

```

SA KTRB      -MNKVHKP-----LYFYLMFFSTTIIGALLLYLPFTG 32
SA KTRD      -MSIFSQFLK-----RSSPQQGIVLYYIVAIVIAFLLLNLPYVH 38
BS KTRB      -MTLQKDK-----VIKVVRFPPQVLAIGFFLTIIIGAVLLMLPIST 41
BS KTRD      MRLKFGKLIQ-----ALSPAQLIALYFLAVTVAVILLSLPAAH 39
VA KTRB      -MTYFHQKGVFYAPDQKQEKAKGNEPRIILLSFGLVLLPSAVLLTLPVFS 49
          .          : : . . : ** **

SA KTRB      KK--PISFLDALFIASSAFTVTGLSPVDIGSQFNILGEIVILLIQLIGGL 80
SA KTRD      KPGVEVNPIDTLFVAVSGISVTCGLSPISIVDTYSTFGQLIILVILNIGGI 88
BS KTRB      TK--PLSWIDALFTAASATTVTGLAVVDTGTQFTVFGQTVIMGLIQLIGGL 89
BS KTRD      KPGADWTFIDALFTAASSVSVTGLTVVDTADTFSTIGIFILAFVLQFGGI 89
VA KTRB      VT--GLSITDALFTATS AISVTGLGVVDTGQHFTLAGKILLMFLMQIGGL 97
          .  *:* * * . :**** :. :. * : : : : : :

SA KTRB      GIVTVTLLTLVFLNRKISMKNRFLIMVTWNIDEPPGVIKLIKHLAIYSLV 130
SA KTRD      GVMAIGTMLWVVLGKHIGIRERQLIMLDNNKNTMSGTVKLIIDIVKSIFV 138
BS KTRB      GFMTFAVLIVMILGKKIGLKERMLVQEALNQPTIGGVIGLVKVLFLFSIS 139
BS KTRD      GIMTLGTFIWLIMGKRIGLKERKLMVDQNSQFSGIVNLMKQVLFILW 139
VA KTRB      GQMTLSAVLLYMFVRLSLRQALAKEALQDRQVNLNRLVKRIVVFAVL 147
          * : : . . : : : : : * . . * : : :

SA KTRB      TELIGMICLCLSFIPKFG-IGKGLFSLFTSVSAFNNA GFALFKNNLIDY 179
SA KTRD      IELVGAMLLAFYFYRDNPDLYAIMQGVFVSIATNGGLDITGKSLIPY 188
BS KTRB      IELIAALILSIRLVPQYG-WSSGLFASLFHAISAFNNA GFSLWPDNLSY 188
BS KTRD      IEFFGGLILGTYFLTYDYSYQEAYLHGFFASISATNGGFDITGNSMIPF 189
VA KTRB      AETIGFIFLSIRWVPEMG-WQTMFYALFHAISAFNNA GFALFSDSMSF 196
          * . . : * . : . * : : * . * : : : : :

SA KTRB      SSDPIVIITISILIIFGGIGHFVVIDFINCKK---LSKLSLHSKLVLT 225
SA KTRD      AHDYFVQAIIVIFLIILGSIGFPVLEVKAYIQNRVTNFRSLFTKITTST 238
BS KTRB      VGDPTVNLVITFLFITGGIGFTVLFVDMKNRR---FKTFSLHTKMLMTG 234
BS KTRD      RHDYFVQFITMLLIIFGAIGFPVLEVKDFLFSKHRRYPFTLFTKITTIA 239
VA KTRB      VNDPLVIFTLAGLFI FGGLGFTVVGD IWLNRK--GFHFLHLHTKIMLIA 244
