Supplemental Material

Table S1. MIC values of antibiotics against bacterial strains used in this study

Strains	MIC (mg/L)														
	CST	TGC	IPM	MEM	ETP	CMZ	CAZ	CTX	FEP	TZP	SCF	CZA	ATM	CIP	AMK
K. pneumoniae Y4	0.25	1	>128	>128	>128	>128	64	>128	>256	>256/4	>256/128	4/4	>128	>32	>256
K. pneumoniae Y4-ΔmgrB	16	1	64	>128	>128	>128	64	>128	>256	>256/4	>256/128	4/4	>128	>32	>256
K. pneumoniae Y17	64	8	64	64	>128	128	32	>128	>256	>256/4	>256/128	2/4	>128	>32	>256
K. pneumoniae Y17-ΔtetA	64	2	64	128	>128	128	32	>128	>256	>256/4	>256/128	2/4	>128	>32	>256
K. pneumoniae Y17-ΔramR	64	64	64	>128	>128	>128	32	>128	>256	>256/4	>256/128	2/4	>128	>32	>256

CST, colistin; TGC, Tigecycline; IPM, Imipenem; MEM, Meropenem; ETP, Ertapenem; CMZ, Cefmetazole; CAZ, Ceftazidime; CTX, Cefotaxime; FEP, Cefepime; TZP, Piperacillin-tazobactam; SCF, Cefoperazone-sulbactam; CZA, Ceftazidime-avibactam; ATM, Aztreonam; CIP, Ciprofloxacin; AMK, Amikacin.

Table S2. Relative fold change in gene expression level of the RND efflux pumps and their transcriptional regulators in bacterial strains used in this study

Strains	ΔΔCq Expression (mean ± StdDev)							
	acrA	*acrB*	*oqxA*	*oqxB*	*ramA*	*rarA*	*marA*	*soxS*
K. pneumoniae Y17	1.571±0.266	1.645±0.143	0.083±0.007	2.045±0.278	0.880±0.037	0.005±0.000	0.462±0.030	8.741±0.638
K. pneumoniae Y17-ΔramR	7.982±0.431	10.559±1.145	0.780±0.011	3.535±0.498	46.886±5.945	0.004±0.000	0.246±0.010	2.392±0.104
K. pneumoniae Y17-ΔtetA	1.183±0.018	1.400±0.222	0.101±0.016	1.844±0.175	0.553±0.047	0.013±0.001	0.289±0.019	3.512±0.132

Table S3. Strains and Plasmids used in this study.

Strain or Plasmid	Description or genotype	Reference
E. coli DH5alpha	F– Φ80lacZΔM15 Δ(lacZYA-argF) U169 recA1 endA1 hsdR17 (rK–, mK+) phoA supE44 λ– thi-1 gyrA96 relA1	Lab Stock
K. pneumoniae Y4	Wild-type clinically isolated *K. pneumoniae* strain Y4	[1]
K. pneumoniae Y4-ΔmgrB	*K. pneumoniae* Y4 *⊿mgrB*	This Study
K. pneumoniae Y17	Wild-type clinically isolated *K. pneumoniae* strain Y17	[1]
K. pneumoniae Y17-ΔramR	*K. pneumoniae* Y17 *⊿ramR*	This Study
K. pneumoniae Y17-ΔtetA	*K. pneumoniae* Y17 *tetA* W301 mutation to stop codon	This Study
pCasKP-apr	Thermosensitive plasmid expresses Cas9 and lambda Red proteins in *K. pneumoniae* (Apr^r)	[2]
pSGKP-spe	Plasmid expressing sgRNA in *K. pneumoniae* (Spe^r)	[2]
pSGKP-spe_mgrB	pSGKP-spe derivative with *mgrB* spacer	This Study
pSGKP-spe_ramR	pSGKP-spe derivative with *ramR* spacer	This Study
pBECKP-spe	*K. pneumoniae* base editing vector (Spe^r)	[2]
pBECKP-spe_tetA	pBECKP-spe derivative with *tetA* spacer	This Study

Table S4. Primers used in this study.

Gene	Primer	Sequence(5'~3')	Purpose
mgrB gene deletion in the *K. pneumoniae* Y4 strain	mgrB-spacer-F	tagtGCTTAACGTAATGTGCGACC	*mgrB* spacer for gene deletion
	mgrB-spacer-R	aaacGGTCGCACATTACGTTAAGC	*mgrB* spacer for gene deletion
	mgrB-seq-F	TAACCGCTCAATAATACGCC	amplification of *mgrB* locus from genome and sequencing of *mgrB* locus
	mgrB-seq-R	GCTCAGCCACATCCTCTTTC	amplification of *mgrB* locus from genome
	mgrB-ssDNA	GGCCGTGCTATCCTGGCGACATTGCGTACTGATGCGGAGAGTGGAT AAGACATTTTTCTGCCGACTGATTTCCTTCTGCGCCGCGGGTGG	90 nt *mgrB* repair template for gene deletion
ramR gene deletion in the *K. pneumoniae* Y17 strain	ramR-spacer-F	tagtAAGAGAACGCGCGCAATATC	*ramR* spacer for gene deletion
	ramR-spacer-R	aaacGATATTGCGCGCGTTCTCTT	*ramR* spacer for gene deletion
	ramR-seq-F	GTTGCTGCTGCGAGTAACAT	amplification of *ramR* locus from genome and sequencing of *ramR* locus
	ramR-seq-R	TTCGGTAAACGGGTAGGTCA	amplification of *ramR* locus from genome
	ramR-ssDNA	GTGGCTCGTCCAAAGAGTGAAGATAAAAAGCAAGCGTTACTGGAA AGAGCTTTCCGGAGCTCAACGAAATGTGCCAGCTGTCGGTGAAAG	90 nt *ramR* repair template for gene deletion
tetA gene W301 to stop in the *K. pneumoniae* Y17 strain	tetA-spacer-F	tagtCATCCATCCCCGTGTCGCGA	*tetA* spacer for its W301 mutation to stop codon
	tetA-spacer-R	aaacTCGCGACACGGGGATGGATG	*tetA* spacer for its W301 mutation to stop codon
	tetA-seq-F	CCAACAGACCCCTGATCGTA	amplification of *tetA* locus from genome and sequencing of *tetA* locus
	tetA-seq-R	GGCAGGCAGAGCAAGTAGAG	amplification of *tetA* locus from genome

