Table S1. Bacterial strains and plasmids used in the study

Bacterial Strains/ Plasmids	Genotype/ relevant characteristics	Reference
***Pseudomonas aeruginosa* reference strains**		
PAO1	Laboratory reference strain	[1]
PAO1 mutants		
Δ*mexZ*	Deletion of *mexZ* gene in PAO1	[2]
Δ*mexXY*	Deletion of *mexXY* genes in PAO1	[2]
mexY$_{G287S}$	Engineered mutation in the genome of PAO1 that replaced amino acid G with S at *mexY* codon 287	This study
fusA1$_{R680C}$	Engineered mutation in the genome of PAO1 that replaced amino acid R with C at codon 680 of *fusA1*	This study
fusA1$_{Y683D}$	PAO1 with spontaneous mutation that replaced amino acid Y with D at codon 683 of *fusA1*	[3]
fusA1$_{D327S}$	PAO1 with spontaneous mutation that replaced amino acid D with S at codon 327 of *fusA1*	[3]
amgS$_{V121G}$	Engineered mutation in the genome of PAO1 that replaced amino acid V with G at codon 121 of *amgS*	This study
Δ*mexZ mexY*$_{G287S}$	Δ*mexZ mexY*$_{G287S}$ double mutant	This study
Δ*mexZ fusA1*$_{R680C}$	Δ*mexZ fusA1*$_{R680C}$ double mutant	This study
Δ*mexXY fusA1*$_{R680C}$	Δ*mexXY fusA1*$_{R680C}$ double mutant	This study
Δ*mexZ fusA1*$_{Y683D}$	Δ*mexZ fusA1*$_{Y683D}$ double mutant	This study
Δ*mexZ fusA1*$_{D327S}$	Δ*mexZ fusA1*$_{D327S}$ double mutant	This study
Δ*mexZ amgS*$_{V121G}$	Δ*mexZ amgS*$_{V121G}$ double mutant	This study
Δ*mexXY amgS*$_{V121G}$	Δ*mexXY amgS*$_{V121G}$ double mutant	This study
mexY$_{G287S}$ *amgS*$_{V121G}$	*mexY*$_{G287S}$ *amgS*$_{V121G}$ double mutant	This study
mexY$_{G287S}$ *fusA1*$_{R680C}$	*mexY*$_{G287S}$ *fusA1*$_{R680C}$ double mutant	This study
fusA1$_{R680C}$ *amgS*$_{V121G}$	*fusA1*$_{R680C}$ *amgS*$_{V121G}$ double mutant	This study
Δ*mexZ amgS*$_{V121G}$ *fusA1*$_{R680C}$	Δ*mexZ amgS*$_{V121G}$ *fusA1*$_{R680C}$ triple mutant	This study
Δ*mexXY amgS*$_{V121G}$ *fusA1*$_{R680C}$	Δ*mexXY amgS*$_{V121G}$ *fusA1*$_{R680C}$ triple mutant	This study
Δ*mexZ fusA1*$_{R680C}$ *mexY*$_{G287S}$	Δ*mexZ fusA1*$_{R680C}$ *mexY*$_{G287S}$ triple mutant	This study
Δ*mexZ amgS*$_{V121G}$ *mexY*$_{G287S}$	Δ*mexZ amgS*$_{V121G}$ *mexY*$_{G287S}$ triple mutant	This study

fusA1$_{R680C}$ *amgS*$_{V121G}$ *mexY*$_{G287S}$	*fusA1*$_{R680C}$ *amgS*$_{V121G}$ *mexY*$_{G287S}$ triple mutant	This study
Δ*mexZ fusA1*R_{680C} *amgS*$_{V121G}$ *mexY*$_{G287S}$	Δ*mexZ fusA1*R_{680C} *amgS*$_{V121G}$ *mexY*$_{G287S}$ quadruple mutant	This study
Strains of *Escherichia coli*		
JM83	*ara*, Δ(*lac-proAB*) *rpsL*(*strA*), φ80, *lacZ*ΔM15	[4]
ST18	S 17 λ*pir*Δ*hemA*	[5]
Plasmids used in the study		
pEX18Tc	pMB1 replicon, *oriT* ,*sacB*, Tc^R , allelic exchange vector	[6]
pEX18Tc::Δ*mexXY*	pEX18Tc derivative carrying *mexXY* (*PA2018-PA2019*) flanking regions	[2]
pEX18Tc::Δ*mexZ*	pEX18Tc derivative carrying *mexZ* (*PA2020*) flanking regions	[2]
pEX18Tc::*mexY*$_{G287S}$	pEX18Tc containing DNA fragment with a mutation that encodes amino acid S instead of G at residue 287 in MexY	This study
pEX18Tc:: *fusA1*$_{R680C}$	pEX18Tc containing DNA fragment with a mutation that encodes amino acid C instead of R at residue 680 in FusA1	This study
pEX18Tc:: *amgS*$_{V121G}$	pEX18Tc containing DNA fragment with a mutation that encodes amino acid G instead of V at residue 121 in AmgS	This study

Table S2. Primers used in the study.