          * * * : * * . * : : . : : * : : :

SA KTRB      TSILIIIGAITFFLLEQFN--TMQHMGLVEKIGNSFFQSVTTRTAGFN 273
SA KTRD      YLFLFIVGVLAILLFEHNN--AFKGLSWHQSLFYSLFQSATTRSAGLQTI 286
BS KTRB      TLMLNAIAMLTVFILEYSNPGTLGHLHIVDKLWASYFQAVTPRTAGFNSL 284
BS KTRD      FGSLVLFGAIGIFALEANH--AFAGKSWHDILFSLFQSTATRSGGLATI 287
VA KTRB      TPILLLVGTMLFWLLERSNPNTMASLPATGQWLAAFFQSASARTAGFNSV 294
          * . . : . : * : : : : : : * : : : : : :

SA KTRB      DIASINKSTALMLMLLMFIGGAPLSAAGGIKITTFAVAFIFVLNYIR--K 321
SA KTRD      DVTTLSDPTNIMGILMFIGSSPSSVGGGIRTTTFAILILFLINFSNNAD 336
BS KTRB      DFGSMREGTIVFTLLMFIGAGSASTASGIKLTTFIVILTSVIAYLR--G 332
BS KTRD      DISQLSDSTLFFICALMFIGASPSVGGGIRTTTFALNLLALFHFAR--G 335
VA KTRB      DLALFTQPALLVMIVMLLIGAGSTSTGGGIKVSTFAVAFMATWTFLR--Q 342
          * . : . : . * : * : . * : : : * : : : : .

SA KTRB      ENNVSVFNKEISDKHIKLSIVTINISFLFISITFILSIIN-PNISLIKL 370
SA KTRD      KTSIKVYNREVHIMDIQRSFAVFTMATILTFLGMLIISATENGKLTFLQV 386
BS KTRB      KKETVIFRRSIKYPYIIKALAVSVTSLFIVFLGIFALTIT--EQAPFLQI 380
BS KTRD      NKAVKVKRELHPADLMKSLVVTMMAILLVFGATLILTITE--KHSLEL 383
VA KTRB      KKHVVMFKRTINWQTVTKSLAIIVVSGAILTSAMFLLMLT--EKASFDKV 390
          : . : : : : : : : : : : : : : : :

SA KTRB      LFEVVSFAFGTVGLSMNLTTEYHGITKIIIFVMLCGKVGLLTLRFTFIPP 420
SA KTRD      FFEVMSAFGTCGLSLGVTSDISDISKVVLMLMFIGRVLISFIMIAGR 436
BS KTRB      VFETFSAFGTVGLTMGLTPELTTAGKCIIVIMFIGRIGPLTFVFSFAKT 430
BS KTRD      LFEVCSAFGTTGLSLGITADLSSVGKCVIMIVMFIGRIGILTFLYLIGRK 433
VA KTRB      MFETISAFATVGLTAGLTAELSEPGKYIMIVVMIIGRIGPLTAYMLARP 440
          . * . * * * * * : : : * : : : : * : : : : :

SA KTRB      KSPKNRYRTKQIYL- 435
SA KTRD      REPDKFHYPKERIQIG 452
BS KTRB      E-QSNIRYPDGEVFTG 445
BS KTRD      EIEANYHYPKERVIG 449
VA KTRB      E-PTLVKYPEDAULTG 455
          . : * . . :