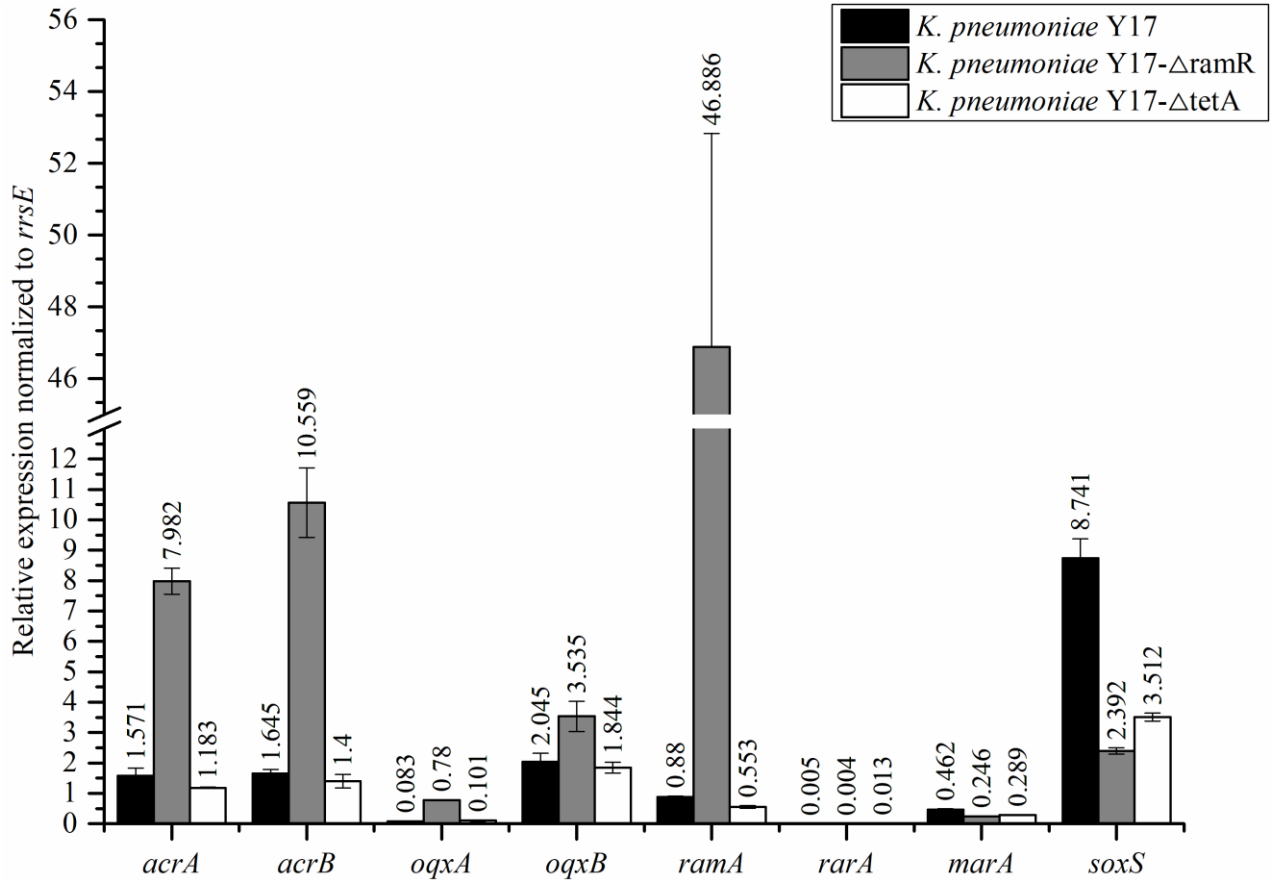

Figure S1. Relative fold change in gene expression level of the RND efflux pumps and their transcriptional regulators in bacterial strains used in this study.

References

1. **Zhang R, Dong N, Huang Y, Zhou H, Xie M, Chan EW, Hu Y, Cai J, Chen S.** 2018. Evolution of tigecycline- and colistin-resistant CRKP (carbapenem-resistant Klebsiella pneumoniae) in vivo and its persistence in the GI tract. Emerging microbes & infections **7:**127.
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