Supplemental Materials

Table S1. *P. aeruginosa* efflux liability of antibiotics tested in this manuscript.

Antibiotics	MICs (μg/mL)			
	K767 (WT)	K1455 (↑*mexAB-oprM*)	K2415 (↑*mexXY-oprM*)	K3698 (Δ*oprM*)
Cefpirome	2	8	8	0.5
Imipenem#	2	1	1	2
Levofloxacin	0.5	4	4	0.031
Cotrimoxazole	64	>256	64	4
Doxycycline	32	≥256	32	1
Minocycline	64	>256	32	1
Chloramphenicol	128	>256	128	8
Imipenem#	2	1	1	2

* Not a substrate of RND efflux pumps in P. aeruginosa.

Table S2. Cumulative susceptibility study of *P. aeruginosa* clinical isolates to TXA09155, levofloxacin and the combination.

Drug	Cumulative Susceptibility % Strains												MIC Range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% R[1]
	>32	32	16	8	4	2	1	0.5	0.25	0.12	0.06	≤0.03				
TXA09155	**100**	**36**	**9**	6	3	2	2	-	-	-	-	-	1 - >32	>32	>32	-
LVX	100	98	93	86	78	68	58	47	14	2	-	-	0.03 - >32	1	16	32
LVX/ TXA09155[2]	100	100	100	98	95	92	88	83	78	65	45	13	0.03 - 32	0.12	2	8

[1] Percent resistant per breakpoints (2); LVX ≤1S 2I ≥4R; [2] TXA09155 concentration: 6.25 μg/ml n: 209; LVX: levofloxacin.

Table S3. Cumulative susceptibility study of *P. aeruginosa* clinical isolates to TXA09155, levofloxacin and the combination.

Drug	Cumulative Susceptibility % Strains												MIC Range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% R[1]
	>32	32	16	8	4	2	1	0.5	0.25	0.125	0.06	≤0.03				
TXA09155	**100**	**13**	**9**	7	7	6	2	-	-	-	-	-	0.25 - >32	>32	>32	-
LVX	100	95	89	84	83	78	69	60	27	4	1	-	0.06 - >32	0.5	32	22
LVX/ TXA09155[2]	100	98	94	92	89	88	84	81	78	70	54	18	≤0.03 - >32	0.06	8	12

[1] Percent resistant per breakpoints (2); LVX ≤1S 2I ≥4R; [2] TXA09155 concentration: 6.25 μg/ml n: 300; LVX: levofloxacin.

Table S4. Cumulative susceptibility percentage of *P. aeruginosa* clinical isolates to TXA09155, levofloxacin and the combination.

Drug	Cumulative Susceptibility % Strains												MIC Range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% R[1]
	128	64	32	16	8	4	2	1	0.5	0.25	0.125	0.06	≤0.03				
LVX	**100**	**91**	**62**	38	29	21	15	9	9	0	0	0	0	0.5 - 128	32	64	85
LVX/	100	91	62	44	32	21	15	9	9	0	0	0	0	0.5 - 128	32	64	85

MC-04,124[2]																	
LVX/ TXA01182[2]	100	100	100	94	76	53	44	29	24	18	15	6	3	0.03 - 32	4	16	56
LVX/ TXA09155[2]	100	100	100	100	94	76	65	47	41	24	21	18	9	≤0.03 - 16	2	8	35

[1] Percent resistant per breakpoints (2); LVX ≤1S 2I ≥4R; [2] EPI concentration: 6.25 μg/ml n: 34; LVX: levofloxacin.

Table S5. Levofloxacin potentiation comparison between TXA09155, TXA01182 and MC-04,124 on multidrug-resistant clinical isolates of *P. aeruginosa*.

