**Supplementary data**

**Table S1.** Primers used in this study

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Substitution introduced</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>CLONING AND RANDOM MUTAGENESIS</strong></td>
<td></td>
</tr>
<tr>
<td>preQnrA1</td>
<td>5’-CGGCAGTTAAAAATTGGGGCT-3’</td>
<td></td>
</tr>
<tr>
<td>postQnrA1</td>
<td>5’-ACGCCGAGTCCCCGACCAGACTGC-3’</td>
<td></td>
</tr>
<tr>
<td>preQnrB1</td>
<td>5’-ATGACGCCATTACTGTATAA-3’</td>
<td></td>
</tr>
<tr>
<td>postQnrB1</td>
<td>5’-CTAACCAATCACCGCGATGCC-3’</td>
<td></td>
</tr>
<tr>
<td>preQnrS1</td>
<td>5’-CCACTTTAAAAACAGGTAAATTG-3’</td>
<td></td>
</tr>
<tr>
<td>postQnrS1</td>
<td>5’-TACATGGTTGTCCCTATGTC-3’</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>INTERNAL PRIMERS FOR RT-PCR AND NORTHERN BLOT ASSAYS</strong></td>
<td></td>
</tr>
<tr>
<td>qnrA1</td>
<td>5’-GGGTATGGATATTATTGATAAAG-3’</td>
<td></td>
</tr>
<tr>
<td>qnrA2</td>
<td>5’-CTAATCCGGCCAGCACTATTA-3’</td>
<td></td>
</tr>
<tr>
<td>qnrB1</td>
<td>5’-ATGACGCCATTACTGTATAA-3’</td>
<td></td>
</tr>
<tr>
<td>qnrB2</td>
<td>5’-GATCGCAATGTGTGAAGTTT-3’</td>
<td></td>
</tr>
<tr>
<td>qnrS1</td>
<td>5’-CAATCATACATATCGGCACC-3’</td>
<td></td>
</tr>
</tbody>
</table>
qnrS2  5’-AGTTCTTGCTGCCAGGCTGC-3’

SITE-DIRECTED MUTAGENESIS

QnrA1

QnrA1 G56- F  5´-GC GGC GCC GTT GAA --- TGT CAC TTC AGC-3´  G56-
QnrA1 G56- R  5´-GCT GAA GTG ACA --- TTC AAC GGC GCC GC-3´
QnrA1 G56D F  5´-GCG GCG CCG TTG AA GAC TGT C ACT TCA GC-3´  G56D
QnrA1 G56D R  5´-GCT GAA GTG ACA GTC TTC AAC GGC GCC GC-3´
QnrA1 G56E F  5´-GGC GCC GTT GAA GAG TGT CAC TTC AGC-3´  G56E
QnrA1 G56E R  5´-GCT GAA GTG ACA GTC TTC AAC GGC GCC GC-3´
QnrA1 C72Y F  5´-GT TTC AAG GCC TAC CGT CTG TCT TT-3´  C72Y
QnrA1 C72Y R  5´-AA AGA CAG ACG GTA GGC CTT GAA AC-3´
QnrA1 C92Y F  5´-GTTCAGGGAGTACGATCTCAAGG-3´  C92Y
QnrA1 C92Y R  5´-CCTTGAGATCTGATACTCCCTGAAC-3´
QnrA1 G96D F  5´-TGC GAT CTC AAG GAC GCC AAC TTT TCC-3´  G96D
QnrA1 G96D R  5´-GGA AAA GTT GCC GTC CTT GAG ATC GCA-3´
QnrA1 F114D F  5´-CAT AAG ATG TAC GAC TGC TCG GCT TA-3´  F114D
QnrA1 F114D R  5´-TA AGC CGA GCA GTA CAT CTT ATG-3´
QnrA1 C115Y F  5´-GATGTACTTCTACTCGGCTTATATC-3´  C115Y
QnrA1 C115Y R  5´ GATATAAGCCGAGTGAAGTACATC-3´
QnrA1 S116D F  5´ ATG TAC TTC TGC GAC GCT TAT ATC TCA-3´   S116D
QnrA1 S116D R  5´-TGA GAT ATA AGC GTC GCA GAA GTA CAT-3´
QnrA1 A117C F  5´-G TAC TTC TGC TCG TGT TAT ATC TCA GGT-3´   A117C
QnrA1 A117C R  5´-ACC TGA GAT ATA ACA CGA GCA GAA GTA C-3´
QnrA1 A117V F  5´-G TAC TTC TGC TCG GTT TAT ATC TCA GGT-3´   A117V
QnrA1 A117V R  5´-ACC TGA GAT ATA AAC CGA GCA GAA GTA C-3´
QnrA1 L159D F   5´-G GGC TCA GAT GAC AGC CGC GGC AC-3´   L159D
QnrA1 L159D R   5´-GT GCC GCG GCT GTC ATC TGA GCC C-3´
QnrA1 D185Y F   5´-C TTT GCC GAT CTG TAT GGG CTC GAC CCC-3´   D185Y
QnrA1 D185Y R   5´-GGG GTC GAG CCC ATA CAG ATC GGC AAA G-3´

