Supplemental Material

Table S1. Primers used to verify location of transposon insertions in PA14 NR transposon insertion library mutants

MexT, MexE, MexF, and other RND efflux pumps				
PA14 NR set plate position	**Gene name**	**Locus name PA14**	**Primer 5' - 3'**	**Product size**
PAMr_nr_mas_12_1:E6	*mexT*	PA14_32410	TACGCGGGTTGACCTGGTTGT	1586 bp
PAMr_nr_mas_13_3:E11	*mexE*	PA14_32400	TACGCGGGTTGACCTGGTTGT	308 bp
PAMr_nr_mas_07_1:B11	*mexF*	PA14_32390	CGATAGGAAGAACCGATGAA	423 bp
PAMr_nr_mas_06_3:E9	*czcA*	PA14_31010	TCGACGTTGGAAACGATGA	379 bp
PAMr_nr_mas_08_2:E2	*muxB*	PA14_31890	CTGGCCAAGGGTTACTCCCT	402 bp
PAMr_nr_mas_13_3:G10	*mexQ*	PA14_18780	CAGATGATGAAGGCCACTGA	374 bp
PAMr_nr_mas_02_3:D3	*mexK*	PA14_16820	GACATCCATTCCAGCGAGAT	311 bp
PAMr_nr_mas_05_1:E4	*mexI*	PA14_09520	AACACCAGGGTGTTGCCT	348 bp
PAMr_nr_mas_10_4:E12	*mexW*	PA14_56890	TTACCGATCCGTTCATCCGT	353 bp
PAMr_nr_mas_03_1:A9	*mexD*	PA14_60830	TCGAAGGTGACGACGATCTC	314 bp
PAMr_nr_mas_02_2:C3	*triC*	PA14_01970	TCCGAACGGAAGTTGCCGGA	368 bp
PAMr_nr_mas_02_4:E12*	*mexA*	PA14_05530	TATTGGCTACCGTCCTCCAG	594 bp
PAMr_nr_mas_03_1:G11	*mexN*	PA14_45890	TCCGATCATGATGACCACCCT	334 bp
PAMr_nr_mas_11_4:G11	*mexY*	PA14_38410	AAGCGAGTACGGCTTCGTCT	435 bp
RND-associated outermebrane porins				
PA14 NR set plate position	**Gene name**	**Locus name PA14**	**Primer 5' - 3'**	**Product size**
PAMr_nr_mas_13_4:B8	*oprN*	PA14_32380	TGAACCAGTTGGTCGAACAGT	370 bp
PAMr_nr_mas_11_1:F3	*opmB*	PA14_31920	TTTCCCCGACCTCACCCTGA	653 bp
PAMr_nr_mas_12_3:F3	*opmI*	PA14_13520	AACGAGAGCTGGACGTAGGC	380 bp
PAMr_nr_mas_05_2:F12	*opmG*	PA14_68120	TGCAACACGTCGCTGGTGTG	242 bp
PAMr_nr_mas_09_1:F4	*opmH*	PA14_65750	ACTGGCAGGGTGTGGCGCAT	302 bp
PAMr_nr_mas_08_3:D1	*oprM*	PA14_05550	ACGAGGCGTCCAAGCAGCAA	293 bp
PAMr_nr_mas_05_4:A5	*opmE*	PA14_18790	GCCCTGGAGTCTCTGATCCA	380 bp
PAMr_nr_mas_12_1:D6	*oprJ*	PA14_60820	CGTGCTGTTCGTACCCATCT	353 bp
PAMr_nr_mas_11_3:F4	*opmD*	PA14_09500	TTCGGTGCTCGCTGCCAGTT	215 bp
PA14_31970 **(not available)**	*czcC*	PA14_31970		
Tn7 transposon-specific primer				
PMFLGM.GB-3a			TACAGTTTACGAACCGAACAGGC	-

*the location of the Tn7 insertion in the *mexB* mutant PAM_nr_mas_06_2:H10 could not be validated by PCR

Table S2. Primer sequences used for inverse PCR reactions and sequencing to map Tn5 insertions in PA14*nfxC*