Strain	Levofloxacin MIC (μg/mL), (fold difference)				Resistance mechanisms
	No EPI	**+ MC-04,124 (6.25 μg/mL)**	**+ TXA01182 (6.25 μg/mL)**	**+ TXA09155 (50 μg/mL)**	
AR-0229	64	64, (1)	8, (8)	2, (32)	*nalC-G71E, mexR-V126Q, gyrA-T83I, OXA-50, PAO*
AR-0230	64	64, (1)	16, (4)	8, (8)	*nalC-G71E, T83I, aac(3)-Id, aadA2, cmlA1, dfrB5, OXA-4, OXA-50, PAO, tet(G), VIM-2*
AR-0231	64	64, (1)	32, (2)	16, (4)	*nalC-G71E, gyrA-T83I, aac(6')-Iic, KPC-5, OXA-2, OXA-50, PAO*
AR-0232	8	8, (1)	1, (8)	0.5, (16)	*nalC-G71E, mexR-V126Q, gyrA-T83I, aadA6, OXA-50, PAO, strA, strB, sul1, tet©*
AR-0233	4	4, (1)	0.5, (8)	0.125, (32)	*nalC-G71E, mexR-V126Q, catB7, OXA-50, PAO*
AR-0234	8	8, (1)	1, (8)	0.5, (16)	*nalC-G71E, mexR-V126Q, gyrA-T83I, aadA6, OXA-50, PAO, strA, strB, tet©*
AR-0236	32	16, (2)	8, (4)	4, (8)	*nalC-G71E, mexR-V126Q, gyrA-T83I, aadB, aph(3')-Iib, OXA-50, PAO*
AR-0239	64	64, (1)	8, (8)	4, (16)	*nalC-G71E, mexR-V126Q, gyrA-T83I, aac(6')-Iia, aadB, aph(3')-Ic, cmlA1, dfrB5, GES-1, OXA-10, OXA-50, strA, strB, tet(G), VIM-11*
AR-0240	128	128, (1)	16, (8)	8, (16)	*nalC-G71E, mexR-V126Q, gyrA-T83I, aadA6, OXA-50, PAO, sul1, VIM-2*
AR-0241	64	64, (1)	16, (4)	8, (8)	*nalC-G71E, mexR-V126Q, gyrA-T83I, aac(6')-Iic, aadA7, catB7, IMP-1, OXA-101, OXA-50, OXA-9, PAO, sul1*
AR-0242	16	8, (2)	2, (8)	1, (16)	*nalC-G71E, gyrA-T83I, aac(3)-Id, aadA2, cmlA1, dfrB5, OXA-4, OXA-50, PAO, VIM-2*
AR-0244	128	128, (1)	8, (16)	2, (64)	*nalC-G71E, mexR-V126Q, gyrA-T133H, OXA-50*
AR-0245	128	128, (1)	32, (4)	16, (8)	*nalC-G71E, mexR-V126Q, gyrA-T83I, aph(3'), OXA, PAO, sul1, VIM-2*
AR-0246	64	64, (1)	16, (4)	8, (8)	*nalC-G71E, mexR-V126Q, gyrA-T83I, aadB, NDM-1, OXA-10, OXA-50, PAO, rmtD2, tet(G), VEB-1*
AR-0247	2	2, (1)	0.031, (64)	≤0.008, (256)	*nalC-G71E, mexR-V126Q, gyrA-T83I, OXA-50, PAO*
AR-0248	16	16, (1)	2, (8)	0.5, (32)	*nalC-G71E, gyrA-T83I, aac(3)-Id, aadA2, cmlA1, dfrB5, OXA-4, OXA-50, PAO, tet(G), VIM-2*
AR-0249	64	64, (1)	8, (8)	2, (32)	*nalC-G71E, gyrA-T83I, aac(3)-Id, aadA2, cmlA1, dfrB5, OXA-4, OXA-50, PAO, tet(G), VIM-2*
AR-0250	64	64, (1)	16, (4)	8, (8)	*nalC-G71E, mexR-V126Q, gyrA-T83I, aadB, NDM-1, OXA-10, OXA-50, PAO, rmtD2, tet(G), VEB-1*
AR-0252	32	32, (1)	2, (16)	0.5, (64)	*nalC-G71E, mexR-V126Q, gyrA-T83I, aadA1, aadA6, OXA-2, OXA-50, PAO, sul1*
AR-0253	2	2, (1)	0.125, (16)	0.031, (64)	*nalC-G71E, mexR-V126Q, gyrA-T83I, catB7, OXA-50, PAO*