QnrB1
QnrB1 G53- F   5´-CGT GAA AGC CAG AAA -- TGC AAT TTT AGT C-3´   G53-
QnrB1 G53- R   5´-G ACT AAA ATT GCA -- TTT CTG GCT TTC ACG-3´
QnrB1 G53D F   5´-CGT GAA AGC CAG AAA GAC TGC AAT TTT AGT C-3´   G53D
QnrB1 G53D R   5´-G ACT AAA ATT GCA GTC TTT CTG GCT TTC ACG-3´
QnrB1 G53E F   5´-GAA AGC CAG AAA GAG TGC AAT TTT AGT C-3´   G53E
QnrB1 G53E R   5´-CT AAA ATT GCA CTC TTT CTG GCT TTC-3´
QnrB1 C69Y F   5´-GCC ATT TTT AAA AGC TAT GAT TTA TCC-3´   C69Y
QnrB1 C69Y R   5´-GGA TAA ATC ATA GCT TTT AAA AAT GGC-3´
QnrB1 C89Y F 5′-CGCCACTACCCGCGCACAAGG-3′ C89Y
QnrB1 C89Y R 5′-CCTTGTGCGCGGCTAGTGCGG-3′

QnrB1 G93D F 5′-CGC GCA CAA GAC GCA GAT TTC C-3′ G93D
QnrB1 G93D R 5′-G GAA ATC TGC GTC TTG TGC GCG-3′

QnrB1 F111D F 5′-G CGC ACC TGG GAT TGT AGC GCA T-3′ F111D
QnrB1 F111D R 5′-A TGC GCT ACA ATC CCA GGT GCG-3′

QnrB1 C112Y F 5′-GCACCTGGTGTATAGCGCATATATCACG-3′ C112Y
QnrB1 C112Y R 5′-CGTGATATATGCGCTATA AAACCAGGTGC-3′

QnrB1 S113D F 5′-C TGG TTT TGT GAC GCA TAT ATC-3′ S113D
QnrB1 S113D R 5′-GAT ATA TGC GTC ACA AAA CCA G-3′

QnrB1 A114C F 5′-GG TTT TGT AGC TGC TAT ATC ACG A-3′ A114C
QnrB1 A114C R 5′-T CGT GAT ATA GCA GCT ACA AAA CC-3′

QnrB1 A114V F 5′-GG TTT TGT AGC GTA TAT ATC ACG A-3′ A114V
QnrB1 A114V R 5′-T CGT GAT ATA TAC GCT ACA AAA CC-3′

QnrB1 L156D F 5′-GGT TCA GAT GAC TCC GGC GGC G-3′ L156D
QnrB1 L156D R 5′-C GCC GCC GGA GTC ATC TGA ACC-3′

QnrB1 G182Y F 5′-C AAT TCG GAG TTG TAT GAC TTA GAT ATA C-3′ G182Y
QnrB1 G182Y R 5′-G TAT ATC TAA GTC ATA CAA CTC CGA ATT G-3′