```

**Fig. S1.** Alignment of Ktr ion-conducting proteins. Sequences from *Staphylococcus aureus* strain FPR3757 (SA), *Bacillus subtilis* strain 168 (BS), and *Vibrio alginolyticus* strain 12G01 (VA) were aligned using ClustalW2. Conserved K<sup>+</sup> selectivity filter glycine residues highlighted in blue. "\*" (asterisk) indicates a single, fully conserved amino acid. ":" (colon) indicates a group of strongly similar residues (> 0.5 in the Gonnet PAM 250 matrix). "." (period) indicates weakly similar properties (< 0.5 in the Gonnet PAM 250 matrix).

```

SA KTRA      MG----KEYVVIGLGRFGGSIRELNALDMDVMAIDHDENRVNEYSDIATHAVVADTTDE 56
BS KTRC      MK----KEFAVIGLGRFGGSICKALSEEGVEVMAMDIDEDKVNEYAKIASHAVIGDSTDE 56
BS KTRA      MGRIKNKQFAVIGLGRFGGSICKELHRMGHEVLAVDINEEKVNAYASYATHAVIANATEE 60
VA KTRA      MKTG-DKQFAVIGLGRFGLAVCKELQDSGPQVLAVDINEDRVKEAASFVSQAVVANCTHE 59
          *      *:::***** :: : *      . :*:*: * :*:*: :. :. :*:*: :. :*
          *      *:::***** :: : *      . :*:*: * :*:*: :. :. :*:*: :. :*

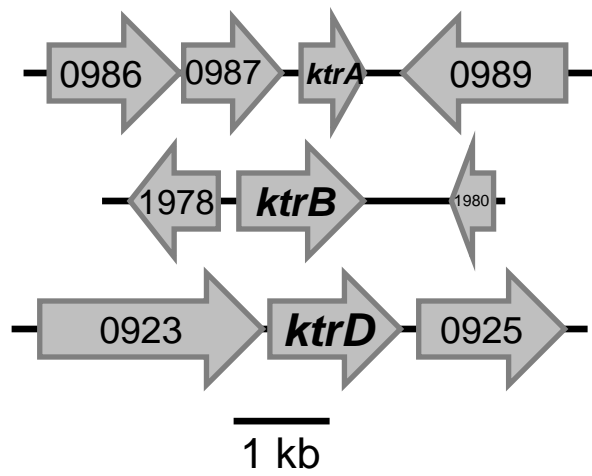