***Nar*I–digested gDNA**	**Primer sequence 5' – 3'**
IPCR primer 1 (Tn*5*-*Nar*I)	CATTCAGGTCGAGGTGGCCCG
IPCR primer 2 (Tn*5*-out2)	CAGAACATATCCATCGCGTCCGCC
Sequencing primer (Tn*5*-out3)	CGGTTTACAAGCATAAAGCTTGC
***Sst*II-digested gDNA**	
IPCR primer 1 (Tn*5*-*Sst*II)	GTCAAAAGGACGATTTCGGTTTGG
IPCR primer 2 (Tn*5*-out)	GATCCCCGGGTACCGAGCTCGAATTC
Sequencing primer (Tn*5*-out-seq)	CCGGGTACCGAGCTCGAATTCG
***Sph*I–digested gDNA**	
IPCR primer 1 (Tn*5*-*Sph*I)	GACATGCGGATGTTATTGTCGCTTGGG
IPCR primer 2 (Tn*5*-out)	GATCCCCGGGTACCGAGCTCGAATTC
Sequencing primer (Tn*5*-out-seq)	CCGGGTACCGAGCTCGAATTCG

Table S3. Inverse PCR reactions and cycling conditions

Reagent	**Volume (µl)**	**Step**	**PCR Cycling Conditions**
Template (Ligation reaction)	2.5	1	95°C for 3 min
10 mM dNTPs (2.5 mM each)	2	2	95°C for 30 sec
10 µM Primer 1	0.5	3	65°C for 1 min (as low as 58°C)
10 µM Primer 2	0.53	4	72°C for 3 min
10 X Invitrogen Buffer	2.5	5	Go to Step 2, 4X
50 mM Invitrogen MgCl2	1	6	95°C for 30 sec
H20	15.9	7	60°C for 1 min (as low as 58°C)
Invitrogen Taq (5 U/ul)	0.125	8	72°C for 3 min
Final Volume	25	9	Go to Step 6, 29X
		10	4°C forever

Supplemental Figure S1.

