Supplementary data

Supplementary methods

PCR amplification and DNA sequencing of the QRDR regions of DNA gyrase and topoisomerase IV

Staphylococci were grown in tryptic soy broth (Becton, Dickinson and Company, Sparks, MD, USA), *S. pneumoniae* in Todd-Hewitt broth (Becton, Dickinson and Company) and *H. influenzae* in *Haemophilus* test medium (Remel, Lenexa, KS, USA) for 18 to 24 h at 37°C. Genomic DNA was extracted from pure bacterial cultures using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. The isolated DNA was then used as the template for PCR amplification.

The QRDRs of the *gyrA*, *gyrB*, *parC* (*grlA*) and *parE* (*grlB*) genes from each isolate were amplified with primers designed with the Clone Manager 9 software package (Sci-Ed Software, Cary, NC, USA), which was also used for sequence analyses. The DNA sequences used for primer design were as follows: *H. influenzae* strains RdKW20 (GenBank accession no. NC_000907), 86-028NP (NC_007146) and PittEE (NC_009566); *S. aureus* strain Newman (NC_009641); *S. epidermidis* strain ATCC 12228 (NC_004461); and *S. pneumoniae* strains R6 (NC_003098) and TIGR4 (NC_003028).

PCR was carried out in a TC-3000 thermal cycler (Techne, Burlington, NJ, USA) in a 50 µL volume containing 0.4 µM primers (Table S1; Integrated DNA Technologies, Coralville, IA, USA), 200 µM deoxynucleoside triphosphates (Omega Bio-Tek, Norcross, GA, USA), 1 U of Vent DNA polymerase and reaction buffer containing 2 mM magnesium sulphate (New England Biolabs, Ipswich, MA, USA). An initial denaturation cycle (10 min at 94°C) preceded 30 cycles consisting of 30 s of denaturation at 94°C, 30 s of annealing at 45-55°C and 1 min of elongation at 72°C, and was followed by a final elongation cycle (10 min at 72°C). Amplification products were purified using the

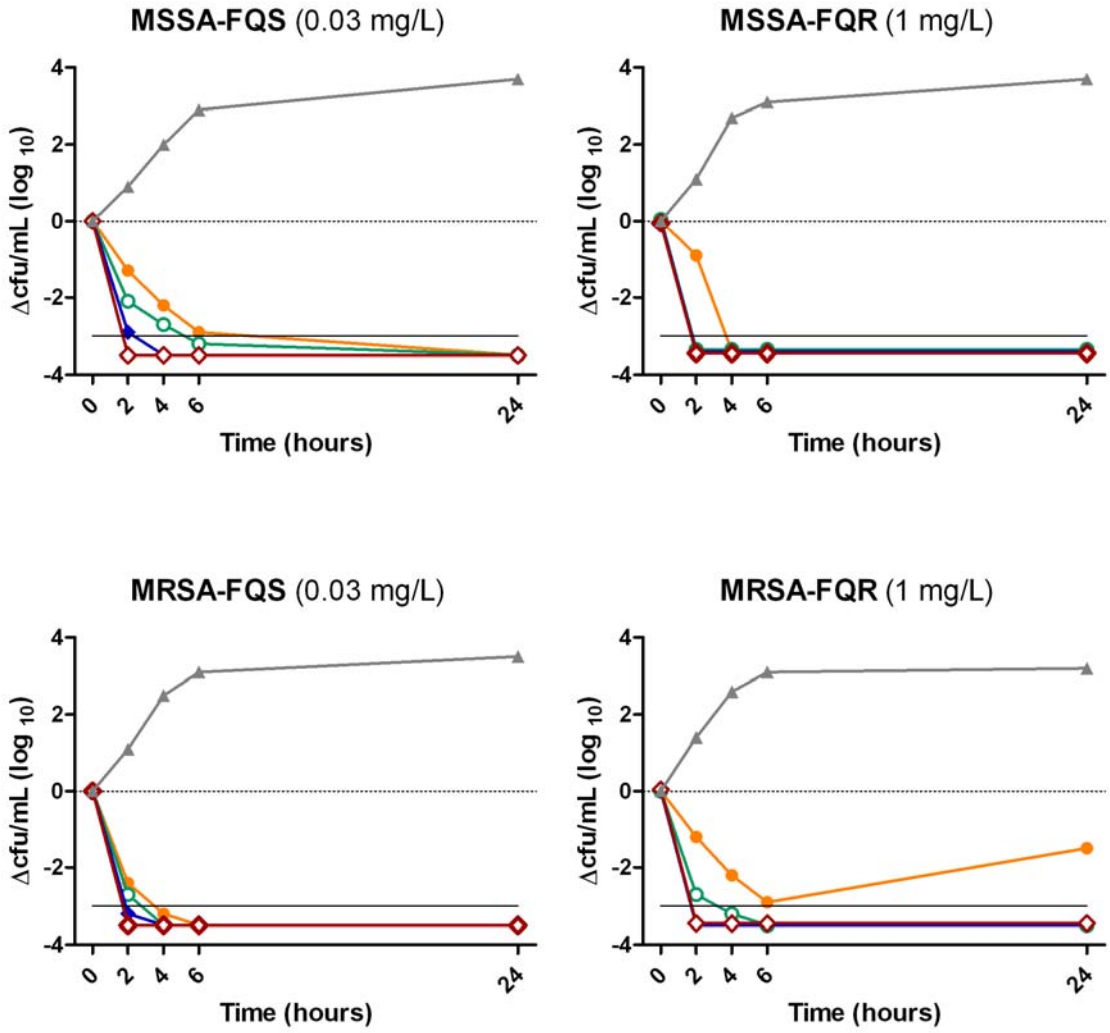
QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA) and sequenced by Macrogen USA (Rockville, MD, USA).

