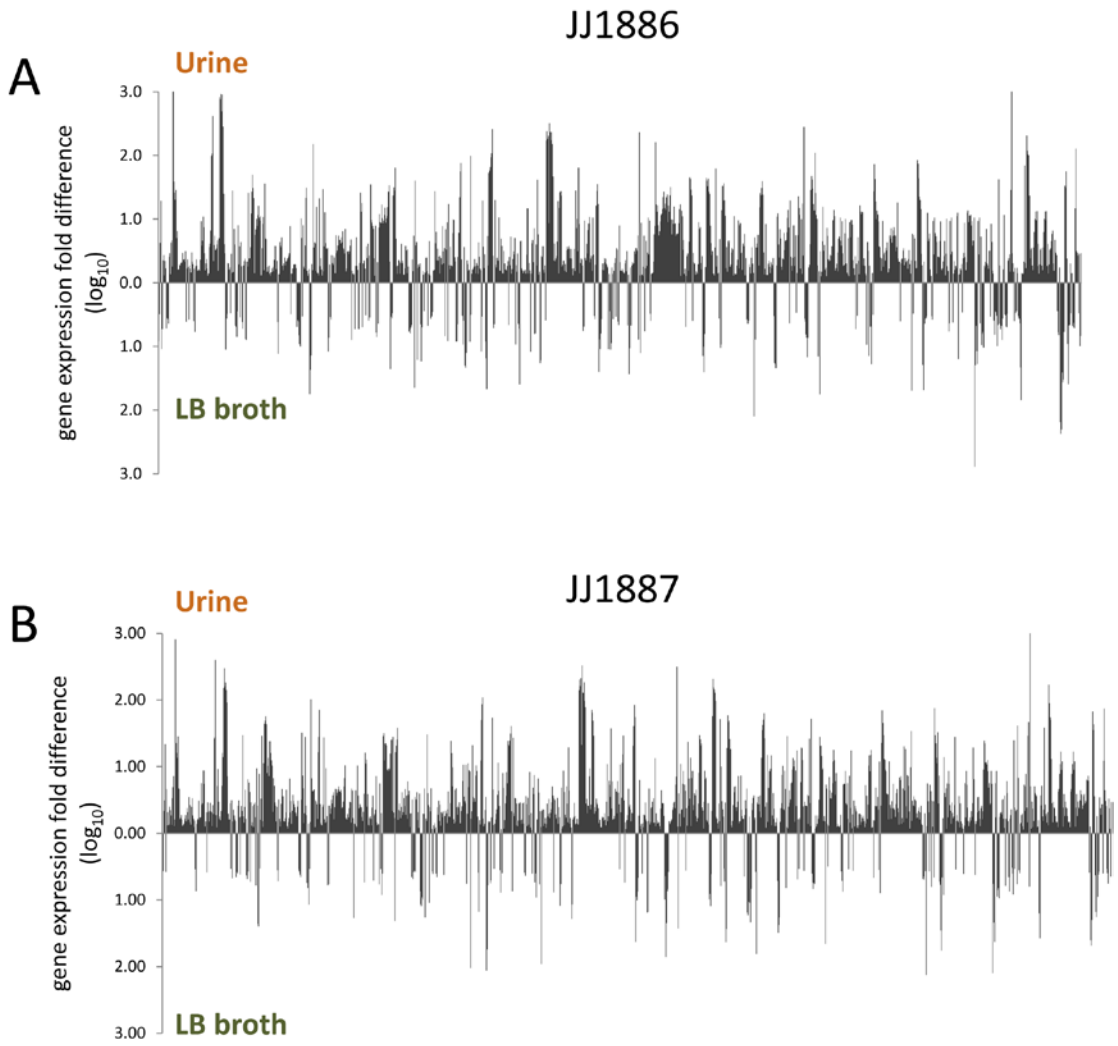

Figure S4. Differential gene expression of two sisters' strains under diverse growth conditions. Gene expression in bacteria grown in urine or LB broth was determined by whole-transcriptome analysis. The X-axis represents genes with differential expression in urine vs LB broth, ordered by chromosomal position. The Y-axis shows the log10 of the fold difference in gene expression level for growth in urine vs. LB broth. The upper Y-axis shows genes that are relatively upregulated in urine; the lower Y-axis shows genes that are relatively upregulated in LB broth. Data are shown only for annotated genes. Average data for three biological replicates are shown.