SA KTRA      AVMKSLGIRNFDHVIVAIGENIQSSTLTTLLIKELGVKKVTAKAQNDYHAKILNKIGADT 116
BS KTRC      SVLKNLGLRNFDHVIVAIGENIQASILTTTLLIKELGVHTITVKAQNDYHEKVLSKIGADH 116
BS KTRA      NELLSLGIRNFEYVIVAIGANIQASTLTTLLIKELDIPNIWVKAQNYYHHKVLEKIGADR 120
VA KTRA      ETVAELKLDDDYDMVMIAIGADVNASILATLIAKEAGVKSVWVKANDRFQARVLQKIGADH 119
          : . * : : : : * : : * * : : * * : : * * : : . : . * * : : : : * . * * * *

SA KTRA      VVHPERDMGRRIAHNVASASVLDYLELADEHSIVELKATEKMAGQSIIDLDIRAQYGINI 176
BS KTRC      IVHPERDMAKRIAHNIVSNNVLDYLELSEEHSIVEIVANSRLAGNTLLDDIRAKYGINI 176
BS KTRA      IIHPEKDMGVKIAQSLSDENVLNYIDLSDEYSIVELLATRKLDSKSIIDLNVRAKYGCTI 180
VA KTRA      IIMPERDMGIRVARKMLDKRVLEFHPLGSGLAMTEFVIGSRWMGKTLSDLALCKVDGVQV 179
          :: * : * * . : : * : : . * * : : * . : : . * : : : : * * : : * :

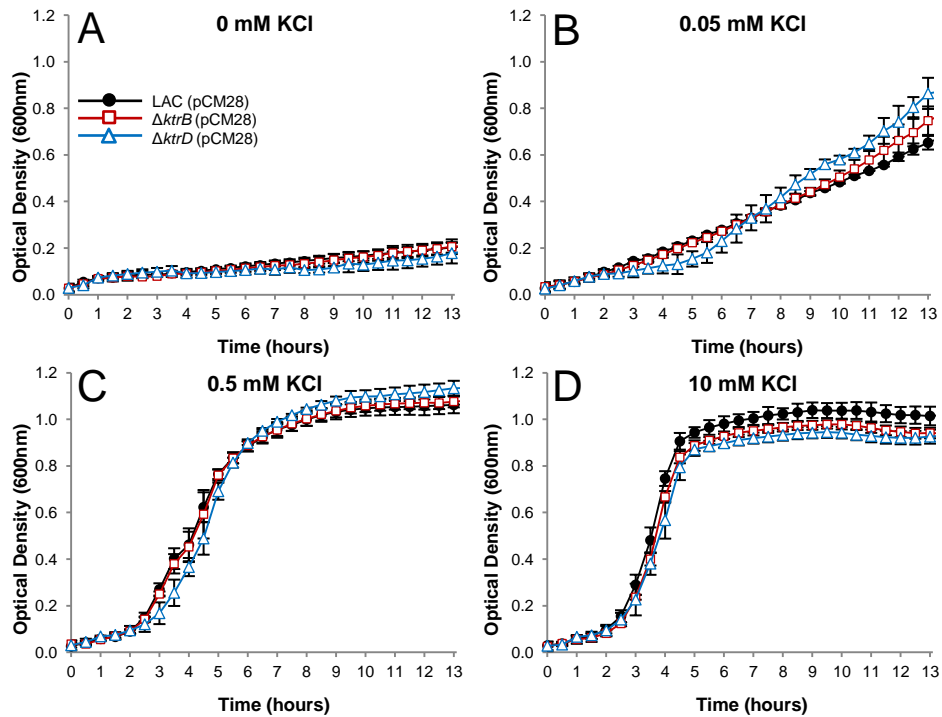
SA KTRA      IAIKRGKEFIISPNPNINLEIGDILIMIGHDNDLNRFEKNIATR- 220
BS KTRC      VAIKRGKEVIVSPLATEVIHQEDILIVIGSVTDISRFEKRVLHTK 221
BS KTRA      LAIKHHGDICLSPAPEDIIREQDCLVIMGHKKDIKRFENEGM--- 222
VA KTRA      LGFKRGPEITKAPDLNTTLEIGDLIIVVGPDALANKLKSL---- 220
          : : * : : . : * : : : * : : . : :

