Table S2. Strains, plasmids, and primers used in this study

Strains

Strain	Genotype or description	Reference
A. baumannii		
ATCC 17978	cerebrospinal fluid isolate	(1)
EGA738	ATCC 17978 Δ*elsL* (ACX60_RS03475)	(2)
EGA739	ATCC 17978 Δldt_{Ab} (ACX60_RS05685)	(2)
EGA740	ATCC 17978 Δ*elsL* Δldt_{Ab}	This study
YDA350	ATCC 17978 with pYDE153 (vector)	This study
YDA475	EGA738 (Δ*elsL*) with pEGE305 (vector)	This study
YDA515	EGA738 (Δ*elsL*) with pYDE350 (P(IPTG)-*elsL*-3xFLAG)	This study
YDA516	EGA738 (Δ*elsL*) with pYDE351 (P(IPTG)-*ldcA*-3xFLAG)	This study
YDA519	EGA739 (Δldt_{Ab}) with pEGE305 (vector)	This study
YDA518	EGA739 (Δldt_{Ab}) with pYDE240 (P(IPTG)-ldt_{Ab})	This study
YDA004	ATCC 17978 *attTn7*::*tet*R-*tet*P-*dcas9-rrnB*T1-T7Te Gm^r	(3)
YDA007	YDA004 with pYDE007 (non-targeting control)	(3)
YDA232	YDA004 with pYDE044 (sgRNA$_{ldtAb}$)	This study
YDA549	YDA004 with pYDE381 (sgRNA$_{blhA}$)	This study
YDA535	YDA004 with pYDE376 (sgRNA$_{RS13190}$)	This study
YDA533	YDA004 with pYDE374 (sgRNA$_{mlaF}$)	This study
YDA186	ATCC 17978 Δ*elsL attTn7*::*tet*R-*tet*P-*dcas9-rrnB*T1-T7Te Gm^r	This study
YDA281	YDA186 with pYDE007 (non-targeting control)	This study
YDA284	YDA186 with pYDE044 (sgRNA$_{ldtAb}$)	This study
YDA550	YDA186 with pYDE381 (sgRNA$_{blhA}$)	This study
YDA539	YDA186 with pYDE376 (sgRNA$_{RS13190}$)	This study
YDA537	YDA186 with pYDE374 (sgRNA$_{mlaF}$)	This study
YDA315	EGA740 (Δ*elsL* Δldt_{Ab}) *ampG*(W99*)	This study
YDA331	EGA740 (Δ*elsL* Δldt_{Ab}) *mpl*(A335T)	This study
YDA380	EGA740 (Δ*elsL* Δldt_{Ab}) *ltgF::IS*	This study
EGA516	ATCC 17978 Δ*ampG*	(4)
EGA692	ATCC 17978 Δ*pbp2*	(4)
YDA414	ATCC 17978 Δ*elsL* Δldt_{Ab} Δ*ampG*	This study
YDA411	ATCC 17978 Δ*elsL* Δ*ampG*	This study
AB5075-UW	bone isolate/osteomyelitis	(5)
AB07436	AB5075-UW *ltgF*118::T26	(5)
AB03078	AB5075-UW *ampG*117::T26	(5)
E. coli		
DH5α	*supE44* Δ*lacU169* (ϕ80lacZΔM15) *hsdR17 recA1 endA1 gyrA96 thi*-1 *relA1*	(6)
DH5λpir	DH5α (λpir) *tet*::Mu *recA*	(7)
XL1-blue	*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F′ *proAB* $lacI^qZ$ΔM15 Tn10 Tc^r)]	Stratagene