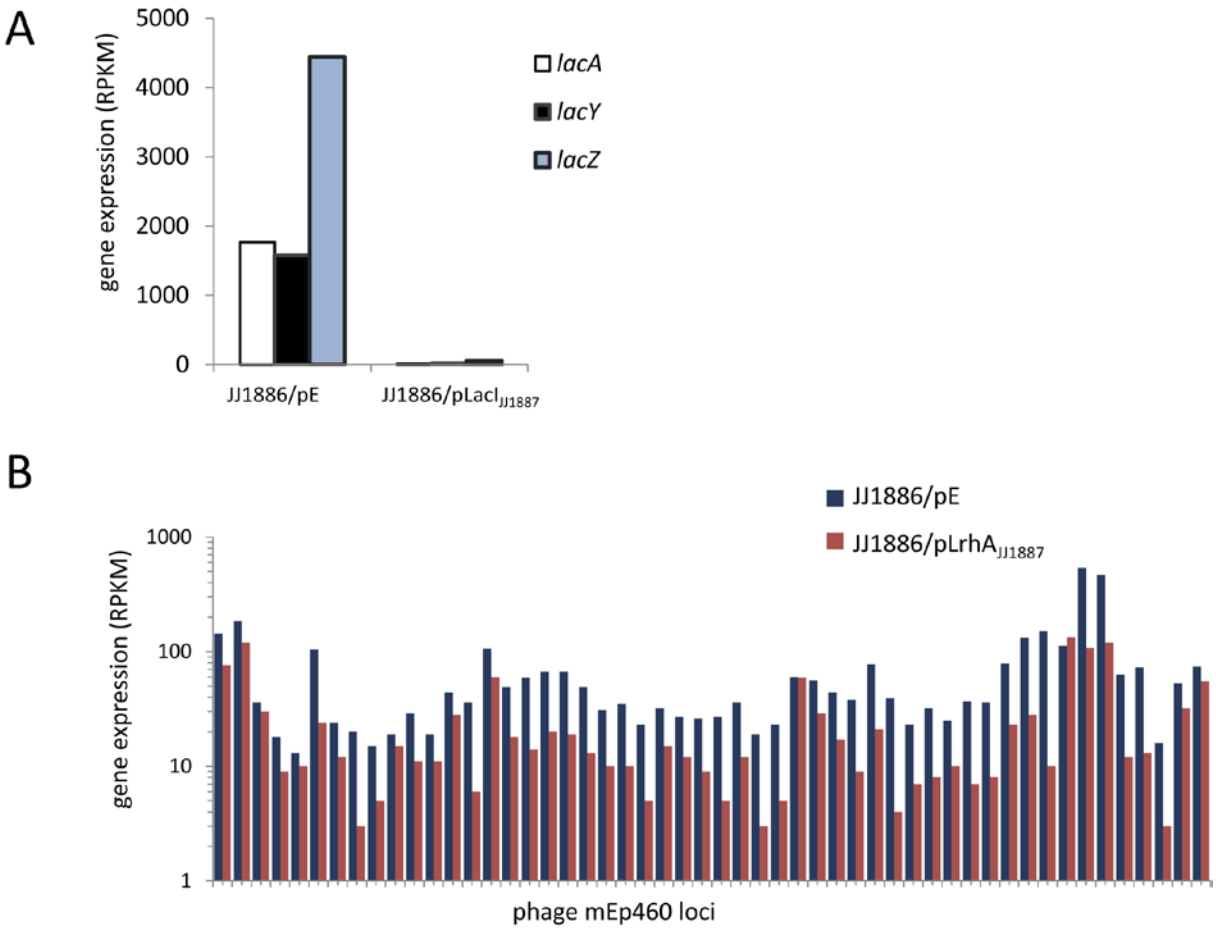

Figure S5. Differential expression of selected genes in JJ1886 transformants carrying pACYC184, pLacIJJ1887, and pLrhAJJ1887. Expression of lac operon genes (A) and mEp460 phage genes (B) in derivatives of JJ1886 transformed with an empty pACYC184 plasmid (pE; control) or pACYC184 carrying lacI$_{JJ1887}$ or lrhA$_{JJ1887}$. Gene expression levels in bacteria grown in urine, as determined by RNAseq, are shown as RPKM (reads per kilobase per million mapped reads). Average data for two biological replicates are shown.