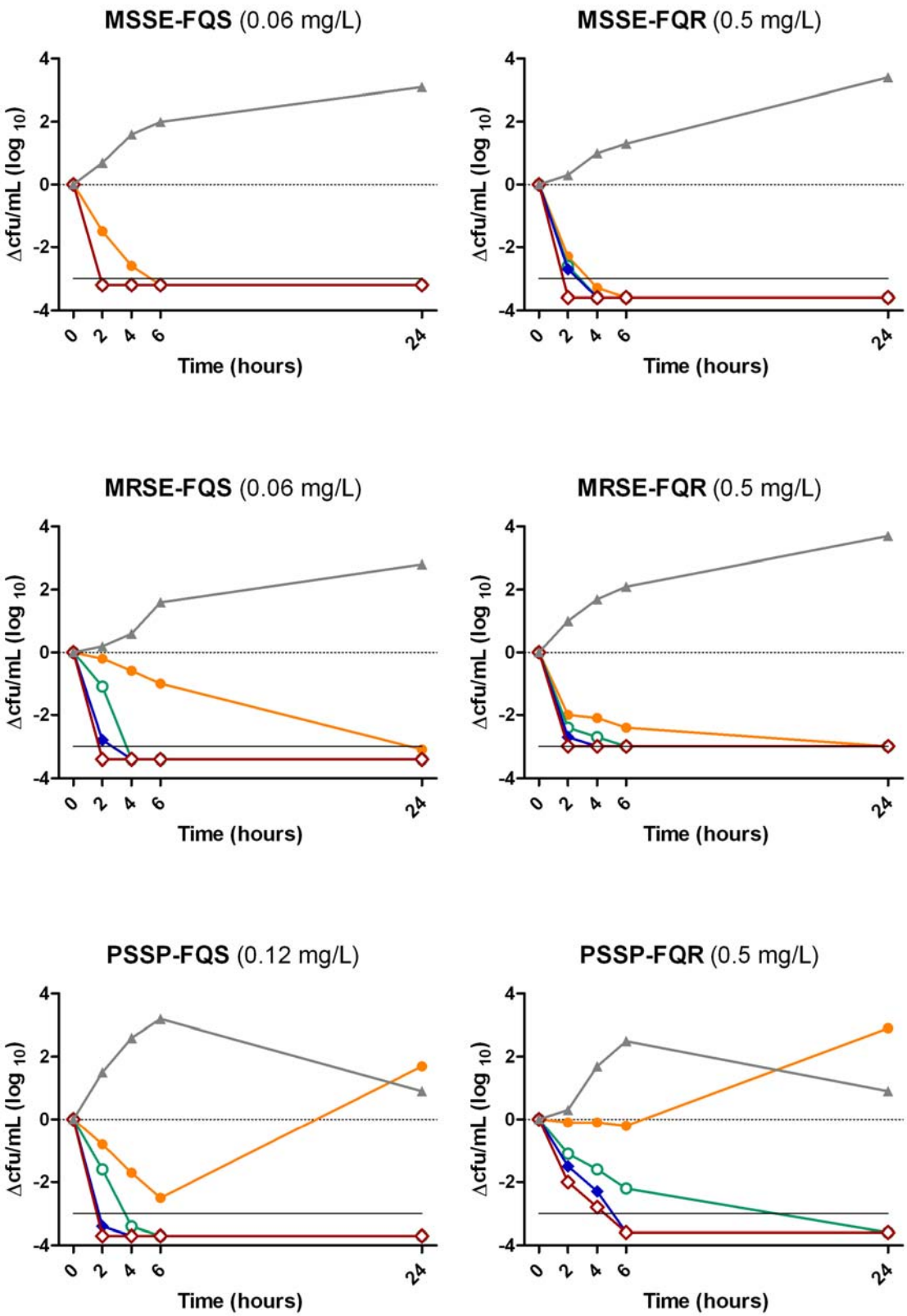
Table S1. Oligonucleotide primers used for PCR amplification of QRDR sequences

