# SmQnrR, a DeoR Type Transcriptional Regulator, Negatively Regulates the Expression of Sm*qnr* and Sm*tcrA* in *Stenotrophomonas maltophilia*

Yi-Chih Chang[1,2], Mei-Jung Tsai[2], Yi-Wei Huang[3], Tung-Ching Chung[1,3*], and Tsuey-Ching Yang[2*]

[1]Department of Veterinary Medicine, National Chung-Hsing University, Taichung, 402, Taiwan

[2]Department of Medical Laboratory Science and Biotechnology, China Medical University, Taichung, Taiwan

[3]Graduate Institute of Microbiology and Public Health, National Chung-Hsing University, Taichung, 402, Taiwan

Running title: Sm*tcrA*-Sm*qnrR*-Sm*qnr* regulon of *Stenotrophomonas maltophilia*

* To whom correspondence should be sent

Mailing address: 91 Hsueh-Shih Rd., Taichung. Taiwan, 40402, R.O.C.

Tel: 886-4-2205-3366 ext. 7203

Fax: 886-4-2205-7414

e-mail: tcyang@mail.cmu.edu.tw

Keywords: quinolone resistance, tetracycline resistance, major facilitator superfamily

**Synopsis**

***Objectives:*** To characterize the Sm*tcrA*-Sm*qnrR*-Sm*qnr* regulon of *Stenotrophomonas maltophilia* KJ.

***Methods:*** Sm*qnrR*, a DeoR-type regulator gene, situates between a quinolone resistance gene (Sm*qnr*) and a major facilitator superfamily (MFS) transmembrane transporter gene (Sm*tcrA*). To assess the regulatory role of SmQnrR in the expression of Sm*qnr* and Sm*tcrA* genes, the transcripts of Sm*qnr* and Sm*tcrA* genes were comparatively determined between the wild-type KJ and the Sm*qnrR* isogenic mutant KJΔQnrR. A Sm*qnrR* polar mutant, KJQnrRΩ, was constructed to investigate the possibility of the Sm*qnrR*-Sm*tcrA* operon. The contribution of Sm*qnr* and Sm*tcrA* genes to the intrinsic and acquired resistances of *S. maltophilia* was evaluated by the susceptibility test among the wild-type KJ and its derived mutants.

***Results:*** SmQnrR acted as a repressor for the expression of Sm*qnr* and Sm*tcrA* genes. Sm*qnrR* and Sm*tcrA* genes formed an operon, which was negatively autoregulated by SmQnrR. Sm*qnr* and Sm*tcrA* hardly contributed to the intrinsic resistance. Nevertheless, overexpression of Sm*qnr* and Sm*tcrA* by inactivating Sm*qnrR* conferred to the acquired slight quinolone resistance and significant tetracycline resistance in *S. maltophilia*.

***Conclusions:*** The SmQnrR protein is a transcriptional repressor for its contiguous,

divergently transcribed Sm*qnr* gene. A negative autoregulation phenomenon occurs in Sm*qnrR*, which, in turn, renders SmQnrR that negatively regulates the expression of the Sm*qnrR*-Sm*tcrA* operon. Inactivation of Sm*qnrR* contributes to the acquired quinolone and tetracycline resistance of *S. maltophilia*.

## Introduction

Many quinolone resistance mechanisms have been characterized in Gram-negative bacteria, including mutation in DNA gyrase, low level permeability of outer membrane, overexpression of efflux pumps, fluoroquinolone-modifying enzyme, fluoroquinolone efflux pump QepA, and DNA gyrase protection proteins encoded by quinolone resistance (*qnr*) determinants.[1-4] The *qnr* determinants have been found either in a plasmid[4] or in the chromosome.[5-6] *Stenotrophomonas maltophilia* has emerged as an important nosocomial pathogen and displays resistance to a variety of antimicrobials. The SmQnr protein encoded by the chromosomal *qnr* determinant of *S. maltophilia* has been shown to contribute to the intrinsic quinolone resistance of *S. maltophilia*.[7-8]

Although several studies have shown the relevance of *qnr* determinants to the quinolone resistance, the regulatory mechanism of *qnr* determinants is still unknown. A silico analysis of *S. maltophilia* K279a genome[9] revealed that a DeoR type transcriptional regulator gene (Smlt1070), annotated as Sm*qnrR* herein, is contiguous to the Sm*qnr* gene (Smlt1071) but divergently transcribed (Fig. 1). The genomic module of Sm*qnrR*-Sm*qnr* indicates that Sm*qnrR* can be a crucial regulator for the expression of Sm*qnr*. In addition, a putative major facilitator superfamily (MFS) transmembrane transporter gene (Smlt1069), annotated as Sm*tcrA* thereafter, was

77 found to locate downstream the Sm*qnrR* gene (Fig. 1). Both the Sm*qnrR* and Sm*tcrA*
78 genes have the same orientation and an 11-bp gap between them. MFS transporters
79 are generally recognized to be involved in the efflux of a wide range of structurally
80 dissimilar compounds. According to the observation of the genomic organization of
81 Sm*tcrA*-Sm*qnrR*-Sm*qnr*, whether Sm*qnrR* is a dual regulator for the expression of
82 Sm*tcrA* and Sm*qnr* genes is of great interest.

