Supplementary Figure and Tables

Gene-gene interactions dictate ciprofloxacin resistance in *P. aeruginosa* and facilitate prediction of resistance phenotype from genome sequence data.

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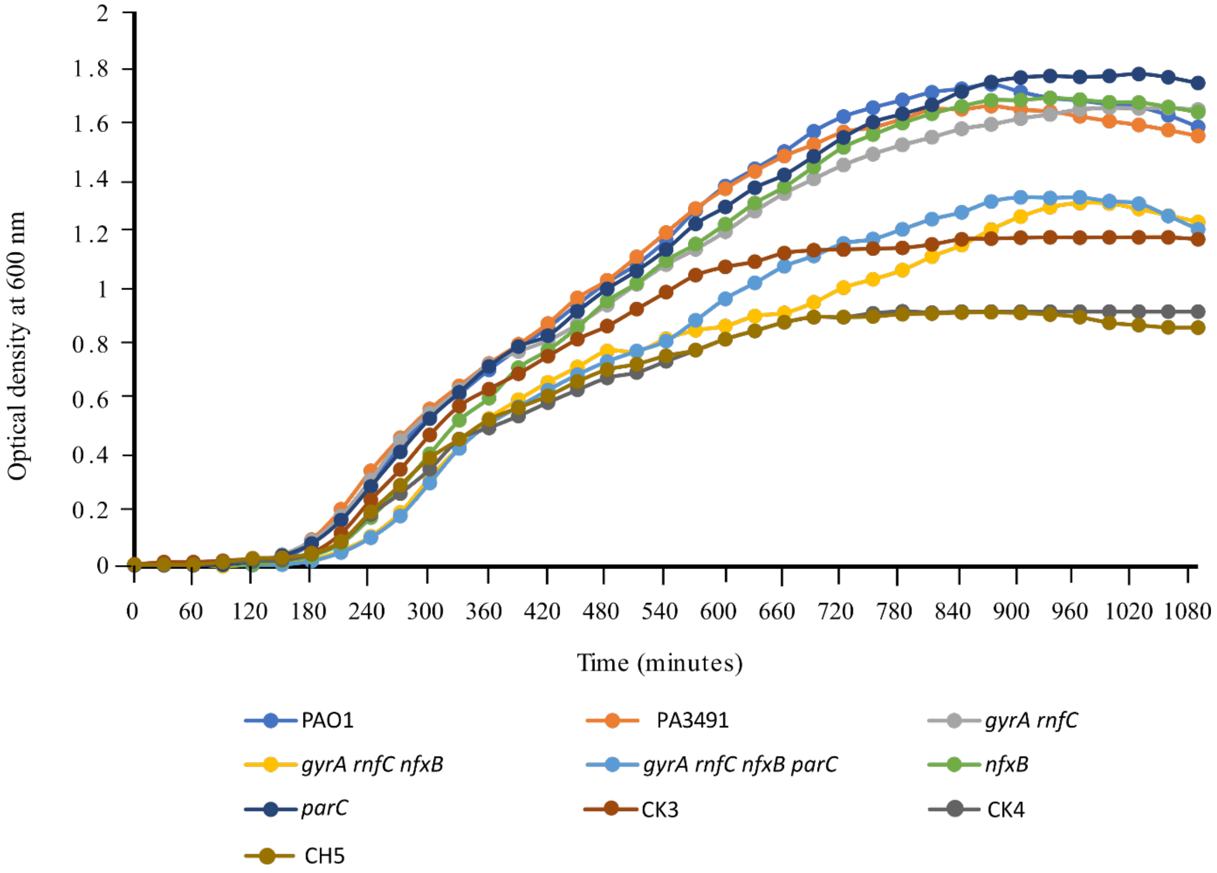

Figure S1: Growth of engineered and lab evolved mutants. Bacteria were grown in L-broth and A600 measured (on y-axis) at 30-minute intervals (on x-axis). Mean value of ODs of 3 biological replicates are shown.

Table S1: Ciprofloxacin MICs of mutants of *P. aeruginosa* PAO1.

Strain	***MIC (mg/L)**
PAO1 wild-type	0.06265 (S)
Experimentally evolved mutants	
PAO1-CF3 *gyrA* $_{\mathrm{T83I}}$ *nfxB*$_{\mathrm{T39P}}$ *rnfC*$_{\mathrm{H87P}}$	4 (R)
PAO1-CF3 *gyrA* $_{\mathrm{T83I}}$ *nfxB*$_{\mathrm{T39P}}$ *rnfC*$_{\mathrm{H87P}}$	4 (R)
PAO1-CK3 *gyrA* $_{\mathrm{T83I}}$ *nfxB*$_{\mathrm{T39P}}$ *rnfC*$_{\mathrm{H87P}}$	4 (R)
PAO1-CC6 *gyrA*$_{\mathrm{T83I}}$ *nfxB*$_{\mathrm{T39P}}$ *rnfC* $_{\mathrm{H87P}}$ *parC*$_{\mathrm{S87L}}$	32 (R)
PAO1-CH5 *gyrA*$_{\mathrm{T83I}}$ *nfxB*$_{\mathrm{T39P}}$ *rnfC* $_{\mathrm{H87P}}$ *parC*$_{\mathrm{S87L}}$	32 (R)
PAO1-CI5 *gyrA*$_{\mathrm{T83I}}$ *nfxB*$_{\mathrm{T39P}}$ *rnfC* $_{\mathrm{H87P}}$ *parC*$_{\mathrm{S87L}}$	32 (R)
PAO1-CK4 *gyrA*$_{\mathrm{T83I}}$ *nfxB*$_{\mathrm{T39P}}$ *rnfC* $_{\mathrm{H87P}}$ *parC*$_{\mathrm{S87L}}$	32 (R)
Engineered mutants	
PAO1 *gyrA*$_{\mathrm{T83I}}$	1 (I)
PAO1 *gyrA*$_{\mathrm{D87G}}$	0.5 (S)
PAO1 *gyrA*$_{\mathrm{D87N}}$	0.25 (S)
PAO1 *nfxB*$_{\mathrm{T39P}}$	0.0625 (S)
PAO1 *rnfC*$_{\mathrm{H87P}}$	0.0625 (S)
PAO1 *parC*$_{\mathrm{S87L}}$	0.0625 (S)
PAO1 *parE*$_{\mathrm{V460G}}$	0.0625 (S)
PAO1 *rnfC* $_{\mathrm{H87P}}$ *nfxB*$_{\mathrm{T39P}}$	0.0625 (S)
PAO1 *rnfC* $_{\mathrm{H87P}}$ *nfxB*$_{\mathrm{T39P}}$ *parC*$_{\mathrm{S87L}}$	0.0625 (S)
PAO1 *rnfC* $_{\mathrm{H87P}}$ *nfxB*$_{\mathrm{T39P}}$ *parE*$_{\mathrm{V460G}}$	0.0625 (S)
PAO1-CH5 *nfxB*$_{\mathrm{T39P}}$ *rnfC* $_{\mathrm{H87P}}$ *parC*$_{\mathrm{S87L}}$ GyrA^{+} knock-in	0.25 (S)
PAO1-CK4 *nfxB*$_{\mathrm{T39P}}$ *rnfC* $_{\mathrm{H87P}}$ *parC*$_{\mathrm{S87L}}$ GyrA^{+} knock-in	0.25 (S)
PAO1 *gyrA*$_{\mathrm{T83I}}$ *parC*$_{\mathrm{S87L}}$	4 (R)
PAO1 *gyrA*$_{\mathrm{T83I}}$ *parE*$_{\mathrm{V460G}}$	2 (R)
PAO1 *gyrA*$_{\mathrm{T83I}}$ *rnfC*$_{\mathrm{H87P}}$	1 (I)
PAO1 *gyrA*$_{\mathit{T83I}}$ *nfxB*$_{\mathrm{T39P}}$	1 (I)
**PAO1 *gyrA*$_{\mathrm{T83I}}$ *nfxB*$_{\mathrm{T39P}}$ *rnfC*$_{\mathrm{H87P}}$	4 (R)