Organism	Target gene	Primer sequence (5′ to 3′)	Product size (bp)	Position
Staphylococcus aureus[a]	*gyrA*	GCCACCGTTGTATAAACTGAC	885	6663–7547
		ATACCTACCGCGATACCTGATG		
	gyrB	TCTGGTGAAGATACACGTGAAG	834	6004–6837
		CTCAGGGTTCATTGTTGTTTCC		
	parC	TTGGTATGCAAGAGGACCAAAG	841	1394067–1394907
		ACGTCCATTGCGTAAAGTTTC		
	parE	GCTGTTGTGTCTGTTCGTATTC	707	1393127–1393833
		GTCTGTCCAAGCGTATTCAACTC		
Staphylococcus epidermidis[b]	*gyrA*	GATCTCTTGATGGCTGAATTAC	827	6907–7733
		ATACGCGCTTTATTGACTTG		
	gyrB	GAGGAAGTTAGAGAAGACTCATATC	892	5602–6493
		GTATATGTGCACCATCAACATC		
	parC	AAAGTTGAGTACGCTTGGACTG	816	1044535–1045350
		TCGCTGGTAATACCATTGGTTC		
	parE	TTGGCACTTCAGAAGCAAGG	764	1043915–1044678
		CCGAGTTTCTGGATTCATGGTAG		
Streptococcus pneumoniae[c]	*gyrA*	AGCACCATCACCGACAAG	771	1150102–1149332
		ATCCCAACCGCGATACCAG		
	gyrB	CAAGATTACCAATCGCCTCTTC	791	761552–762,342
		CTGCTTCTGCAGCATCATCTAC		
	parC	GAGTTGAACACGCCCTAGATAC	804	752142–752945
		CCCAGTCTCATAGGCTTTCTTG		
	parE	GAGGGACTAGCGGCCGTTCTTTC	726	750850–751575
		TTCTCCGTCCGTCCAAGCGTAGG		
Haemophilus influenzae[d]	*gyrA*	ATGACGGATTCAATCCAATCATC	648	1739444–1740091
		TGGGCCCGGAATATGTTG		
	gyrB	TGAAATGACGCGCCGTAAAG	805	27686–28490
		GCGTTCCGCGTTAAATTGTG		
	parC	CAAGCGAAAGGCGATCAG	831	1600152–1600982
		TATTGAGCCACGACCTTG		
	parE	CCTACGCCATTTATTAAGAGC	907	1598837–1599743
		GACTTTACCATAGCGAAGTTG		

DNA reference strain used for primer design (GenBank accession number): [a]Newman (NC_009641); [b]ATCC 12228 (NC_004461); [c]TIGR4 (NC_003028) and R6 (NC_003098); and [d]86-028NP (NC_007146), PittEE (NC_009566) and RdKW20 (NC_00907).