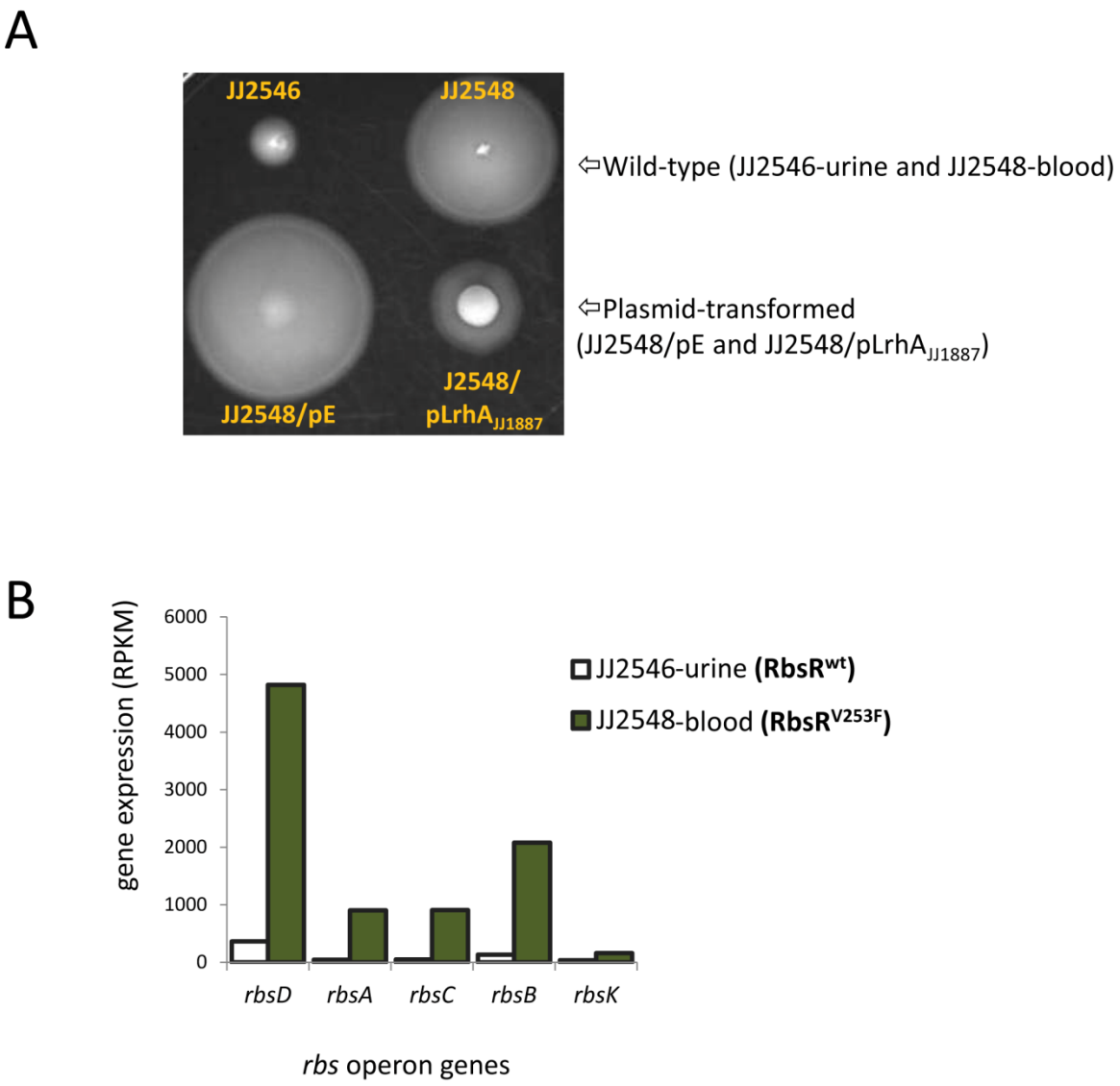

Figure S6. Functional validation of selected mutations detected in household #2 strains.

(A) Motility of wild-type strains (JJ2546 and JJ2548) and plasmid-complemented derivatives of strain JJ2548 (carrying pE or pLrhA$_{JJ1887}$), grown for 6h on LB 0.2 % agar. (B) Expression of rbs operon genes (as determined by RNAseq) in wild-type strains JJ2546 and JJ2548 grown in urine. Gene expression is shown as RPKM (reads per kilobase per million mapped reads). Average data for two biological replicates are shown.

Table S1. Frequency of rifampicin-resistant mutants in household #1 and #2 isolates.

Strain	Frequency of Rif^R mutants per 10^8 cells
MG1655	0.7
JJ1886	0.8
JJ1887	1.0
JJ2546	0.6
JJ2547	1.9
JJ2548	1.5
JJ2963	1.1
JJ2974	0.3

Average frequency of rifampicin-resistant (Rif^R) mutants were determined for at least two biological replicates.

Table S2. Differential expression of plasmid genes in strains JJ1886 and JJ1887.

Medium	Plasmid	Gene[a]	Product	Fold difference[b]
Urine	**110,040 bp** (pJJ1886_5 = pJJ1887_4)	P423_26110	endoribonuclease	7.7
		P423_25600	hypothetical protein	6.6
		P423_26105	dihydrodipicolinate synthase	8.7
		P423_25690	hypothetical protein	-3.4
		P423_25685	single-stranded DNA-binding protein	-2.9
		P423_25995	NADH:quinone oxidoreductase	-2.8
		P423_26115	2-keto-3-deoxygluconate permease	3.2
LB	**5,631 bp** (pJJ1886_3 = pJJ1887_3)	P423_25190	mobilization protein	2