PAO1 *gyrA*$_{\text{T83I}}$ *parC*$_{\text{S87L}}$ *rnfC*$_{\text{H87P}}$	4 (R)
PAO1 *gyrA*$_{\text{T83I}}$ *nfxB*$_{\text{T39P}}$ *parC*$_{\text{S87L}}$	4 (R)
PAO1 *gyrA*$_{\text{T83I}}$ *nfxB*$_{\text{T39P}}$ *parE*$_{\text{V460G}}$	4 (R)
**Knock in WT *rnfC* in PAO1-CK3	2 (R)
**PAO1 *gyrA*$_{\text{T83I}}$ *nfxB*$_{\text{T39P}}$ *rnfC*$_{\text{H87P}}$ *parC*$_{\text{S87L}}$	32 (R)
PAO1 *gyrA*$_{\text{T83I}}$ *nfxB*$_{\text{T39P}}$ *rnfC*$_{\text{H87P}}$ *parE*$_{\text{V460G}}$	32 (R)

*S denotes Susceptible, I denotes Intermediate and R denotes Resistant strains. ** Mutants constructed twice, with the same MIC each time.

Table S2. Relative quantification of *mexD* in different mutants*

Strain	Replicate 1	Replicate 2	Replicate 3	SEM
PAO1	3.81E-3	3.01E-3	2.83E-3	0.0003
PAO1 *gyrA*$_{T83I}$	4.23E-3	3.60E-3	3.60E-3	0.0002
PAO1 *nfxB*$_{T39P}$	0.1150	0.2016	0.1222	0.027
PAO1 gyrA $_{T83I,}$ *nfxB* $_{T39P}$	0.1114	0.1855	0.1215	0.023

*Expression of *mexD* was quantified by RT-qPCR using the method described in the text. Values represent target/reference ratios of crossing points after normalisation of data in three biological replicates. SEM denotes standard error of the mean.

Table S3: Tukey's HSD (Honestly significant difference) test p-values for multiple comparison of expression of *mexD* between mutants*

	PAO1	***gyrA***	***nfxB***
PAO1			
gyrA	1.00		
nfxB	**0.00429**	**0.00446**	
gyrA*, *nfxB	**0.00665**	**0.00691**	0.9

* Input values are from Table S2. Significant p-values ($p < 0.01$) are shown in bold text.

Table S4: Average area under the growth curve of engineered mutants.

Engineered mutants	Area under curve	Average area under curve	±SEM
PAO1 wildtype	1112.477	1123.2	6.2
	1134.12		
	1122.9		
rnfC	1129	1106.4	16.3
	1115.29		
	1074.7		
nfxB	1049.6	1040.5	5.7
	1030		
	1042		
parC	1125	1102	18.6
	1115		
	1065		
gyrA+rnfC	1021	1041.4	18.8
	1079.04		
	1024.12		
gyrA+rnfC+nfxB	721.45	784.7	32.5
	803.64		
	829.04		
gyrA+rnfC+nfxB+parC	851.78	820.5	18.4
	788.08		
	821.51		

Table S5. Mutations in experimentally-evolved PA14**.**

Genes	**Mutant strain***				
	PA14_CB4	PA14_C2	PA14_CC4	PA14_D2	PA14_E4
gyrA	T83I		T83I		T83A
gyrB	Δ 6 bp			$(TCC)_{2\ \text{to}\ 3}$	
parC	S87L		S87L		
nfxB			Y101*		Δ 13 bp
mexS (PA14_32420, Oxidoreductase)		Δ 11 bp		Δ 11 bp	
parE	P394L				

**Five independent ciprofloxacin-resistant mutants of *P. aeruginosa* PA14 were evolved using the gradient selection method (1). Mutations were identified following whole genome sequencing and comparison with the parental PA14 genome. Mutations known to be related to ciprofloxacin resistance are listed.

Table S6: *P. aeruginosa* isolates and genomes used in this study

Isolates IDs	*BioProject	Host	Isolate_country
37	PRJNA325248	Human	Canada
38	PRJNA325248	Human	Canada
65	PRJNA325248	Human	Canada
66	PRJNA325248	Human	Canada
88	PRJNA325248	Human	Denmark
89	PRJNA294726	Human	Belgium
90	PRJNA325248	Human	USA
111	PRJNA325248	Human	Canada
112	PRJNA325248	Human	Canada
113	PRJNA325248	Human	Canada
114	PRJNA325248	Human	Canada
115	PRJNA325248	Human	Canada
116	PRJNA325248	Human	Canada
117	PRJNA325248	Human	Canada
118	PRJNA325248	Human	Canada
119	PRJNA325248	Human	Canada
120	PRJNA325248	Human	Canada
121	PRJNA325248	Human	Canada
122	PRJNA325248	Human	Canada
123	PRJNA325248	Human	Canada
124	PRJNA325248	Human	Canada
125	PRJNA325248	Human	Canada
126	PRJNA325248	Human	Canada
127	PRJNA325248	Human	Canada
128	PRJNA325248	Human	Canada
129	PRJNA325248	Human	Canada
130	PRJNA325248	Human	Canada
131	PRJNA325248	Human	Canada
132	PRJNA325248	Human	Canada
133	PRJNA325248	Human	Canada