AR-0256	0.5	0.5, (1)	0.062, (8)	0.031, (16)	*nalC-G71E, catB7, OXA-50, PAO*
AR-0258	0.5	0.5, (1)	0.125, (4)	0.062, (8)	*nalC-G71E, mexR-V126Q, OXA-50*
AR-0259	0.5	0.5, (1)	0.125, (1)	0.062, (8)	*nalC-G71E, catB7, OXA-50, PAO*
AR-0260	64	64, (1)	16, (4)	8, (8)	*nalC-G71E, gyrA-T83I, catB7, OXA-50, PAO*
AR-0261	8	8, (1)	0.5, (16)	0.25, (32)	*nalC-G71E, mexR-V126Q, OXA-50, PAO*
AR-0262	4	4, (1)	0.25, (16)	0.063, (64)	*nalC-G71E, catB7, OXA-50, PAO*
AR-0264	32	32, (1)	4, (8)	1, (32)	*nalC-G71E, D87Y, OXA-50, PAO*
AR-0265	64	64, (1)	8, (8)	4, (16)	*nalC-G71E, gyrA-T83I, aadB, catB7, OXA-50, PAO*
AR-0266	32	32, (1)	8, (4)	2, (16)	*nalC-G71E, mexR-V126Q, gyrA-T83I, catB7, OXA-50, PAO*
AR-0267	32	32, (1)	2, (16)	0.5, (64)	*nalC-G71E, gyrA-T83I, catB7, OXA-50, PAO*
AR-0269	32	32, (1)	4, (8)	2, (16)	*nalC-G71E, mexR-V126Q, gyrA-T83I, catB7, OXA-50, PAO*
AR-0270	32	16, (2)	2, (16)	0.5, (64)	*nalC-G71E, gyrA-T83I, catB7, OXA-50, PAO*
AR-0271	32	32, (1)	8, (4)	4, (8)	*nalC-G71E, mexR-V126Q, gyrA-T83I, catB7, OXA-50, PAO*
AR-0272	16	16, (1)	4, (4)	2, (8)	*nalC-G71E, mexR-V126Q, gyrA-T83I, catB7, OXA-50, PAO*

The *nalC-G71E* mutation is associated with MexAB-OprM overexpression [46-48]. The *mexR-V126Q* mutation is associated with MexAB-OprM overexpression [49-50]. The *gyrA-T83I or gyrA-T133H* mutations lead to fluoroquinolone resistance [26, 51-52].

Table S6. Susceptibility of *P. aeruginosa* mutants resistant to TXA09155 or TXA09155/levofloxacin combination to various antimicrobials.

Strain	MIC (μg/mL)								
	TXA	**LVX**	**DXC**	**CAZ**	**TGC**	**PMB**	**AMK**	**MEM**	**AZM**
ATCC 27853[#]	50	1	32	2	8	2	8	1	64
EPIR1S	200	1	16	2	8	4	8	2	64
EPIR9S	100	1	16	2	8	2	2	1	64
EPIR20L	200	1	16	2	8	4	8	2	64
EPIR43	ND	0.125	1	0.125	2	4	1	0.125	16
EPIR24L	ND	ND	ND	ND	ND	ND	ND	ND	ND

[#] Parent strain; TXA, TXA09155; LVX, levofloxacin; DXC, doxycycline; CAZ, ceftazidime; TGC, tigecycline; PMB, polymyxin B; AMK, amikacin; MEM, meropenem; AZM, azithromycin; ND, not determined.