QnrS1
QnrS1 G56- F   5´-G GGT GAT ATC GAA --- TGC CAC TTT GAT-3´   G56-
QnrS1 G56- R   5´-ATC AAA GTG GCA --- TTC GAT ATC ACC C-3´
QnrS1 G56D F   5´-GGG TGA TAT CGA A GAC TGC CAC TTT GAT-3´    G56D
QnrS1 G56D R   5´-ATC AAA GTG GCA GTC T TCG ATA TCA CCC-3´
QnrS1 G56E F   5´-GGT GAT ATC GAA GAG TGC CAC TTT G-3´    G56E
QnrS1 G56E R   5´-C AAA GTG GCA CTC TTC GAT ATC ACC-3´
QnrS1 C72Y F   5´-GT TTC CAA CAA TACCAA CTT GCG ATG-3´    C72Y
QnrS1 C72Y R   5´-CAT CGC AAG TTG GTA TTG TTG GAA AC-3´
QnrS1 C92Y F   5´-GAGTTC CGTGTCGTATGATTTAAAAAGG-3´    C92Y
QnrS1 C92Y R   5´-CCTTT TAAATCATACGCACGGAACTC-3´
QnrS1 G96D F   5´-GT GAT TTA AAA GAC GCC AAC TTT TCC-3´    G96D
QnrS1 G96D R   5´-GGA AAA GTT GGC GTC TTT TAA ATC AC-3´
QnrS1 F114D F   5´-CGT ATG TAC GAT TGC TCA GCA T-3´    F114D
QnrS1 F114D R   5´-A TGC TGA GCA ATC GTA CAT ACG-3´
QnrS1 C115Y F   5´-CGT ATG TAC TTT TAC TCA GCA TTT ATT TC-3´   C115Y
QnrS1 C115Y R   5´-GA AAT AAA TGC TGA GTA AAA GTA CAT ACG-3´
QnrS1 S116D F   5´-G TAC TTT TGC GAC GCA TTT ATT TC-3´    S116D
QnrS1 S116D R   5´-GA AAT AAA TGC GTC GCA AAA GTA C
QnrS1 A117C F   5´-C TTT TGC TCA TGT TTT ATT TCT GG-3´    A117C
QnrS1 A117C R   5´-CC AGA AAT AAA ACA TGA GCA AAA G-3´
<table>
<thead>
<tr>
<th></th>
<th>Site</th>
<th>Primer Sequence</th>
<th>Underlined Changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>QnrS1 A117V F</td>
<td>5’-C TTT TGC TCA GTA TTT ATT TCT GG-3’</td>
<td>A117V</td>
<td></td>
</tr>
<tr>
<td>QnrS1 A117V R</td>
<td>5’-CC AGA AAT AAA TAC TGA GCA AAA G-3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>QnrS1 L159D F</td>
<td>5’-GAG TCA GAC GAC AGT CGA GGT G-3’</td>
<td>L159D</td>
<td></td>
</tr>
<tr>
<td>QnrS1 L159D R</td>
<td>5’-C ACC TCG ACT GTC GTC TGA CTC-3’</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Changes introduced to site-directed mutagenesis primers are underlined.
Figure S1. Amino acid sequence comparisons of plasmid-mediated QnrA1, QnrB1 and QnrS1 proteins with the chromosome-encoded proteins of Gram-positive bacteria (Rodriguez-Martinez JM, Velasco C, Briales A et al. Qnr-like pentapeptide repeat proteins in Gram-positive bacteria. J Antimicrob Chemother 2008; 61: 1240-3) and Vibrionaceae (Poirel L, Liard A, Rodriguez-Martinez JM et al. Vibrionaceae as a possible source of Qnr-like quinolone resistance determinants. J Antimicrob Chemother 2005; 56: 1118-21). EfsQnr, EfmQnr, LmQnr, CpQnr, BcQnr and BsQnr determinants are from Enterococcus faecalis ATCC 29212, Enterococcus faecium ATCC 3566, Listeria monocytogenes ATCC 7644, Clostridium perfringens ATCC 10388, Bacillus cereus ATCC 11778 and Bacillus subtilis ATCC 12432, respectively. VvQnr is from Vibrio vulnificus CIP103196, VpQnr is from Vibrio parahaemolyticus CIP71.2, VfQnr is from Vibrio fischeri ES114 (GenBank accession no. AAW85819) and PpQnr is from Photobacterium profundum CIP106289. BfQnr is from Bacteroides fragilis NCTC 9343 (GenBank accession no. YP_210825). The proteins were aligned using the CLUSTALW program. Symbols: asterisk, identical amino acids; colon, strongly similar amino acids; period, weakly similar amino acids. Amino acid lengths are indicated at the end of each line. The residues selected for site-directed mutagenesis in QnrA1, QnrS1 and QnrB1, and residue D185, associated with increased quinolone resistance in the S-RM-3 mutant from QnrS1, are indicated in grey.