Isolates IDs	*BioProject	Host	Isolate_country
134	PRJNA325248	Human	Canada
135	PRJNA325248	Human	Canada
139	PRJNA325248	Human	Canada
140	PRJNA325248	Human	Canada
141	PRJNA325248	Human	Canada
142	PRJNA325248	Human	Canada
143	PRJNA325248	Human	Canada
144	PRJNA325248	Human	Canada
145	PRJNA325248	Human	Canada
146	PRJNA325248	Human	Canada
147	PRJNA325248	Human	Canada
148	PRJNA325248	Human	Canada
149	PRJNA325248	Human	Canada
150	PRJNA325248	Human	Canada
151	PRJNA325248	Human	Canada
152	PRJNA325248	Human	Canada
153	PRJNA325248	Human	Canada
154	PRJNA325248	Human	Canada
155	PRJNA325248	Human	Canada
156	PRJNA325248	Human	Canada
157	PRJNA325248	Human	Canada
158	PRJNA325248	Human	Canada
159	PRJNA325248	Human	Canada
161	PRJNA325248	Human	Canada
162	PRJNA325248	Human	Canada
163	PRJNA325248	Human	Canada
164	PRJNA325248	Human	Canada
166	PRJNA325248	Human	Canada
167	PRJNA325248	Human	Canada
168	PRJNA325248	Human	Canada
169	PRJNA325248	Human	Canada
170	PRJNA325248	Human	Canada

Isolates IDs	***BioProject**	**Host**	**Isolate_country**
171	PRJNA325248	Human	Canada
172	PRJNA325248	Human	Canada
173	PRJNA325248	Human	Canada
174	PRJNA325248	Human	Canada
175	PRJNA325248	Human	Canada
176	PRJNA325248	Human	Canada
177	PRJNA325248	Human	Canada
178	PRJNA325248	Human	Canada
179	PRJNA325248	Human	Canada
180	PRJNA325248	Human	Canada
181	PRJNA325248	Human	Canada
182	PRJNA325248	Human	Canada
183	PRJNA325248	Human	Canada
184	PRJNA325248	Human	Canada
185	In process	Human	Canada
186	In process	Human	Canada
369	PRJNA325248	Environmental	Australia
532	PRJNA325248	Human	Australia
534	PRJNA325248	Human	Australia
664	PRJNA325248	Hospital Environment	Columbia
669	PRJNA325248	Human	Panama
677	PRJNA325248	Human	Bulgaria
688	PRJNA325248	Human	France
689	PRJNA325248	Human	France
690	PRJNA325248	Human	France
691	PRJNA325248	Human	France
692	PRJNA325248	Human	France
693	PRJNA325248	Human	France
694	PRJNA325248	Human	France
695	PRJNA325248	Human	France
696	PRJNA325248	Animal	France
698	PRJNA325248	Environmental	Mexico

Isolates IDs	*BioProject	Host	Isolate_country
699	PRJNA325248	Environmental	Belgium
700	PRJNA325248	clinical isolate	Canada
703	PRJNA325248	Plant	United Kingdom
705	PRJNA325248	Human	Georgian Republic
706	PRJNA325248	Human	Georgian Republic
710	PRJNA325248	Human	Belgium
713	PRJNA325248	Human	Belgium
714	PRJNA294726	Human	Belgium
715	PRJNA325248	Human	Canada
718	PRJNA325248	Human	Colombia
720	PRJNA325248	Animal	India
723	PRJNA325248	Animal	United Kingdom
725	PRJNA325248	Animal	United Kingdom
727	PRJNA325248	Human	USA
728	PRJNA325248	Human	United Kingdom
729	PRJNA325248	Human	United Kingdom
730	PRJNA325248	Human	United Kingdom
731	PRJNA325248	Human	United Kingdom
733	PRJNA325248	Human	USA
734	PRJNA325248	Human	United Kingdom
736	PRJNA325248	Human	United Kingdom
737	PRJNA325248	Human	United Kingdom
738	PRJNA325248	Human	United Kingdom
740	PRJNA325248	Environmental	Tunisia
742	PRJNA325248	Animal	USA
744	PRJNA325248	Animal	USA
747	PRJNA325248	Environmental	Japan
748	PRJNA325248	Environmental	Japan
749	PRJNA325248	Environmental	Japan
750	PRJNA325248	Environmental	Japan
751	PRJNA325248	Environmental	Japan
752	PRJNA325248	Environmental	Japan

Isolates IDs	*BioProject	Host	Isolate_country
754	PRJNA325248	Environmental	Japan
755	PRJNA325248	Environmental	Japan
756	PRJNA325248	Animal	Japan
757	PRJNA325248	Environmental	Japan
758	PRJNA325248	Environmental	Japan
759	PRJNA325248	Environmental	Japan
760	PRJNA325248	Environmental	Japan
761	PRJNA325248	Environmental	Japan
762	PRJNA325248	Environmental	Japan
769	In process	Animal	Portugal
775	PRJNA325248	Animal	Portugal
776	PRJNA325248	Animal	Portugal
780	PRJNA325248	Animal	Portugal
782	PRJNA325248	Animal	Portugal
785	PRJNA325248	Animal	Portugal
786	PRJNA325248	Animal	Portugal
789	PRJNA325248	Animal	Portugal
790	PRJNA325248	Animal	Portugal
792	In process	Animal	Portugal
796	PRJNA325248	Animal	Portugal
797	PRJNA325248	Animal	Portugal
810	PRJNA294726	Human	United Kingdom
811	PRJNA294726	Human	United Kingdom
824	PRJNA325248	Environmental	Belgium
825	PRJNA325248	Environmental	Belgium
826	PRJNA325248	Environmental	Belgium
828	PRJNA325248	Environmental	Belgium
830	PRJNA325248	Environmental	Belgium
831	PRJNA325248	Environmental	Belgium
838	PRJNA325248	Human	Belgium
852	PRJNA294726	Human	United Kingdom
856	PRJNA325248	Human	USA

Isolates IDs	***BioProject**	**Host**	**Isolate_country**
858	PRJNA325248	Human	Germany
860	PRJNA325248	Human	USA
867	PRJNA325248	Human	USA
871	PRJNA294726	Human	Belgium
875	PRJNA325248	Environmental	Benin
877	PRJNA325248	Environmental	Puerto Rico
878	PRJNA325248	Environmental	Puerto Rico
879	PRJNA325248	Human	Congo
880	PRJNA325248	Human	Philippines
881	PRJNA325248	Environmental	Germany
884	PRJNA325248	Human	Tunisia
885	PRJNA325248	Human	Tunisia
934	PRJNA325248	Human	Australia
946	PRJNA325248	Human	Australia
948	PRJNA325248	Human	Australia
954	PRJNA325248	Human	Australia
976	PRJNA325248	Human	Australia
977	PRJNA325248	Human	Australia
1053	PRJNA325248	Human	United Kingdom
1074	PRJNA325248	Human	United Kingdom
1108	PRJNA325248	Human	Thailand
1112	PRJNA325248	Human	Thailand
1124	PRJNA325248	Human	Thailand
1130	PRJNA325248	Human	Thailand
1133	PRJNA325248	Human	Thailand
1152	PRJNA325248	Human	Thailand
1180	PRJNA325248	Human	USA
1182	PRJNA325248	Human	USA
1184	PRJNA325248	Human	USA
1241	PRJNA325248	Human	Brazil
1242	PRJNA325248	Human	Brazil
1243	PRJNA325248	Human	Brazil