	110,040 bp (pJJ1886_5 = pJJ1887_4)	P423_25600	hypothetical protein	6.8
		P423_25720	transposase Tn3	5.1
		P423_26145	insertion element protein InsB	4.3
		P423_25725	hypothetical protein	2.9
		P423_25955	conjugal transfer protein TraA	2.9
		P423_25675	hypothetical protein	2.5
		P423_25820	conjugal transfer protein TraI	2.4
		P423_25740	transporter	2
		P423_25815	conjugal transfer protein TraX	2

[a]NCBI gene number as in the JJ1886 genome.

[b]Gene upregulated in JJ1887 (positive values) and gene upregulated in JJ1886 (negative values).The fold difference was calculated from the ratio of the gene expression between strains. Only genes with difference ≥ 2-fold are shown.

Table S3. Intergenic SNPs in the household #2 strains.

Mutated position[a]	Mutation type	Region affected by mutation	Distance (nt) from mutated position to start codon	Reference or source
857299	G-T	promoter of *dps* (P423_04030)	127	(2)
1316931	C-T	promoter of *ycgJ* (P423_06490) encoding hypothetical protein	121	This study[b]
3425079	C-T	upstream region of P423_16705 encoding polysialic acid transporter	44	This study
4057275	C-A	5'UTR of *cspA* (P423_19795) involved in regulation of *cspA* transcription	74	(3)

[a]Chromosomal position as in JJ1886 genome.
[b]Promoter location of the mutation was predicted using the RegulonDB database (http://regulondb.ccg.unam.mx/).

Table S4. Bacterial strains and plasmids used in this study.