Figure S1. Change in cfu/mL over time after the addition of besifloxacin. Strains were treated with 1× (filled circles), 2× (open circles), 4× (filled diamonds) or 8× (open diamonds) the MIC of besifloxacin. A no-treatment control is identified by filled triangles. A 3 log reduction in viable cells is indicated by a continuous horizontal line. 1× besifloxacin MIC values are shown in parentheses. MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*; MSSE, methicillin-susceptible *S. epidermidis*; MRSE, methicillin-resistant *S. epidermidis*; PSSP, penicillin-susceptible *S. pneumoniae*; BLNHI, β-lactamase-negative *H. influenzae*; FQS, fluoroquinolone susceptible; FQNS, fluoroquinolone non-susceptible; FQR, fluoroquinolone resistant.




MSSE-FQS (0.06 mg/L)
MSSE-FQR (0.5 mg/L)
MRSE-FQS (0.06 mg/L)
MRSE-FQR (0.5 mg/L)
PSSP-FQS (0.12 mg/L)
PSSP-FQR (0.5 mg/L)
Δcfu/mL (log 10)
Time (hours)
4
2
0
-2
-4
0
2
4
6
24

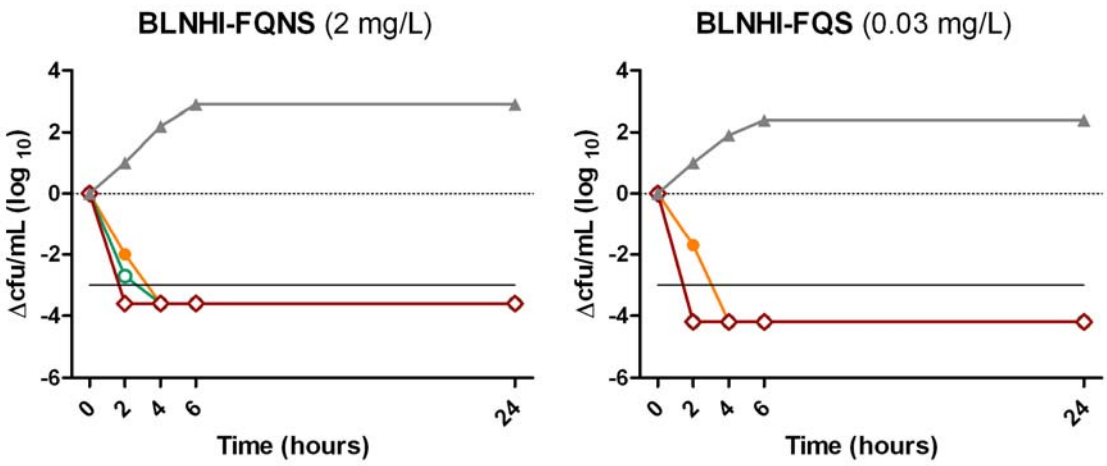
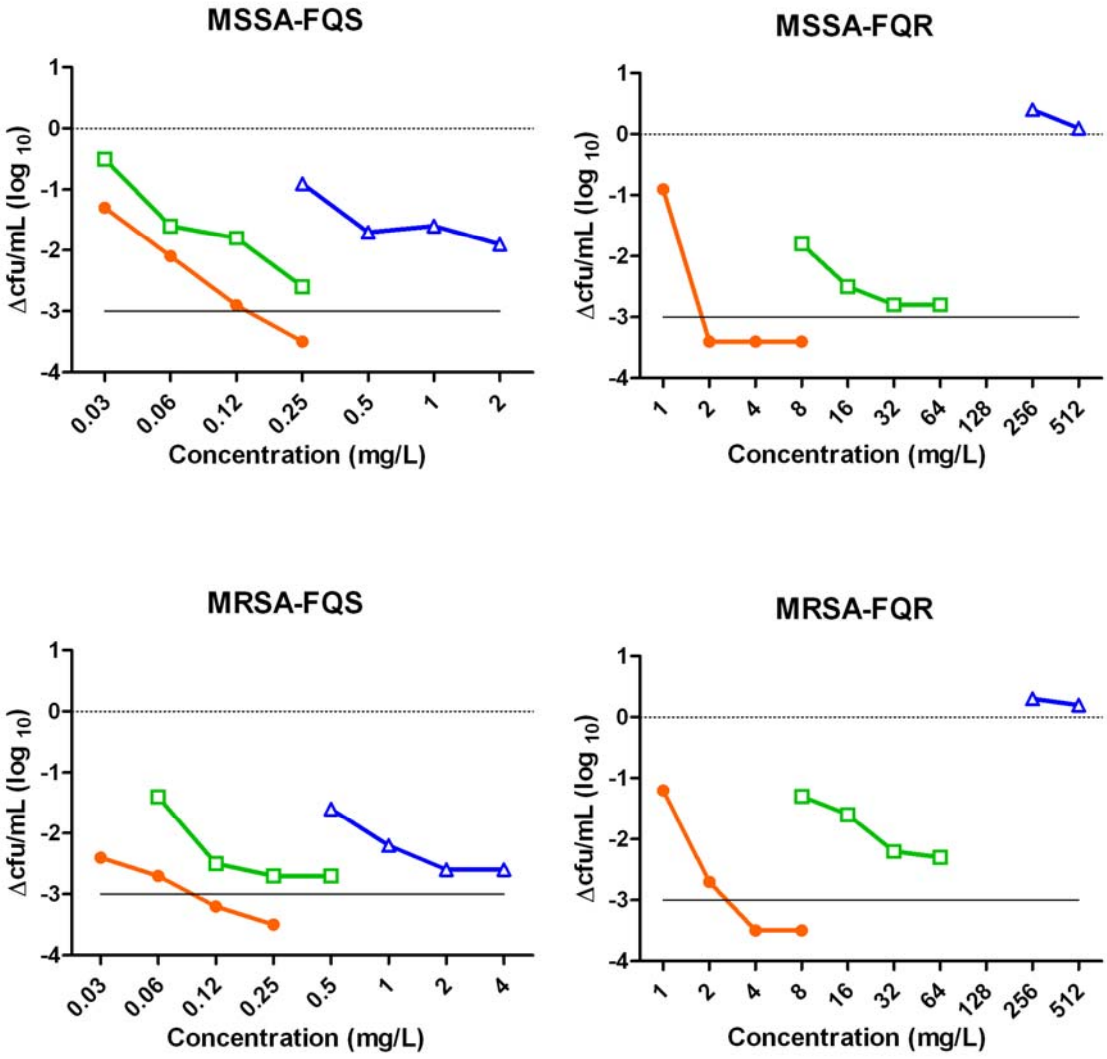
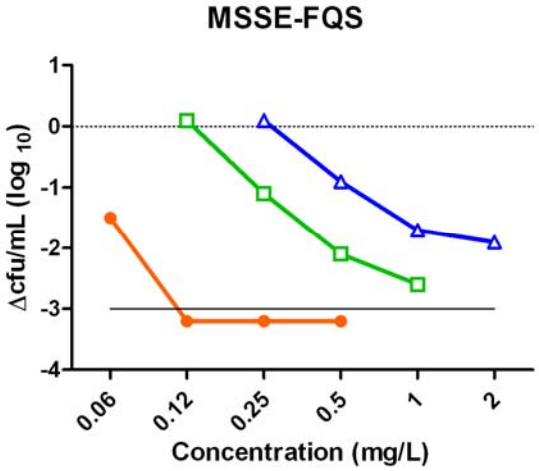
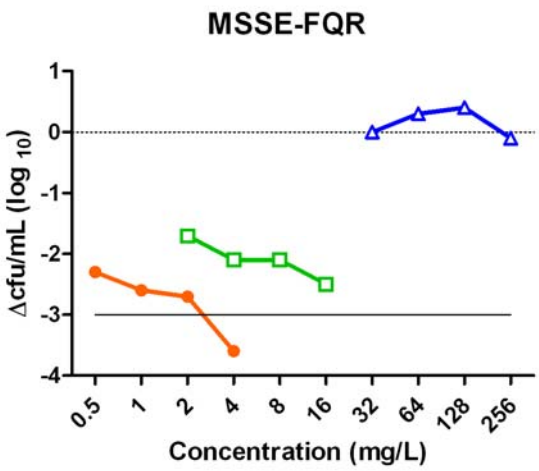
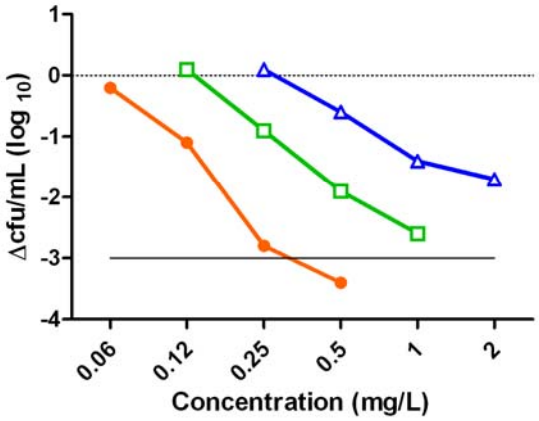