Isolates IDs	***BioProject**	**Host**	**Isolate_country**
1244	PRJNA325248	Human	Brazil
1245	PRJNA325248	Human	Brazil
1269	PRJNA325248	Human	France
1296	PRJNA325248	Human	United Kingdom
1506	PRJNA325248	Human	Canada
1530	PRJNA326244	Human	Italy
1532	PRJNA326244	Human	Italy
1533	PRJNA326244	Human	Italy
1534	PRJNA326244	Human	Italy
1535	PRJNA326244	Human	Italy
1536	PRJNA326244	Human	Italy
1537	PRJNA326244	Human	Italy
1538	PRJNA326244	Human	Italy
1539	PRJNA326244	Human	Italy
1540	PRJNA326244	Human	Italy
1541	PRJNA326244	Human	Italy
1542	PRJNA326244	Human	Italy
1543	PRJNA326244	Human	Italy
1544	PRJNA326244	Human	Italy
1545	PRJNA326244	Human	Italy
1546	PRJNA326244	Human	Italy
1547	PRJNA326244	Human	Italy
1548	PRJNA326244	Human	Italy
1549	PRJNA326244	Human	Italy
1550	PRJNA326244	Human	Italy
1551	PRJNA326244	Human	Italy
1553	PRJNA326244	Human	Italy
1554	PRJNA326244	Human	Italy
1555	PRJNA326244	Human	Italy
1557	PRJNA326244	Human	Italy
1558	PRJNA326244	Human	Italy
1559	PRJNA326244	Human	Italy

Isolates IDs	***BioProject**	**Host**	**Isolate_country**
1560	PRJNA326244	Human	Italy
1561	PRJNA326244	Human	Italy
1562	PRJNA326244	Human	Italy
1563	PRJNA326244	Human	Italy
1564	PRJNA326244	Human	Italy
1565	PRJNA326244	Human	Italy
1576	PRJNA325248	Human	USA
1577	PRJNA325248	Human	USA
1578	PRJNA325248	Human	USA
1579	PRJNA325248	Human	USA
1580	PRJNA325248	Human	USA
1581	PRJNA325248	Human	USA
1582	PRJNA325248	Human	USA
1590	PRJNA325248	Human	Canada
1594	PRJNA325248	Human	Canada
1603	PRJNA325248	Human	Canada
1252420	PRJNA649313	Human	Australia
1254434	PRJNA649313	Human	Australia
1255885	PRJNA649313	Human	Chile
1257147	PRJNA649313	Human	Argentina
1257763	PRJNA649313	Human	Italy
1260410	PRJNA649313	Human	Czech Republic
1260715	PRJNA649313	Human	Turkey
1260990	PRJNA649313	Human	Greece
1265577	PRJNA649313	Human	Poland
1266654	PRJNA649313	Human	Russia
1268230	PRJNA649313	Human	Spain
1271047	PRJNA649313	Human	Germany
1271701	PRJNA649313	Human	Israel
1271954	PRJNA649313	Human	Israel
1272032	PRJNA649313	Human	Czech Republic
1275655	PRJNA649313	Human	Mexico

Isolates IDs	*BioProject	Host	Isolate_country
1275666	PRJNA649313	Human	Mexico
1279293	PRJNA649313	Human	United Kingdom
1280466	PRJNA649313	Human	Spain
1281476	PRJNA649313	Human	United States
1292777	PRJNA649313	Human	Venezuela
1295835	PRJNA649313	Human	Italy
1300097	PRJNA649313	Human	Mexico
1301482	PRJNA649313	Human	Mexico
1313352	PRJNA649313	Human	Malaysia
1324459	PRJNA649313	Human	Romania
1344658	PRJNA649313	Human	Turkey
1417546	PRJNA649313	Human	Mexico
1419840	PRJNA649313	Human	Colombia
1420275	PRJNA649313	Human	Mexico
1423253	PRJNA649313	Human	Belgium
1449019	PRJNA649313	Human	United States
1459372	PRJNA649313	Human	Philippines
1586981	PRJNA649313	Human	Belgium
1586994	PRJNA649313	Human	Belgium
1607533	PRJNA649313	Human	Russia
1679579	PRJNA649313	Human	Hungary
1690076	PRJNA649313	Human	Mexico
1709299	PRJNA649313	Human	Brazil

*Isolates in Bioproject PRJNA325248 were from (2), (3), (4), Project PRJNA294726 were from (5) and Project PRJNA326244 were from (6). Details of *P. aeruginosa* isolates and genome data are described in the International Pseudomonas Consortium Database (https://ipcd.ibis.ulaval.ca).

Table S7. MICs and presence or absence of genetic variations in analysed genes of *P. aeruginosa* isolates.

Isolates with actual MIC the same as predicted MIC										
No analysed resistant alleles										
	Clinical isolate's IDs	**Actual MICs**	***gyrA* (T83I)**	***parC* (S87L)**	***parE***	***nfxB***	***gyrB***	**CrpP**	**Corresponding engineered mutants**	**Predicted MIC**
1.	116	0.05	no	no	no	no	no	no	WT *gyrA*	S (<1)
2.	122	0.5	no	no	no	no	no	no	WT *gyrA*	S (<1)
3.	124	0.125	no	no	no	no	no	no	WT *gyrA*	S (<1)
4.	125	0.125	no	no	no	no	no	no	WT *gyrA*	S (<1)
5.	127	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
6.	130	0.5	no	no	no	no	no	no	WT *gyrA*	S (<1)
7.	145	0.5	no	no	no	no	no	no	WT *gyrA*	S (<1)
8.	146	0.5	no	no	no	no	no	no	WT *gyrA*	S (<1)
9.	149	0.5	no	no	no	no	no	no	WT *gyrA*	S (<1)
10.	152	0.125	no	no	no	no	no	no	WT *gyrA*	S (<1)
11.	158	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
12.	161	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
13.	162	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
14.	163	0.5	no	no	no	no	no	no	WT *gyrA*	S (<1)
15.	164	0.05	no	no	no	no	no	no	WT *gyrA*	S (<1)
16.	167	0.125	no	no	no	no	no	no	WT *gyrA*	S (<1)