Name	Characteristics and/or use				Reference or source
Wild-type strains					
	Specimen	**Patient ID**	**Other**	**Year of isolation**	
JJ1887	urine	older sister (index patient), household #1		2007	(4)
JJ1886	blood	younger sister, household #1		2007	(4)
JJ2546	urine	father (index patient), household #2		2008	(5)
JJ2547	abscess	daughter, household #2		2008	(5)
JJ2548	blood	daughter, household #2		2008	(5)
JJ2963	urine	mother, household #2		2014	(5)
JJ2974	feces	mother, household #2		2014	(5)
MVAST2639	urine	1173	LrhA mutant (R17G)	2014	J.R. Johnson, Minneapolis, MN
MVAST2641	blood	1173	LrhA mutant (R17G)	2014	J.R. Johnson, Minneapolis, MN
MG1655	NA	NA	reference strain	NA	(6)
CD358	NA	NA	reference strain	2007	(7)
JJ2555	NA	NA	reference strain	2007	(7)
JJ1887 plasmid cured	TET-sensitive derivative of JJ1887 that spontaneously lost plasmid pJJ1887_5				This study
Recombinant strains					
JJ1886/pE	JJ1886 transformed with plasmid pACYC184				This study
JJ1886/pRpoS$_{JJ1886}$	JJ1886 transformed with plasmid pACYC184 carrying *rpoS*$_{JJ1886}$				This study
JJ1886/pRpoS$_{JJ1887}$	JJ1886 transformed with plasmid pACYC184 carrying *rpoS*$_{JJ1887}$				This study
JJ1886/pLacI$_{JJ1886}$	JJ1886 transformed with plasmid pACYC184 carrying *lacI*$_{JJ1886}$				This study
JJ1886/pLacI$_{JJ1887}$	JJ1886 transformed with plasmid pACYC184 carrying *lacI*$_{JJ1887}$				This study
JJ1886/pLrhA$_{JJ1887}$	JJ1886 transformed with plasmid pACYC184 carrying *lrhA*$_{JJ1887}$				This study
JJ2548/pE	JJ2548 transformed with plasmid pACYC184				This study
JJ2548/pLrhA$_{JJ1887}$	JJ2548 transformed with plasmid pACYC184 carrying *lrhA*$_{JJ1887}$				This study
E.coli XL1-Blue	Host used for gene amplification and cloning				Stratagene
Recombinant plasmids					
pE	plasmid pACYC184 used for gene expression in wild-type *E. coli* strains with chloramphenicol selection (Cm^R)				
pRpoS$_{JJ1886}$	pACYC184 containing *rpoS*$_{JJ1886}$ cloned using XbaI/XhoI, Cm^R				This study
pRpoS$_{JJ1887}$	pACYC184 containing *rpoS*$_{JJ1887}$ cloned using XbaI/XhoI, Cm^R				This study
pLacI$_{JJ1886}$	pACYC184 containing *lacI*$_{JJ1886}$ cloned using XbaI/XhoI, Cm^R				This study
pLacI$_{JJ1887}$	pACYC184 containing *lacI*$_{JJ1887}$ cloned using XbaI/XhoI, Cm^R				This study
pLrhA$_{JJ1887}$	pACYC184 containing *lrhA*$_{JJ1887}$ cloned using XbaI/XhoI, Cm^R				This study

TET, tetracycline; Cm^R, chloramphenicol resistant; NA, not applicable.

Table S5. Primers used in this study.