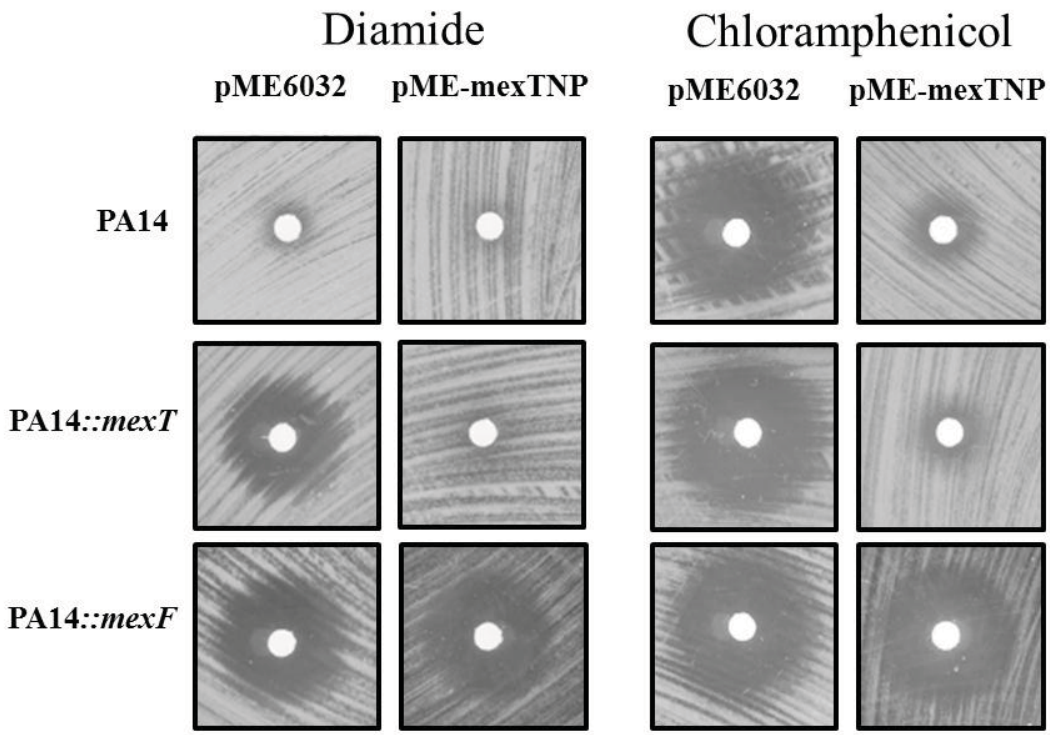

Figure S1. Complementation of diamide susceptibility phenotype. The impact of *mexT* overexpression on diamide and chloramphenicol susceptibility was determined in PA14, PA14*::mexT*, and PA14*::mexF* by disc diffusion as described in materials and methods. Agar was supplemented with 25 μg ml^{-1} tetracycline to select for the pME6032-derived *mexT* overexpression construct and vector control and 1mM IPTG was added to drive expression of *mexT*. Overexpression of *mexT* complemented the diamide resistance phenotype of PA14*::mexT* but not PA14*::mexF* suggesting that *mexT* mediates this effect via *mexF*. In addition, while disruption of *mexT* had no noticeable effect on chloramphenicol resistance levels, *mexT* overexpression leads to increased chloramphenicol resistance which is dependent on *mexF* as previously reported (1). This supports the role of *mexF* in mediating intrinsic diamide resistance via a MexT-dependent induction pathway. Images are representative of three independent experiments.

Supplemental Figure S2.

Chloramphenicol MIC (µg/ml)				
PA14	**PA14*nfxC***	**PA14*nfxC::mexT***	**PA14*nfxC::mexF***	**PA14*nfxC::oprN***
75	>1200	75	75	600

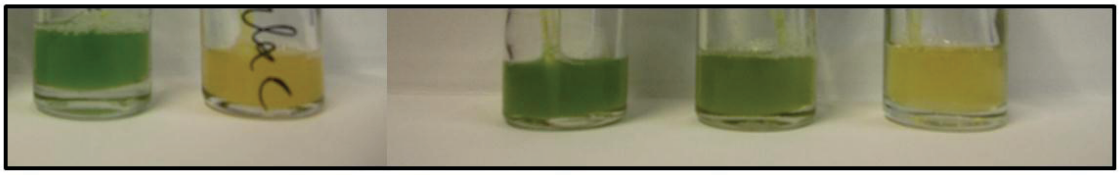

Figure S2. Phenotypic properties of mutants from Tn5 mutagenesis screen for restoration of chloramphenicol susceptibility in PA14*nfxC*. Mutants retrieved from the Tn5 screen with insertions which mapped to *mexT*, *mexF* and *oprN* were investigated. Chloramphenicol susceptibility and phenazine production (green pigment) were restored to wild type levels in PA14*nfxC::mexT* and PA14*nfxC::mexF*. However, Chloramphenicol susceptibility was not fully restored to wild type levels in PA14*nfxC::oprN*, which also exhibited reduced phenazine production compared to PA14*nfxC*.

Supplemental Figure S3.