17.	169	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
18.	171	0.5	no	no	no	no	no	no	WT *gyrA*	S (<1)
19.	177	0.05	no	no	no	no	no	no	WT *gyrA*	S (<1)
20.	178	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
21.	184	0.25	T83A	no	no	no	no	no	WT *gyrA*	S (<1)
22.	185	0.05	no	no	no	no	no	no	WT *gyrA*	S (<1)
23.	186	0.5	no	no	no	no	no	no	WT *gyrA*	S (<1)
24.	**369**	0.125	no	no	no	no	no	no	WT *gyrA*	S (<1)
25.	688	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
26.	689	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
27.	690	0.5	no	no	no	no	no	no	WT *gyrA*	S (<1)
28.	691	0.5	no	no	no	no	no	no	WT *gyrA*	S (<1)
29.	692	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
30.	693	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
31.	694	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
32.	695	0.25	no	no	no	A170T	no	no	WT *gyrA*	S (<1)
33.	696	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
34.	698	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
35.	699	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
36.	703	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
37.	710	0.5	no	no	no	no	no	no	WT *gyrA*	S (<1)
38.	714	0.5	no	no	no	no	no	no	WT *gyrA*	S (<1)
39.	725	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
40.	727	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)

41.	728	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
42.	729	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
43.	730	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
44.	731	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
45.	733	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
46.	736	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
47.	738	0.5	no	no	no	no	no	no	WT *gyrA*	S (<1)
48.	742	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
49.	749	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
50.	750	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
51.	751	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
52.	752	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
53.	754	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
54.	755	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
55.	757	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
56.	758	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
57.	759	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
58.	760	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
59.	761	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
60.	762	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
61.	769	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
62.	775	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
63.	780	0.5	no	no	no	no	no	no	WT *gyrA*	S (<1)
64.	785	0.5	no	no	no	no	no	no	WT *gyrA*	S (<1)

65.	786	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
66.	790	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
67.	792	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
68.	796	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
69.	824	0.5	no	no	no	no	no	no	WT *gyrA*	S (<1)
70.	828	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
71.	831	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
72.	856	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
73.	860	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
74.	867	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
75.	875	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
76.	877	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
77.	881	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
78.	884	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
79.	885	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
80.	**1108**	0.125	no	no	no	no	no	no	WT *gyrA*	S (<1)
81.	1533	0.5	no	no	no	no	no	no	WT *gyrA*	S (<1)
82.	1535	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
83.	1539	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
84.	1579	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
85.	1580	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
86.	1581	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
87.	1252420	0.064	no	no	no	no	no	no	WT *gyrA*	S (<1)
88.	1254434	0.064	no	no	no	no	no	no	WT *gyrA*	S (<1)

89.	1265577	0.064	no	no	no	no	no	no	WT *gyrA*	S (<1)
90.	1271047	0.064	no	no	no	no	no	no	WT *gyrA*	S (<1)
91.	1275666	0.064	no	no	no	no	no	no	WT *gyrA*	S (<1)
92.	1280466	0.064	no	no	no	no	no	no	WT *gyrA*	S (<1)
93.	1301482	0.064	no	no	no	no	no	no	WT *gyrA*	S (<1)
94.	1313352	0.25	no	no	no	no	no	no	WT *gyrA*	S (<1)
95.	1417546	0.38	no	no	no	no	no	no	WT *gyrA*	S (<1)
96.	1419840	0.5	no	no	no	no	no	no	WT *gyrA*	S (<1)
97.	1459372	0.094	no	no	no	no	no	no	WT *gyrA*	S (<1)
98.	1679579	0.38	no	no	no	no	no	no	WT *gyrA*	S (<1)
99.	1292777	0.064	no	no	no	no	no	no	WT *gyrA*	S (<1)
100.	120	1 (I)	no	no	no	no	no	no	WT *gyrA*	S (<1)
101.	121	1 (I)	no	no	no	no	no	no	WT *gyrA*	S (<1)
102.	128	1 (I)	no	no	no	no	no	no	WT *gyrA*	S (<1)
103.	129	1 (I)	no	no	no	no	no	no	WT *gyrA*	S (<1)
104.	133	1 (I)	no	no	no	no	no	no	WT *gyrA*	S (<1)
105.	170	1(I)	no	no	no	no	no	no	WT *gyrA*	S (<1)
106.	715	1 (I)	no	no	no	no	no	no	WT *gyrA*	S (<1)
107.	720	1 (I)	no	no	no	no	no	no	WT *gyrA*	S (<1)
108.	782	1 (I)	no	no	no	no	no	no	WT *gyrA*	S (<1)
109.	880	1 (I)	no	no	no	no	no	no	WT *gyrA*	S (<1)
110.	1550	1 (I)	no	no	no	no	no	no	WT *gyrA*	S (<1)
111.	1564	1 (I)	no	N170S	R30C+H59R	no	no	no	WT *gyrA*	S (<1)
112.	1577	1 (I)	no	no	no	no	no	no	WT *gyrA*	S (<1)

***gyrA* T83I and *parC* S87**										
1.	**89**	>4	yes	yes	no	no	no	no	*gyrA + parC*	R (4)
2.	**664**	>4	yes	yes	no	no	no	no	*gyrA + parC*	R (4)
3.	**669**	>4	yes	yes	no	no	no	no	*gyrA + parC*	R (4)
4.	**706**	>4	yes	yes	no	no	no	no	*gyrA + parC*	R (4)
5.	**718**	>4	yes	yes	no	no	no	no	*gyrA + parC*	R (4)
6.	**871**	>4	yes	yes	no	no	no	yes	*gyrA + parC*	R (4)
7.	1053	4	yes	yes	no	no	no	no	*gyrA + parC*	R (4)
8.	**1112**	>4	yes	yes	no	no	no	no	*gyrA + parC*	R (4)
9.	**1122**	4	yes+D87H	yes	E215Q	no	no	no	*gyrA + parC*	R (4)
10.	**1124**	>4	yes	yes	E215Q	no	no	no	*gyrA + parC*	R (4)
11.	**1130**	>4	yes	yes	no	no	no	no	*gyrA + parC*	R (4)
12.	**1133**	4	yes	yes	no	no	no	no	*gyrA + parC*	R (4)
13.	**1152**	4	yes+D87N	yes	no	no	no	yes	*gyrA + parC*	R (4)
14.	1182	4	yes	yes	no	no	no	no	*gyrA + parC*	R (4)
15.	**1184**	4	yes	yes	no	no	no	no	*gyrA + parC*	R (4)
16.	**1241**	>4	yes	yes	no	no	no	yes	*gyrA + parC*	R (4)
17.	**1242**	>4	yes	yes	no	no	no	yes	*gyrA + parC*	R (4)
18.	**1243**	>4	yes	yes	no	no	no	yes	*gyrA + parC*	R (4)
19.	**1244**	>4	yes	yes	no	no	no	yes	*gyrA + parC*	R (4)
20.	**1245**	>4	yes	yes	no	no	no	yes	*gyrA + parC*	R (4)
21.	**1269**	4	yes	yes	no	no	no	no	*gyrA + parC*	R (4)
22.	1255885	R	yes	yes	no	no	no	no	*gyrA + parC*	R (4)
23.	1257147	R	yes	yes	no	no	no	no	*gyrA + parC*	R (4)