Primer name	Sequence 5'-3'
gene cloning	
lrhA_xbaI_F	GGAGTCTAGAACAGGAGGAATTAACCATGATAAGTGCAAATCGTCCG
lrhA_xhoI_R	CACACTCGAGTTACTCGATATCCCTTTCAATCAAC
lacI_xbaI_F	GGAACGTCTAGAACAGGAGGAATTAACCATGAAACCAGTAACGTTATACG
lacI_xhoI_R	GAGGAACTAGTCTCGAGTCACTGCCCGCTTTCCAGTCGG
rpoS_xbaI_F	GGAGTCTAGAACAGGAGGAATTAACCATGAGTCAGAATACGCTGAAAG
rpoS_xhoI_R	GTGGCTCGAGTTATTCGCGGAACAGCGCTTC
gene sequencing	
lrhA_F	CACAGCATTAACCAGCTCAGT
lrhA_R	CCCAGCGGTTCGTTTTTACAC
lacI_F	CATCTTCCGGCGCTGCAAC
lacI_R	CCTCTTCGCTATTTCGCCAG
rpoS_F	CAACCATGGGTAGCACC
rpoS_R	CCAATGTGCTTGCGTCAACC
deoR_F	CTCAACACGATTCACCTCT
deoR_R	CGGTCCAGTCTTGCGCC
cspE_F	CCTCTTCCGGTTCTACTGCC
cspE_R	CTGTTTTCCGCCTCAACCGC
dapA_F	ATGCCGAAAACCATTGAAG
dapA_R	TTCAGGCGTGAAATTAACCGC
ompN_F	ATGAAAAGCAAAGTACTG
ompN_R	GGTAGAGATGCCGTTGGCAGCGTAG
gadA_F	ATGGACCAGAAGCTGTTAAC
gadA_R	TCAGGTGTGTTTAAAGC
P423_00080_F	TGGGCTACGATGGCTTTGG
P423_00080_R	TCGGCTCGTGGAGTGGGTTG
degS_F	GAGTTTTCTCTGGGATTTGG
degS_R	GCTGCACATTTTCCAGTACAGG
ykgC_F	CTACGTCTGCCTTCTCCC
ykgC_R	AATATCGGCTGTATCCCAACC
nfsB_F	GCAGCGGATTTGGCAACACG
nfsB_R	GCAAGGAACGGATACG
ydbk_F	GCAGCGGATTTGGCAACACG
ydbk_R	GCAAGGAACGGATACG
lipA_F	CCTCTTCCGGTTCTACTGCC
lipA_R	CTATTTGTACCCGCCGTTGCCC
dps_F	CGTCATCTTTCGCTTCGCC
dps_R	CGCTACTTTTCCTCTACACC
ycgJ_F	CCGCACCCTAATCGTGACAGC
ycgJ_R	AGCCCGCCAGCGTCATTTCC
dnaC_F	GGCAGTTGGGGTTCTTG
dnaC_R	GTTCATGGTCATGCGGT
ydbH_F	GTTCGGGTAATGGTCGCTGG
ydbH_R	GCTCCAGCCATGCCTGGAG
yebC_F	CACCGTTATGGTAAACTTCC
yebC_R	CAGACATCGTAAAGCTGCGC
yfaL_F	GGATCTGTGAAGATCACTG
yfaL_R	GTTACCGTAAACCAGGGCG
P423_12990_F	CACGATTGTCCACCGACC

Table S5. Continued.