Primers	Sequences	Reference
***mexXY* deletion Primers**		
*mexXY*_F1	5’ CCCCC GGTACC GAGTCGGCTGATG ACCTACA 3’	[2]
*mexXY*_R1	5'GGGGG TCTAGA GTACCGCTGTTCTTCCTGGT 3'	[2]
*mexXY*_F2	5’ CCCCC TCTAGAGTCCCTCGATTC GTGAACT 3’	[2]
*mexXY*_R2	5' GGGGG AAGCTT GCTCTACATCGAC GGCAAG 3'	[2]
***mexXY* deletion screening Primers**		
*PA2017*_F	5' GCA GCC TGT ACG TGG TCA 3'	[2]
*mexZ*_R	5' GGG TTT TCT GGG ATT CCT CT 3'	[2]
***mexZ* deletion Primers**		
*mexX*_F	5' CCCCC GAATTC GTTCTCGACGATCACCCACT 3'	[2]
*mexX*_R	5' GGGGG TCTAGA GGGTTTTCTGGGATTCCTCT 3'	[2]
*PA2022*_F	5' CCCCC TCTAGA CGCAGTTCTCCCTACCTGTT 3'	[2]
*PA2022*_R	5' GGGGG AAGCTT CGCAGTATCTGGCTGTCGTA 3'	[2]
***mexZ* deletion screening Primers**		
*mexZ_SR*_F	5' GTGTCCCTCGATTCGTGAAC 3'	[2]
*mexZ_SR*_R	5' CGTGAAGCTACCGTGACAGA 3'	[2]
***mexY*$_{G287S}$ mutation engineering primers**		
*mexY*_F	5' GGGGAATTCCCTGTTCCGCAATCCGCATC 3'	This study
*mexY*_R	5'GGGGGATCCAGTCCTTCAGGGTGGCGAAG 3'	This study
***mexY*$_{G287S}$ mutation screening primers**		
*mexY_scr*_F	5' CTCGTCCAACGTGTTGCAGG 3'	This study
*mexY_scr*_R	5' AACGCCGAGGTGTCATAGGG 3'	This study
***fusA1*$_{R680C}$ mutation engineering primers**		
*fusA1*_F	5' GGGGG TCTAGA TTACTCGATGATCTTGGCAAC 3'	This study
fusA1_R	5' CCCCC AAGCTT GAAGCCGAGATCAAGGAAGG 3'	This study
***fusA1*$_{R680C}$ mutation screening primers**		
*fusA1_scr*_F	5' GTATTCAACGTGCGAGGTGT 3'	This study

*fusA1_scr*_R	5' GTTCAAGATCGCTGCTTCCA 3'	This study
***amgS*$_{V121G}$ mutation engineering primers**		
*amgS*_F	5'CCCCC GAATTC TTCTCCGTAAGCACGAGAGG 3'	This study
*amgS*_R	5' CCCCC TCTAGA AGCAGATGGATCGCCTTCTA 3'	This study
***amgS*$_{V121G}$ mutation screening primers**		
*amgS_scr*_F	5' ATACACCTCGGCCATTTCAC 3'	This study
*amgS_scr*_R	5' CTGATGAACGAGGACGTGAT 3'	This study
RT-qPCR Primers		
*mexX_RT*_F	5′ GGC CCT GGT CGC CCT ATT C 3′	[7]
*mexX_RT*_R	5′ TCC TCG TAC AGG CGA CGG 3′	[7]
clpX_F	5′ GTG GGC GAG GAT GTC GAG AAC 3′	[7]
clpX_R	5′ CGG TAC CCT CGA TGA GCT TCA G 3′	[7]
oprL_F	5′ CCA ACA GCG GTG CCG TTG A 3′	[7]
oprL_R	5′ GCC ATA TTG TAC TCG CGG GT 3′	[7]
Universal primers		
M13_F	GTA AAA CGA CGG CCA GT	Universal
M13_R	CAG GAA ACA GCT ATG AC	Universal

[a]Introduced restriction sites are underlined.

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