Plasmids

Plasmid	Description	Reference
pUC18	*oriColE1* MCS Cb^r	(8)
pSR47S	Conditionally replicating allele exchange plasmid, Km^r (*oriTRP4 oriR6K sacB* Km^r)	(9)
pJB4648	Conditionally replicating allele exchange plasmid, Gm^r (*oriTRP4 oriR6K sacB* Gm^r)	(9)
pEGE305	P(IPTG) shuttle vector (*ori-pBR322 ori-pWH1277 bla*::*lacI*^{q}-T5*lac*P, Tc^r)	(4)
pYDE153	P(IPTG) shuttle vector (*ori-pBR322 ori-pWH1277 bla*::*lacI*^{q}-T5*lac*P-MCS, Tc^r)	(2)
pDL1100	*Himar1 mariner* (Km^r) delivery plasmid, C9 transposase, *ori pSC101* Cb^r	(2)
pYDE009	*dcas*9 miniTn7 delivery plasmid (*oriColE1* miniTn7::*tetR*-tetP-*dcas9-rrnB*T1-T7Te, Gm^r, Cb^r)	(3)
pYDE007	sgRNA delivery plasmid non-targeting control guide (*ori-pBR322 ori-pWH1277* P_{J23119}-$sgRNA_{mrfp}$-terminator, Cb^r)	(3)
pYDE044	pYDE007 derivative with $sgRNA_{ldtAb}$	This study
pYDE381	pYDE007 derivative with $sgRNA_{blhA}$	This study
pYDE376	pYDE007 derivative with $sgRNA_{RS13190}$	This study
pYDE374	pYDE007 derivative with $sgRNA_{mlaF}$	This study
pEGE268	pSR47S::Δ*elsL* allele exchange construct	(2)
pEGE271	pSR47S::Δldt_{Ab} allele exchange construct	(2)
pYDE388	pJB4648::Δ*ltgF* allele exchange construct	This study
pYDE231	pUC18::ldt_{Ab}	This study
pYDE240	pEGE305::ldt_{Ab} (P(IPTG)-ldt_{Ab})	This study
pYDE350	pEGE305::*elsL-3xFLAG* (P(IPTG)-*elsL-3xFLAG*)	This study
pYDE351	pEGE305::*ldcA-3xFLAG* (P(IPTG-*ldcA-3xFLAG*)	This study
pYDE342	pUC18::*elsL* (XbaI hexameric sequence in-frame)	This study
pYDE063	pUC18::ldt_{Ab} (XbaI hexameric sequence in-frame)	This study
pYDE343	pUC18::*ldcA* (XbaI hexameric sequence in-frame)	This study
pYDE346	pUC18::*elsL-3xFLAG*	This study
pYDE347	pUC18::*ldcA-3xFLAG*	This study
pYDE386	pUC18::*elsL-msGFP2*	This study
pYDE387	pUC18::ldt_{Ab}-*msGFP2*	This study
pYDE389	pEGE305::*elsL-msGFP2* (P(IPTG)-*elsL-msGFP2*)	This study
pYDE390	pEGE305::ldt_{Ab}-*msGFP2* (P(IPTG)-ldt_{Ab}-*msGFP2*)	This study
pYDE281	pUC18::*elsL-6xHis*	This study
pYDE324	pUC18::*ldcA-6xHis*	This study
pYDE290	pET28b::*elsL-6xHis*	This study
pYDE328	pET28b::*ldcA-6xHis*	This study