BLNHI-FQNS (2 mg/L)
BLNHI-FQS (0.03 mg/L)
Δcfu/mL (log 10)
4
2
0
-2
-4
-6
0
2
4
6
24
Time (hours)

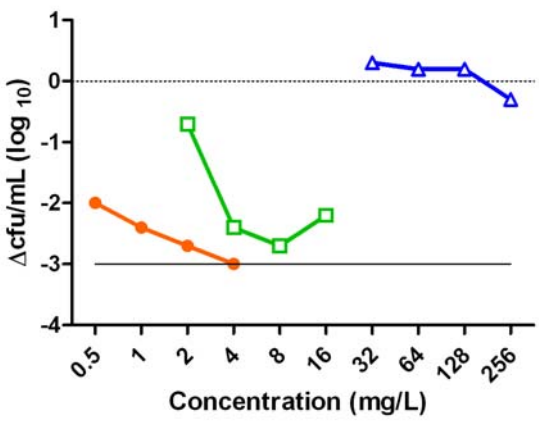
Figure S2. Change in cfu/mL 2 h after the addition of 1×, 2×, 4× or 8× the MIC of besifloxacin (filled circles), moxifloxacin (open squares) or ciprofloxacin (open triangles). A 3 log reduction in viable cells is indicated by a continuous horizontal line. MRSA, methicillin-resistant *S. aureus*; MSSE, methicillin-susceptible *S. epidermidis*; MRSE, methicillin-resistant *S. epidermidis*; PSSP, penicillin-susceptible *S. pneumoniae*; BLNHI, β-lactamase-negative *H. influenzae*; FQS, fluoroquinolone susceptible; FQNS, fluoroquinolone non-susceptible; FQR, fluoroquinolone resistant.