```

**Fig. S2.** Alignment of Ktr regulatory proteins. Sequences from *Staphylococcus aureus* strain FPR3757 (SA), *Bacillus subtilis* strain 168 (BS), and *Vibrio alginolyticus* strain 12G01 (VA) were aligned using ClustalW2. KTN/RCK domain is highlighted in blue. "\*" (asterisk) indicates a single, fully conserved amino acid. ":" (colon) indicates a group of strongly similar residues (> 0.5 in the Gonnet PAM 250 matrix). "." (period) indicates weakly similar properties (< 0.5 in the Gonnet PAM 250 matrix).



**Fig. S3:** Chromosomal organization of the *ktr* genes in *Staphylococcus aureus* strain FPR3757. Numbers represent locus ID.



**Fig. S4.** Growth of *S. aureus* Ktr mutants in low K<sup>+</sup>. Wild-type *S. aureus* (solid black circles) and mutant strains  $\Delta ktrB$  (open red squares) and  $\Delta ktrD$  (open blue triangles) were inoculated from overnight cultures to OD<sub>600</sub>=0.05 in K-CDM supplemented with KCl to the desired concentration.

Data presented represent the mean of at least three independent experiments, with  $\pm$  standard error.

**Table S1.** Bacterial strains, plasmids, and oligonucleotide used in this study.

Strain	Characteristics	Source
<b><i>S. aureus</i></b>		
LAC	Wild-type CA-MRSA (USA300) isolate	(Voyich <i>et al.</i> , 2005)
KB7004	LAC $\Delta ktrB$	This study
KB7005	LAC $\Delta ktrA$	This study
KB7006	LAC $\Delta ktrD$	This study
KB7007	LAC $\Delta ktrB \Delta ktrD$	This study
KB7008	LAC $\Delta kdpFABC$	This study
KB7009	LAC $\Delta ktrA \Delta kdpFABC$	This study
JE2	LAC cured of plasmids LAC-p01 and LAC-p03	(Fey <i>et al.</i> , 2013)
<i>ktrA</i> :: $\Phi$ N $\Sigma$	JE2 <i>ktrB</i> :: $\Phi$ N $\Sigma$	This study
<i>ktrB</i> :: $\Phi$ N $\Sigma$	JE2 <i>ktrA</i> :: $\Phi$ N $\Sigma$	This study
<i>ktrD</i> :: $\Phi$ N $\Sigma$	JE2 <i>ktrD</i> :: $\Phi$ N $\Sigma$	This study
RN4220	Highly transformable restriction-deficient strain	(Kreiswirth <i>et al.</i> , 1983)
<b>Plasmids</b>		
pCM28	Complementing vector, <i>ampR</i> , <i>chmR</i>	(Pang <i>et al.</i> , 2010)
pCL52.2	Temperature sensitive Staphylococcal shuttle vector, <i>tetR</i> , <i>specT</i>	(Sau <i>et al.</i> , 1997)
pJB38	Temperature sensitive Staphylococcal shuttle vector, <i>ampR</i> , <i>chmR</i>	(Bose <i>et al.</i> , 2013)
pCG31	<i>ktrB</i> complement plasmid, created using CG37 and CG38	This study
pCG32	<i>ktrB</i> allelic exchange plasmid, created using CG51/52 and CG53/54	This study
pCG33	<i>ktrA</i> complement plasmid, created using CG39 and CG40	This study
pCG34	<i>ktrA</i> allelic exchange plasmid, created using CG55/56 and CG57/58	This study
pCG35	<i>ktrD</i> complement plasmid, created using CG41 and CG42	This study
pCG36	<i>ktrD</i> allelic exchange plasmid, created using CG27/28 and CG29/31	This study
pCG37	<i>kdpFABC</i> complement plasmid, created using CG74 and CG75	This study
pCG38	<i>kdpFABC</i> allelic exchange plasmid, created using CG 70/71 and CG 72/73	This study
<b>Oligonucleotides</b>		
CG27	CCGAGCTCGCTCAAAAATATACGACAACGATGAAAAAT	This study
CG28	CCGGATCCCACGTTGTTACACCTCAACATTATTTTAGTTTAATATTAATTT	This study
CG29	CCGGATCCTAATATAATAGCAATCTAAGTTTAGTTAATGTAGATTTAACTGGA	This study
CG31	CCGTCGACGATCCTCTTCATACTCATTAAAGTCA	This study
CG37	GGGAGCTCCCATAGCATCTACAACAATTAATCTGGTTT	This study
CG38	GGGAATTCTTATAGATAAATTTGTCCTTTAGTGTAGCGGTAAT	This study
CG39	GGGAGCTCTATCATTACGCATTCAAATTCTGAACAT	This study
CG40	GGGAATTCTTATCTCGTCGCAATATTTTTTCAAAGCGA	This study
CG41	GGGAGCTCAATTGATGCATCAGTAAATCCAGGTAAT	This study
CG42	GGGAATTCTTATCCTATTTGAATACGTTCTTTTGGATAATGGAA	This study
CG51	CCGAGCTCATAATCTTCTGCTTTATATGAA	This study
CG52	GGGGATCCCATTTTAATTTTCATACTCCTTT	This study
CG53	CCGGATCCTAAAATAACTATCCATCTTTAA	This study
CG54	GGGTCGACAGCTGATTCTGAAGAATTTTCT	This study
CG55	CCGAGCTCTTTAAACCCAGTTTGGGAAGTT	This study
CG56	GGGGATCCCATACTCGCCCTCCATTAATG	This study
CG57	CCGGATCCTAATCGGGAGTGGGACAGAAAT	This study
CG58	GGGTCGACTAAAGCTGGTGCAGATGTTATC	This study
CG70	CCGAGCTCACTTGCTTTTACTATCTTT	This study
CG71	GGGGATCCCATCGTAACAACCTTGTTTAT	This study
CG72	CCGGATCCTGATTATAGATGAAAGGCAT	This study

CG73	GGGTCGACGACCCCTTGTGTCATATCAA	This study
CG74	GGGAGCTCTTCTTCAATATCCATATATCG	This study
CG75	GGGAATTCTCAATCTTTCATCTTATCAAG	This study

**a.** Antibiotic resistance abbreviations used: *ampR*, ampicillin resistance (*bla*); *chmR*, chloramphenicol resistance; *tetR*, tetracycline resistance (*tetM*), and *specR*, spectinomycin resistance.

**b.** Oligonucleotides sequences are provided in the 5'-3' orientation. Italics indicate non-homologous sequences added for cloning.