Oligonucleotide primers and gene fragment

Primer or fragment name	Sequence (5' – 3'; restriction site underlined if present)	RE site(s)
Complementation and localization experiments		
Bam-elsL-F	GATATCGGATCCAGTTGAGTTGATATAAGAGAAGAAATTCG	BamHI
Xba-elsL-R	GATCATTCTAGAGGTTAAAGATTGTTCAGACAAATAAAC	XbaI
Bam-ldcA-F	GATATCGGATCCAGTTGAGTTGATATAAGAGAAGAAATTCGATGTCTCTGTTTCACTTAATTGC	BamHI
Xba-ldcA-R	GATCATTCTAGACATTTTAAGAACAGGATGACCGC	XbaI
ldtAb-BamHI-F	GATATCGGATCCGCTTATATGAAAATTCTTAAGGTTG	BamHI
ldtAb-XbaI-R	GATCATTCTAGATTCTAAGAATTTAACAGTTACGCC	XbaI
Ldt-OE-F	TGCATAGAATTCCAAAAGTCTACTGCAAGAAGAAACG	EcoRI
Ldt-OE-R	GCTGAACTGCAGGAAACAGAAGCTAAAATGCAAACC	PstI
elsL-C138S-F	TAGATTCAATTGTGAGCGGATAACAATTTC	
elsL-C138S-R	GATCATTCGAATAGACCCATGTGACATTGGAAC	BstBI
msGFP2_F	GTCAGAATTCTTTAAGAAGGAGATATACATATGGATTCTACTGAATCTTTATTCAC	EcoRI
msGFP2_R	AACTGCAGGTCTGGACATTTAAG	PstI
Gene deletion		
D-RS13100-up-F	TTCGTGGATCCCAGTAATACCGTGGACTTGAACAG	BamHI
D-RS13100-up-R	GGGAGGGTACCTGGTTTATACATAAAAACTTCCAAACAAC	KpnI
D-RS13100-dw-F	CTGAGGGTACCGATATCATTAAAGTGCCTAATTTATAG	KpnI
D-RS13100-dw-R	CTTGCGCGGCCGCCTTTGTAGTCTTTGATATTGTCC	NotI
Overexpression and purification of elsL* and *ldcA		
elsL-NcoI-FOR	TGCATACCATGGCTCAAAATAGTTGAGTTGATATAAGAG	NcoI
elsL-his-EcoRI-REV	GATCATGAATTCTTAGTGGTGGTGGTGGTGGTGGGTTAAAGATTGTTCAGACAAATAAAC	EcoRI
ldcA-NcoI-FOR	TGCATACCATGGCATGATATACATTTCGTTTCTGTTGTC	NcoI
ldcA-his-EcoRI-REV	GATCATGAATTCTTAGTGGTGGTGGTGGTGGTGCATTTTAAGAACAGGATGACCGCT	EcoRI
CRISPRi		
SGR29-F	TTACACTAGTAGCAGATACTTTATTAGGGTTCAGGTTTTAGAGCTAGAAATAGCAAG	SpeI
SGR45-F	TTACACTAGTTTGATAAGCATGACGGACAGCTTCGTTTTAGAGCTAGAAATAGCAAG	SpeI
SGR60-F	TTACACTAGTCGCTGTAATTGTTCTAACATAACGGTTTTAGAGCTAGAAATAGCAAG	SpeI
SGR58-F	TTACACTAGTAGAGAGGAGTTTTATTATTCATAAGTTTTAGAGCTAGAAATAGCAAG	SpeI
SGR-R (BglII) (ref. 3)	AAGTGGGCCCAGATCTAAGCTTCAAAAAAAGCACCGAC	ApaI, BglII
Gene fragment		
msGFP2 (for C-terminal fusion, codon optimized)	AGTTCGTCTAGAGGCGGCGGCGGCGGCATGGATTCTACTGAATCTTTATTCACTGGTGTTGT TCCAATTTTAGTTGAATTAGATGGTGATGTTAACGGTCACAAATTCTCTGTTCGTGGTGAAGG TGAAGGTGATGCTACTAACGGTAAATTAACTTTAAAATTCATTTGTACTACTGGTAAATTACCA GTTCCATGGCCAACTTTAGTTACTACTTTAACTTATGGTGTTCAATGTTTCTCTCGTTATCCAG ATCACATGAAACAACACGATTTCTTCAAATCTGCTATGCCAGAAGGTTATGTTCAAGAACGTA CTATTACTTTCAAAGATGATGGTACTTATAAAACTCGTGCTGAAGTTAAATTCGAAGGTGATA CTTTAGTTAACCGTATTGAATTAAAAGGTATTGATTTCAAAGAAGATGGTAACATTTTAGGTCA CAAATTAGAATATAACTATAACTCTCACAACGTTTATATTACTGCTGATAAACAAAAAAACGGT ATTAAAGCTAACTTCAAAATTCGTCACAACGTTGAAGATGGTTCTGTTCAATTAGCTGATCACT ATCAACAAAACACTCCAATTGGTGATGGTCCAGTTTTATTACCAGATAACCACTATTTATCTAC TCAATCTAAATTATCTAAAGATCCAAACGAAAAACGTGATCACATGGTTTTATTAGAGTTTGTT ACTGCTGCTGGTATTACTGGTGGTTCTGGTGGTTCTTAAATGTCCAGACCTGCAGTTCTG	XbaI, PstI

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