24.	1260990	R	yes	yes	no	no	no	no	*gyrA* + *parC*	R (4)
25.	1275655	6	yes	yes	no	no	no	no	*gyrA* + *parC*	R (4)
26.	1324459	R	yes	yes	no	no	no	no	*gyrA* + *parC*	R (4)
27.	1344658	R	yes + D87	yes	no	no	no	no	*gyrA* + *parC*	R (4)
28.	1420275	R	yes	yes + S197L/E	no	no	no	no	*gyrA* + *parC*	R (4)
29.	1586981	R	yes	yes	no	no	no	no	*gyrA* + *parC*	R (4)
30.	1690076	R	yes	yes + S197L/E	no	no	no	no	*gyrA* + *parC*	R (4)
31.	1268230	R	yes + D87	yes + L168Q	no	no	no	Yes	*gyrA* + *parC*	R (4)
***gyrA* T83I**										
1.	179	1 (I)	yes	no	no	no	no	no	*gyrA* T83I	I (1)
2.	797	1 (I)	yes	no	no	no	no	no	*gyrA* T83I	I (1)
3.	**1074**	1 (I)	yes	no	D142N	no	no	no	*gyrA* T83I	I (1)
4.	**1603**	1 (I)	yes	no	no	no	no	no	*gyrA* T83I	I (1)
5.	1266654	1.5	yes	no	no	no	no	no	*gyrA* T83I	1(I)
6.	811	2 (R)	yes	no	D142N	no	no	no	*gyrA* T83I	I (1)
7.	852	2 (R)	yes	no	no	no	no	no	*gyrA* T83I	I (1)
8.	858	2 (R)	yes	no	no	no	no	no	*gyrA* T83I	I (1)
9.	**934**	2 (R)	yes	no	no	no	no	no	*gyrA* T83I	I (1)
10.	**946**	2 (R)	yes	no	no	no	no	no	*gyrA* T83I	I (1)
11.	**948**	2 (R)	yes	no	no	no	no	no	*gyrA* T83I	I (1)
12.	**65**	0.5 (S)	yes	no	no	A30V	no	no	*gyrA* T83I	I (1)
13.	112	0.5 (S)	yes	no	no	no	no	no	*gyrA* T83I	I (1)
14.	143	0.5 (S)	yes	no	no	A167D	no	no	*gyrA* T83I	I (1)
15.	174	0.5 (S)	yes	no	no	no	no	no	*gyrA* T83I	I (1)

16.	180	0.5 (S)	yes	no	no	no	no	no	*gyrA* T83I	I (1)
17.	181	0.5 (S)	yes	no	no	no	no	no	*gyrA* T83I	I (1)
WT *gyrA*$_{83}$ in combination with other resistance alleles in *gyrB/nfxB/parC/parE*										
1.	**37**	0.25	no	no	no	R21H+A168D+D56G	no	no	*nfxB* T39P	S (<1)
2.	**38**	0.5	no	no	no	R21H+A168D+D56G	no	no	*nfxB* T39P	S (<1)
3.	**88**	0.5	D87N	no	yes	R82L	no	no	*gyrA* D87N	S (<1)
4.	723	0.25	no	no	no	R21H+D56G	no	no	*nfxB* T39P	S (<1)
5.	737	0.25	no	no	A473T	R21H	no	no	*parE* + *nfxB*	S (<1)
6.	740	0.25	no	no	no	R21H+D56G	no	no	*nfxB* T39P	S (<1)
7.	**825**	0.25	no	no	yes	no	no	no	*parE*	S (<1)
8.	**830**	0.25	no	no	yes	no	no	no	*parE*	S (<1)
9.	1578	0.25	no	no	no	R82L	no	no	*nfxB*	S (<1)
10.	111	0.5	no	no	no	no	P749S	no	*parE*	S (<1)
11.	113	0.5	D87N	no	no	no	no	no	*gyrA* D87N	S (<1)
12.	114	0.125	D87N	no	no	no	no	no	*gyrA* D87N	S (<1)
13.	115	0.125	D87N	no	no	no	no	no	*gyrA* D87N	S (<1)
14.	131	0.05	D87Y	no	no	no	no	yes	*gyrA* D87N	S (<1)
15.	132	0.05	D87Y	no	no	no	no	yes	*gyrA* D87N	S (<1)
16.	144	0.5	no	no	no	no	P749S	no	WT *gyrA*	S (<1)
17.	147	0.5	no	no	no	no	P749S	no	WT *gyrA*	S (<1)
18.	153	0.25	no	no	no	no	P749S	no	WT *gyrA*	S (<1)
19.	154	0.25	no	no	no	no	P749S	no	WT *gyrA*	S (<1)
20.	155	0.25	no	no	no	no	P749S	no	WT *gyrA*	S (<1)

21.	156	0.5	no	no	no	no	P749S	no	WT *gyrA*	S (<1)
22.	157	0.5	no	no	no	no	P749S	no	WT *gyrA*	S (<1)
23.	176	0.25	no	no	no	Yes	no	no	*nfxB*	S (<1)
24.	182	0.5	no	no	no	no	P749S	no	WT *gyrA*	S (<1)
25.	183	0.05	no	no	no	no	S466F	no	WT *gyrA*	S (<1)
26.	734	0.25	no	no	E215Q	no	no	yes	WT *gyrA*	S (<1)
27.	744	0.25	no	no	no	Del+E75G	no	no	WT *gyrA*	S (<1)
28.	747	0.25	no	no	no	no	no	yes	WT *gyrA*	S (<1)
29.	748	0.25	no	no	no	no	no	yes	WT *gyrA*	S (<1)
30.	756	0.25	no	no	no	no	no	yes	WT *gyrA*	S (<1)
31.	776	0.25	no	no	no	Del+E75G	no	no	WT *gyrA*	S (<1)
32.	789	0.25	no	no	no	no	no	yes	WT *gyrA*	S (<1)
33.	878	0.25	no	no	no	no	no	yes	WT *gyrA*	S (<1)
34.	1530	0.25	no	no	no	no	P749S	no	WT *gyrA*	S (<1)
35.	1536	0.25	no	no	no	no	P749S	no	WT *gyrA*	S (<1)
36.	1537	0.5	no	no	no	no	P749S	no	WT *gyrA*	S (<1)
37.	1538	0.5	no	no	no	no	P749S	no	WT *gyrA*	S (<1)
38.	1540	0.5	no	no	no	no	P749S	no	WT *gyrA*	S (<1)
39.	1541	0.12	no	no	no	no	P749S	no	WT *gyrA*	S (<1)
40.	1542	0.25	no	no	no	no	P749S	no	WT *gyrA*	S (<1)
41.	1271954	0.064	no	no	A342V	no	no	no	WT *gyrA*	S (<1)
42.	1279293	0.032	no	T364A	no	no	no	no	WT *gyrA*	S (<1)
43.	1709299	0.125	no	A421V	no	no	no	no	WT *gyrA*	S (<1)
44.	123	1 (I)	no	no	no	R82L	no	yes	WT *gyrA*	S (<1)