## References

- Bose, J.L., P.D. Fey & K.W. Bayles, (2013) Genetic tools to enhance the study of gene function and regulation in *Staphylococcus aureus*. *Applied and environmental microbiology* **79**: 2218-2224.
- Fey, P.D., J.L. Endres, V.K. Yajjala, T.J. Widhelm, R.J. Boissy, J.L. Bose & K.W. Bayles, (2013) A genetic resource for rapid and comprehensive phenotype screening of nonessential *Staphylococcus aureus* genes. *mBio* **4**: e00537-00512.
- Kreiswirth, B.N., S. Lofdahl, M.J. Betley, M. O'Reilly, P.M. Schlievert, M.S. Bergdoll & R.P. Novick, (1983) The toxic shock syndrome exotoxin structural gene is not detectably transmitted by a prophage. *Nature* **305**: 709-712.
- Pang, Y.Y., J. Schwartz, M. Thoendel, L.W. Ackermann, A.R. Horswill & W.M. Nauseef, (2010) agr-Dependent interactions of *Staphylococcus aureus* USA300 with human polymorphonuclear neutrophils. *Journal of innate immunity* **2**: 546-559.
- Sau, S., J. Sun & C.Y. Lee, (1997) Molecular characterization and transcriptional analysis of type 8 capsule genes in *Staphylococcus aureus*. *Journal of bacteriology* **179**: 1614-1621.
- Voyich, J.M., K.R. Braughton, D.E. Sturdevant, A.R. Whitney, B. Said-Salim, S.F. Porcella, R.D. Long, D.W. Dorward, D.J. Gardner, B.N. Kreiswirth, J.M. Musser & F.R. DeLeo, (2005) Insights into mechanisms used by *Staphylococcus aureus* to avoid destruction by human neutrophils. *Journal of immunology* **175**: 3907-3919.

**Table S2.** Locus ID and accession numbers of Ktr proteins.

	KtrA	KtrB	KtrD
<i>S. aureus</i> USA300_FPR3757	0988 (YP_493686)	1979 (YP_494630)	0924 (YP_493624)
<i>S. epidermidis</i> ATCC 12228	0786 (NP_764341)	0571 (NP_764126)	0724 (NP_764279)
<i>S. haemolyticus</i> JCSC1435	1867 (YP_253782)	2567 (YP_254482)	1935 (YP_253850)
<i>S. lugdunensis</i> HKU09-01	1788 (YP_003472005)		1845 (YP_003472060)
<i>S. saprophyticus</i> ATCC 15305	1703 (YP_301793)	2353 (YP_302443)	1762 (YP_301852)
<i>S. carnosus</i> TM300	0709 (YP_002633805)	0441 (YP_002633540)	0626 (YP_002633723)
<i>S. pseudintermedius</i> HKU10-03	0795 (YP_004148959)	0870 (YP_004149034)	0719 (YP_004148883)

**Table S3.** Contribution of pH to antibiotic MIC.

<b>A</b> Gentamicin MIC values ( $\mu\text{g/mL}$ )		
pH	LAC (pCM28)	$\Delta ktrA$ (pCM28)
5.35	>16	16
7.01	4	1
8.0	1	.25
9.08	.5	NG

<b>B</b> Polymyxin B MIC values ( $\mu\text{g/mL}$ )		
pH	LAC (pCM28)	$\Delta ktrA$ (pCM28)
5.35	>64	>64
7.01	>64	64
8.0	64	8
9.08	32	NG

<b>C</b> HNP-1 MIC values ( $\mu\text{M}$ )		
pH	LAC (pCM28)	$\Delta ktrA$ (pCM28)
5.35	>5	>5
7.01	>5	>5
8.0	>5	1.25
9.08	2.5	NG

- a.** MIC values reported are the concentration of antibiotic in which no growth was seen following 20 hrs of static incubation at 37°C.
- b.** Results are representative of at least two independent experiments.
- c.** NG=no growth detectable for  $\Delta ktrA$  in 0.5 mM KCl pH 9.08