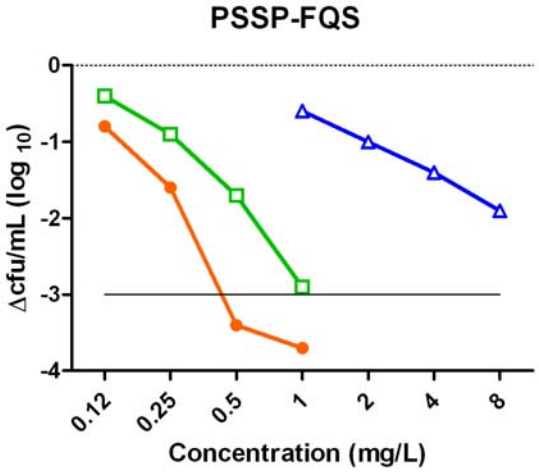


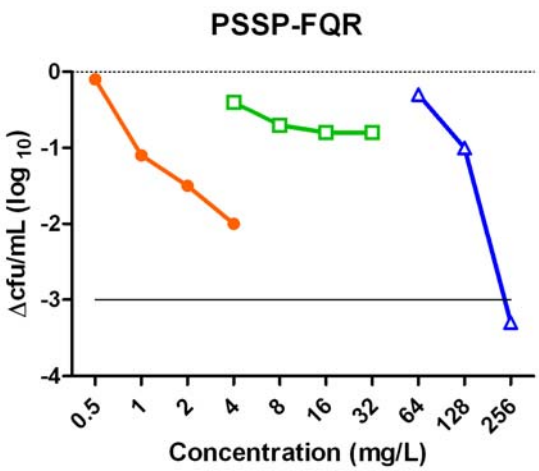

MSSE-FQS
Δcfu/mL (log 10)
1
0
-1
-2
-3
-4
0.06
0.12
0.25
0.5
1
2
Concentration (mg/L)

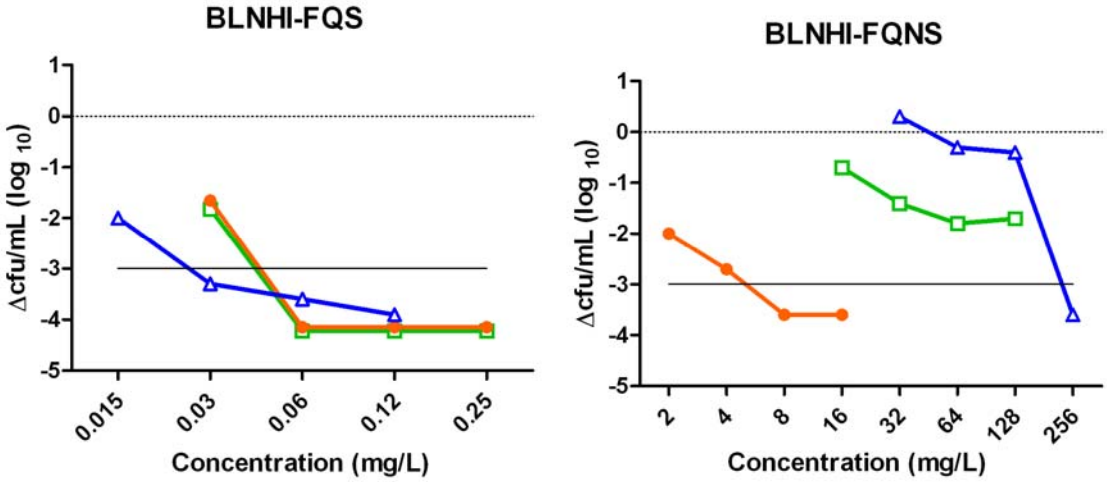

MSSE-FQR
Δcfu/mL (log 10)
1
0
-1
-2
-3
-4
0.5
1
2
4
8
16
32
64
128
256
Concentration (mg/L)


MRSE-FQS
Δcfu/mL (log 10)
1
0
-1
-2
-3
-4
0.06
0.12
0.25
0.5
1
2
Concentration (mg/L)


MRSE-FQR
Δcfu/mL (log 10)
1
0
-1
-2
-3
-4
0.5
1
2
4
8
16
32
64
128
256
Concentration (mg/L)


PSSP-FQS
Δcfu/mL (log 10)
0
-1
-2
-3
-4
0.12
0.25
0.5
1
2
4
8
Concentration (mg/L)


PSSP-FQR
Δcfu/mL (log 10)
0
-1
-2
-3
-4
0.5
1
2
4
8
16
32
64
128
256
Concentration (mg/L)


BLNHI-FQS
Δcfu/mL (log 10)
1
0
-1
-2
-3
-4
-5
0.015
0.03
0.06
0.12
0.25
Concentration (mg/L)
BLNHI-FQNS
Δcfu/mL (log 10)
1
0
-1
-2
-3
-4
-5
2
4
8
16
32
64
128
256
Concentration (mg/L)