45.	**532**	1 (I)	no	no	A473V + 533	no	S466F	no	*parE*	S (<1)
46.	810	1 (I)	no	no	no	P22Q	P749H	no	*nfxB*	S (<1)
47.	117	1 (I)	no	no	no	no	S466F	no	WT *gyrA*	S (<1)
48.	118	1 (I)	no	no	no	no	S466F	no	WT *gyrA*	S (<1)
49.	119	1 (I)	no	no	no	no	S466F	no	WT *gyrA*	S (<1)
50.	126	1 (I)	D87N	no	no	no		no	WT *gyrA*	S (<1)
51.	148	1 (I)	no	no	no	no	R138H	no	WT *gyrA*	S (<1)
52.	150	1 (I)	no	no	no	no	P749S	no	WT *gyrA*	S (<1)
53.	151	1 (I)	no	no	no	no	P749S	no	WT *gyrA*	S (<1)
54.	159	1 (I)	no	no	no	no	P749S	no	WT *gyrA*	S (<1)
55.	713	1 (I)	no	no	no	no	S466Y	yes	WT *gyrA*	S (<1)
56.	1576	1 (I)	no	no	R30C+H59R	no	P749H	no	WT *gyrA*	S (<1)
57.	1300097	1	no	no	no	no	S466F/Y	no	*gyrB*	S (<1)
gyrA$_{\mathbf{T83I}}$ **and** ***parE***$_{\mathbf{473/457/460/438}}$										
1.	**677**	2 (R)	yes	no	Yes (S457C)	no	no	no	*gyrA + parE*	R (≥2)
2.	**954**	4 (R)	yes	no	P438S	no	no	no	*gyrA + parE*	R (≥2)
3.	**1594**	2 (R)	yes	no	yes (S457C)	no	no	no	*gyrA + parE*	R (≥2)
4.	1295835	R	yes	no	P438S	no	no	no	*gyrA + parE*	R (≥2)
gyrA$_{\mathbf{T83I}}$ **+** ***nfxB*** **and** ***gyrA***$_{\mathbf{T83I}}$ **+** ***nfxB*** **+** ***parE***$_{\mathbf{473/457/460/438}}$										
1.	139	2 (R)	yes	no	yes	R42C	no	no	*gyrA + parE + nfxB*	R (≥2)
2.	**1590**	2 (R)	yes	no	no	DEL+E75G	no	no	gyrA T83I + *nfxB* del	R (≥2)
3.	**1506**	1.25 (I)	yes	no	no	no	no	yes	*gyrA* T83I	I (1)

4.	1449019	8	yes	no	no	R82L/H/E	no	no	*gyrA + nfxB*	R (≥2)
5.	1586994	R	yes	yes	no	D138Y	no	no	*gyrA + parC + nfxB*	R (≥2)
Isolates with actual MICs higher than predicted MICs										
No resistance allele in *gyrA*$_{83}$/*nfxB*/*parC*$_{87}$/*parE*$_{473/457/460/438}$										
1.	700	2 (R)	no	no	no	no	no	no	WT *gyrA*	S (<1)
2.	826	4 (R)	no	no	no	no	no	no	WT *gyrA*	S (<1)
3.	838	2 (R)	no	no	no	no	no	no	WT *gyrA*	S (<1)
4.	879	4 (R)	no	no	no	no	no	no	WT *gyrA*	S (<1)
5.	1532	2 (R)	no	N170S	no	no	no	no	WT *gyrA*	S (<1)
6.	1534	2 (R)	no	no	no	no	no	no	WT *gyrA*	S (<1)
7.	1543	2 (R)	no	no(N170S)	no	no	no	no	WT *gyrA*	S (<1)
8.	1544	2 (R)	no	no(N170S)	R30C+H59R	no	no	no	WT *gyrA*	S (<1)
9.	1545	2 (R)	no	no(N170S)	no	no	no	no	WT *gyrA*	S (<1)
10.	1546	2 (R)	no	no	no	no	no	no	WT *gyrA*	S (<1)
11.	1548	2 (R)	no	no	no	no	no	no	WT *gyrA*	S (<1)
12.	1549	2 (R)	no	No	R30C+H59R	no	no	no	WT *gyrA*	S (<1)
13.	1551	2 (R)	no	No	no	no	no	no	WT *gyrA*	S (<1)
14.	1553	2 (R)	no	No	R30C+H59R	no	no	no	WT *gyrA*	S (<1)
15.	1554	2 (R)	no	No	no	no	no	no	WT *gyrA*	S (<1)
16.	1555	2 (R)	no	No	no	no	no	no	WT *gyrA*	S (<1)
17.	1557	2 (R)	no	N170S	no	no	no	no	WT *gyrA*	S (<1)
18.	1558	2 (R)	no	N170S	R30C+H59R	no	no	no	WT *gyrA*	S (<1)
19.	1559	2 (R)	no	No	no	no	no	no	WT *gyrA*	S (<1)

20.	1560	2 (R)	no	No	no	no	no	no	WT *gyrA*	S (<1)
21.	1561	2 (R)	no	N170S	R30C+H59R	no	no	no	WT *gyrA*	S (<1)
22.	1563	2 (R)	no	N170S	R30C+H59R	no	no	no	WT *gyrA*	S (<1)
23.	1565	2 (R)	no	N170S	R30C+H59R	no	no	no	WT *gyrA*	S (<1)
24.	1582	2 (R)	no	no	no	no	no	no	WT *gyrA*	S (<1)
WT *gyrA*$_{83}$ in combination with resistance alleles in gyrB/*nfxB*/*parC*/*parE* or *crpP* present										
1.	168	2 (R)	no	no	no	**R82L**	no	no	WT *gyrA*	S (<1)
2.	1547	2 (R)	no	no	no	no	no	yes	WT *gyrA*	S (<1)
3.	1562	2 (R)	no	N170S	R30C+H59R	no	no	yes	WT *gyrA*	S (<1)
4.	135	2 (R)	D87N	no	no	no	no	no	*gyrA* D87N	S (0.25)
5.)	1423253	3 (R)	no	no	no	R163P	S466F/Y	no	*gyrB*	S (<1)
6.	1281476	3 (R)	no	no	no	no	P749S	no	*gyrB*	S (<1)
Only *gyrA*										
1.	166	8 (R)	yes	no	no	no	no	no	*gyrA* T83I	1 (I)
2.	175	4 (R)	yes	no	no	no	no	no	*gyrA* T83I	1 (I)
3.	**976**	4 (R)	yes	no	no	no	no	no	*gyrA* T83I	I (1)
4.	**1180**	4 (R)	yes	no	no	no	no	no	*gyrA* T83I	I (1)
5.	1271701	1.5	D87N	no	no	no	no	no	*gyrA* D87N	S (<1)
6.	1257763	12 (R)	D87N	no	no	no	no	no	*gyrA* D87N	S (<1)
gyrA* T83I and *parC										
1.	1272032	24 (R)	yes	yes	no	no	no	no		R (4)
gyrA* T83I, *nfxB*, *parC										
1.	1607533	12 (R)	yes	yes	no	R82L	no	no		4 (R)