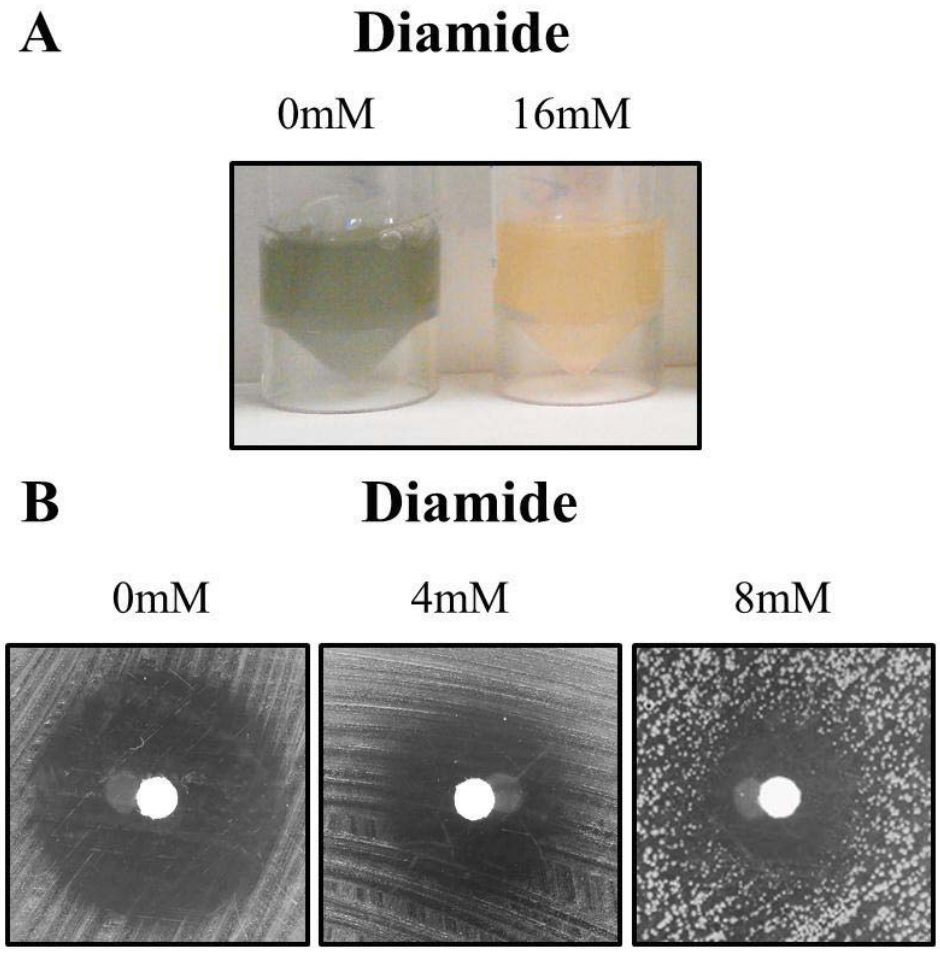

Figure S3. Impact of diamide on phenazine production and chloramphenicol resistance in PA14. Overnight cultures of PA14 grown in LB broth in the presence of 16mM diamide exhibit a marked reduction in phenazine production (A). The inhibitory zone surrounding a chloramphenicol disk (200 μg) was substantially reduced in the presence of 4 or 8 mM diamide (B). Although growth of PA14 on LB agar was somewhat inhibited at 8 mM diamide, a clearly smaller inhibitory zone surrounded the chloramphenicol disk. Results are representative of three independent experiments.

Supplemental Figure S4.

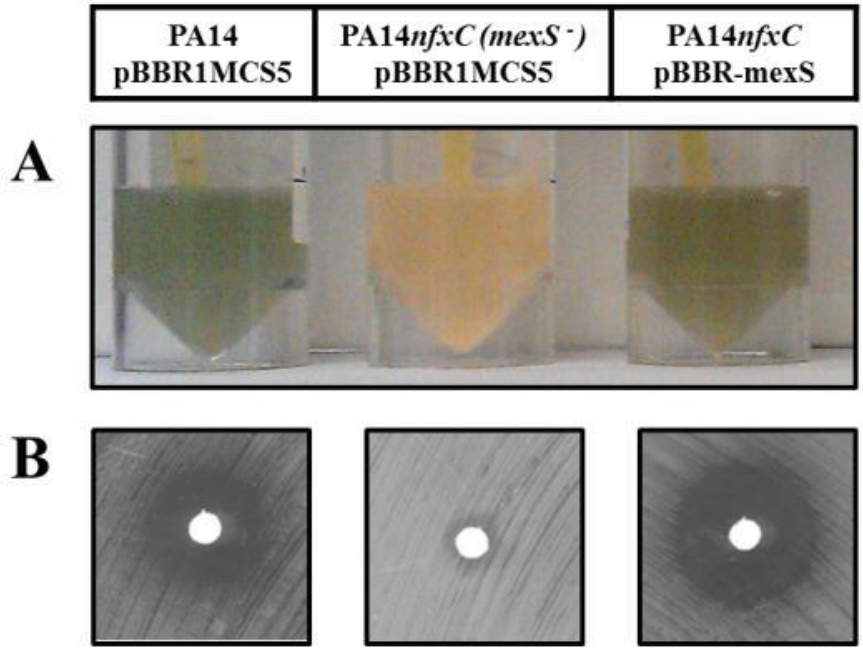

Figure S4. Complementation of phenazine production and chloramphenicol sensitivity in PA14*nfxC*. Overnight cultures of the *mexS* deficient strain PA14*nfxC* grown in LB broth exhibit markedly reduced phenazine production (green pigment), which is restored by complementation with pBBR-mexS (A). The increased chloramphenicol resistance observed in PA14*nfxC* is also complemented when a functional copy of *mexS* is introduced to PA14*nfxC* (B).

1. **Tian, Z. X., M. Mac Aogáin, H. F. O'Connor, E. Fargier, M. J. Mooij, C. Adams, Y. P. Wang, and F. O'Gara.** 2009. MexT modulates virulence determinants in Pseudomonas aeruginosa independent of the MexEF-OprN efflux pump. Microb Pathog **47:**237-41.