Primer name	Sequence 5'-3'
P423_12990_R	CAATTCCGGCGGCAGCC
fadJ_F	CCCCACGCTCGCTCATCTC
fadJ_R	CGCAAACCATCGCCACCAC
P423_13360_F	ACTCTGACACTCTCTTCT
P423_13360_R	TGAACCACCAACCGCTGCC
crr_F	TCATTGCTCCGCTCTCTG
crr_R	CACCTACGGTTACGCTACC
fhlA_F	CTTACGCAGCCACCGTAAGAAC
fhlA_R	CAGCGGCATATCGCCCACTTC
sdaC_F	GGTCAGGCACCTTCCCGG
sdaC_R	GAGATGATCGGAGAGTGGTTG
mltA_F	ACCGGTGCAAAAGATTGCGG
mltA_R	TGGGCACGGTTGTGGCAATG
P423_16465_F	TCAGCAATAACTCCCCTTC
P423_16465_R	CCAGATACACTCGACAAACC
P423_16480_F	CATATGCTCCTGACACCCCCTC
P423_16480_R	TTTATATATCTTCCCTTCTGC
P423_16705_F	CCTGCCATCCAGACGATAAGC
P423_16705_R	CAATTTATCCCTGCCGAAATAATTTCC
rapZ_F	GTACTGATGATCGTCAGCGG
rapZ_R	GCGTCCGATGGCGTGACTGG
malT_F	TGAAATCAAGGACATCAGAG
malT_R	GCAAGATTTGTGCACGGG
ftsY_F	AGATTTTCCCGGCGCTGGG
ftsY_R	GGCAGAATGCGGTAAGCCAGG
P423_19450_F	ATCCCCGCCAGCTCCGC
P423_19450_R	CTCCCTTTGAATTGCCTC
cspA_F	CATCACCCACCAATGCGT
cspA_R	CTGCCGGGCCTTTAGCGCC
glyS_F	CTGCAACAGCGAAATATCCGC
glyS_R	CTTCGGTATCGGCCAGCATC
gltS_F	CGCACCGACCATCGGCAC
gltS_R	ACACTCCACCACACCGAAC
frvA_F	GCAGACATCGGATTGTGCAT
frvA_R	GAATAAAGCGGAGGCGATTG
ilvN_F	CACCGCAATCTTGTTAAACATC
ilvN_R	CGGCGCTGATCCATGTGC
rbsR_F	TGGTGCGTGGCGTTGAACG
rbsR_R	GGTCGGCTGGGTTATCCGATGG
P423_21035_F	TGTAGCGGTACACCCATTGG
P423_21035_R	GACAAGATGGCGAAGAGTGA
P423_21850_F	GAGAAAGCAGTATTTGCG
P423_21850_R	GTCTGCATTATCGATACAGAG
ntrC_F	AGTCATTGGCACTGGCTGG
ntrC_R	GGTTGTGGTAAAGAAGTCG
bsmA_F	GGTACAAAATGGCTTGTGAA
bsmA_R	GGTTAGCAGGAAACGTAATAG
P423_24355_F	CTGGACACATCCTTCACCC
P423_24355_R	CTCTTACACTCCGCGCT

Supplementary data references:

1. Lewis M, Chang G, Horton NC, Kercher MA, Pace HC, Schumacher MA, Brennan RG, Lu P. 1996. Crystal structure of the lactose operon repressor and its complexes with DNA and inducer. Science 271:1247-54.
2. Jeong KC, Baumler DJ, Kaspar CW. 2006. dps expression in Escherichia coli O157:H7 requires an extended -10 region and is affected by the cAMP receptor protein. Biochim Biophys Acta 1759:51-9.
3. Jiang W, Fang L, Inouye M. 1996. The role of the 5'-end untranslated region of the mRNA for CspA, the major cold-shock protein of Escherichia coli, in cold-shock adaptation. J Bacteriol 178:4919-25.
4. Owens RC, Jr., Johnson JR, Stogsdill P, Yarmus L, Lolans K, Quinn J. 2011. Community transmission in the United States of a CTX-M-15-producing sequence type ST131 Escherichia coli strain resulting in death. J Clin Microbiol 49:3406-8.
5. Ender PT, Gajanana D, Johnston B, Clabots C, Tamarkin FJ, Johnson JR. 2009. Transmission of an extended-spectrum-beta-lactamase-producing Escherichia coli (sequence type ST131) strain between a father and daughter resulting in septic shock and Emphysematous pyelonephritis. J Clin Microbiol 47:3780-2.
6. Blattner FR, Plunkett G, 3rd, Bloch CA, Perna NT, Burland V, Riley M, Collado-Vides J, Glasner JD, Rode CK, Mayhew GF, Gregor J, Davis NW, Kirkpatrick HA, Goeden MA, Rose DJ, Mau B, Shao Y. 1997. The complete genome sequence of Escherichia coli K-12. Science 277:1453-62.
7. Johnson JR, Davis G, Clabots C, Johnston BD, Porter S, DebRoy C, Pomputius W, Ender PT, Cooperstock M, Slater BS, Banerjee R, Miller S, Kisiela D, Sokurenko EV, Aziz M, Price LB. 2016. Household Clustering of Escherichia coli Sequence Type 131 Clinical and Fecal Isolates According to Whole Genome Sequence Analysis. Open Forum Infect Dis 3:ofw129.