Isolates with actual MIC less than predicted MIC										
Only *gyrA*T83I										
1.	**66**	0.25 (S)	yes	no	no	A30V	no	no	*gyrA* T83I	I (1)
2.	**142**	0.25 (S)	yes	no	no	A167D	no	no	*gyrA* T83I	I (1)
3.	**172**	0.05 (S)	yes	no	no	no	no	no	*gyrA* T83I	I (1)
4.	**173**	0.05 (S)	yes	no	no	no	no	no	*gyrA* T83I	I (1)
5.	**977**	0.25 (S)	yes	no	no	no	no	no	*gyrA* T83I	I (1)
gyrA* T83I + *parE*+*nfxB										
1.	**141**	0.05 (S)	yes	no	T562A	A167D	no	no	*gyrA* T83I	I (1)
2.	**534**	0.5 (S)	yes	no	yes	no	no	no	*gyrA* + *parE*	R (2)
3.	140	1 (I)	yes	no	yes	R42C	no	yes	*gyrA* T83I + *nfxB*	R (≥2)
4.	**1296**	0.125 (S)	yes	no		Del	no	no	*gyrA T83I*	I (1)
***gyrA* T83I + *parC* S87L**										
1.	**90**	0.125 (S)	yes	yes	no	no	no	no	*gyrA* + *parC*	R (4)

Table S8. Primers used*

Genes	Primer Sequences (5’ to 3’)	Restriction enzymes	*Genome coordinates	References
gyrA	CCCCC**GGATCC**GCCTGGTCAATCGCATCTAC CCCCC**AAGCTT**GGTCGGCGAACTCTTGAT	BamHI HindIII	3559924-3558007	This study
rnfC	CCCCC**GGATCC**GGCTTTTCCCTGGTACTGGT CCCCC**AAGCTT**ATGCAATCGAACAGGTTGTG	BamH1 HindIII	3907697-3909637	
nfxB	GGGG**GGATCC**CAGGATCGCCTGGTTGTC GGGG**AAGCTT**GGGTCGAGGCAATAGTGGTA	BamH1 HindIII	5154491-5156819	
parC	CCCCC**GGATCC**GTTGGGGGACAAGTGACG CCCCC**AAGCTT**AGGTTGAGGAAGGCGATCA	BamH1 HindIII	5575013-5573327	
parE	CCCCC**TCTAGA**GCTGGGTGAATTCGAGTTGT CCCCC**AAGCTT**GGCTATCTCGAGGAGGATCA	XbaI HindIII	5574535-5579100	

Primers used for amplification of mutation-containing DNA region

Gene	Primer Sequences (5’ to 3’)	Product Size (bp)	*Genome coordinates	References
gyrA (3558951)	TCGAAGACGAGCTGAAACAG GCTCATCGACGGTCAGGT	565	3558577- 3559142	This study
nfxB (5155675)	ATGACCCTGATTTCCCATGA CATCGACCATGCCGTAGAC	453	5155561-5156014	
parC (5574227)	CCGATTCCAAGCACAAGAA GACTTCCCGCAGGTTGTG	379	5573902-5574281	
parE (5576539)	AGGTCGAGCGCAAGAAGA TCGTCGAGGGCGTAGTAGAT	451	5576750- 5576299	
rnfC (3908671)	GTACTCAAGGGCCAGACCAT CCACCAGCGTATGGATCTTT	302	3908577- 3908879	

List of primers used to carry out RT-qPCR for expression analysis of genes *mexD*

Gene	Sequences	Product size	Reference
clpX	GTGGGCGAGGTCGAGAAC CGGTACCCTCGATGAGCTTCAG	200	(7)
oprL	CCAACAGCG GTGCCGTTGA GCCATATTGTACTCGCGGGT	200	(7)
mexD	CGAGCGCTATTCGCTGC GGCAGTTGCACGTCGA	165	(8)

*Restriction sites in the primer sequences are shown in bold text. Genome coordinates for expected amplified DNA region based on reference PAO1 genome (NCBI NC_002516.2).

Table S9. Plasmids used.

Plasmid	**Description**	**Reference**
pEX18Tc	Suicide vector with tetracycline resistance, *oriT* and *sacB* gene	(9)
pEX18Tc::*gyrA*$_{T83I}$	pEX18Tc containing 1917bp DNA fragment with mutation that encodes amino acid T instead of I at residue 83 in GyrA	
pEX18Tc::*gyrA*$_{D87G}$	pEX18Tc containing 1917bp DNA fragment with mutation that encodes amino acid D instead of G at residue 87 in GyrA	
pEX18Tc::*gyrA*$_{D87N}$	pEX18Tc containing 1917bp DNA fragment with mutation that encodes amino acid D instead of N at residue 87 in GyrA	
pEX18Tc::*nfxB*$_{T39P}$	pEX18Tc containing 2328 bp DNA fragment with mutation that encodes amino acid T instead of P at residue 39 in NfxB	
pEX18Tc::*rnfC*$_{H87P}$	pEX18Tc containing 1940 bp DNA fragment with mutation that encodes amino acid H instead of P at residue 87 in *rnfC*	
pEX18Tc::*parC*$_{S87L}$	pEX18Tc containing 1686 bp DNA fragment with mutation that encodes amino acid S instead of L at residue 87 in ParC	
pEX18Tc::*parE*$_{V460G}$	pEX18Tc containing 4565 bp DNA fragment with mutation that encodes amino acid V instead of G at residue 460 in ParE	
pEX18Tc::WT *rnfC*	pEX18Tc containing 1755 bp DNA fragment from *rnfC* gene	
pEX18Tc:: WT *gyrA*	pEX18Tc containing 1917 bp DNA fragment from *gyrA* gene	

Supplementary material references

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