83 The DNA sequences of the Sm*tcrA*-Sm*qnrR*-Sm*qnr* module of *S. maltophilia*
84 KJ[10] was PCR amplified using the PCR protocol described previously.[11] Primer pairs
85 TcrA-F/TcrA-R, QnrRC-F/QnrRN-R, and QnrB-F/QnrB-R were used to amplify
86 Sm*tcrA*, Sm*qnrR*, and Sm*qnr* genes, respectively (Table 1). PCR amplicons were
87 cloned into pRKm415 and the resultant plasmids, pRKmTcrA, pRKmQnrR, and
88 pRKmQnr (Table 1, Fig. 1), carrying correctly oriented genes were used for sequences
89 determination and complementary test. The deduced 219-aa SmQnr protein of strain
90 KJ, which displays the feature of pentapeptide family, shared 94-100% identity to
91 those of other *S. maltophilia* strains, in good consistence with the previous studies
92 showing that the SmQnr displays a high intra-specie protein identity.[12] However,
93 SmQnr had 57–62%, 42-45%, and 38-41% inter-species protein identities to QnrB-,
94 QnrA-, and QnrS-type proteins. A 777-bp Sm*qnrR* was included in the 1683-bp PCR
95 amplicon primered by primers QnrRN-F and QnrRC-R (Table 1). A homology search

using the BLAST algorithm revealed that SmQnrR showed a significant sequence identity to members of the DeoR family transcriptional regulators. A Sm*tcrA*-containing PCR amplicon amplified by primers TcrA-F and TcrA-R (Table 1) was obtained. The Sm*tcrA* gene is 1134 bp long and its encoded protein SmTcrA with a significant identity to majority of the MFS proteins, whose functions have not been characterized.

To assess the role of SmQnrR in the regulation of Sm*qnr* and Sm*tcrA* genes, an unmarked deletion mutant KJΔQnrR was constructed. A sucrose selection suicide delivery system was performed for mutant construction.[11] The procedure included constructing a mutagenic plasmid, transferring the plasmid into the target strain, selecting the mutant by tetracycline, norfloxacin, and sucrose, and confirming the correctness of the mutant by PCR. Two nonoverlapping DNA fragments corresponding to the upstream and downstream of the Sm*qnrR* gene were amplified by PCR using pairs of primers, QnrRN-F/QnrRN-R and QnrRC-F/QnrRC-R (Table 1), respectively. The PCR amplicons were sequentially cloned into pEX18Tc, giving rise to a mutagenic plasmid pΔQnrR with a ΔSm*qnrR* allele (Table 1, Fig. 1). Quantitative real-time PCRs (qRT-PCR) of Sm*qnr* and Sm*tcrA* genes were comparatively assayed between strains KJ and KJΔQnrR. The protocols for qRT-PCR were described elsewhere.[11] The transcripts of Sm*qnr* and Sm*tcrA* genes had an approximately

18-fold (18 ± 4%) and 4-fold (4 ± 1%) increment in mutant KJΔQnrR, indicating that the SmQnrR protein plays a repressor role in the expression of Sm*qnr* and Sm*tcrA* genes. A standard twofold serial agar dilution method[11] was performed to determine the MICs of strains KJ and KJΔQnrR. The antimicrobials tested included quinolone (nalidix acid, norfloxacin, and ofloxainc), aminoglycoside (kanamycin, gentamicin, and sisomicin), tetracycline (tetracycline and deoxycycline), and erythromycin. E-test was used for the MIC determination of moxifloxacin. Compared with KJ, KJΔQnrR displayed an increased resistance toward tetracycline (128 versus 16 mg/L), deoxycycline (8 versus 1 mg/L), nalidixic acid (16 versus 8 mg/L), and moxifloxacin (0.094 versus 0.19 mg/L), whereas retained the same susceptibility to the other agents tested. Owing to the presence of two inducible L1 and L2 β-lactamase genes in *S. maltophilia* KJ[10], the impact of ΔSm*qnrR* to the β-lactams resistance may be shielded. Therefore, the ΔSm*qnrR* allele was further introduced into a L1/L2 double mutant KJΔL1ΔL2.[10] KJΔL1ΔL2ΔQnrR and KJΔL1ΔL2 had an indistinguishable β-lactams susceptibility, indicating that inactivation of Sm*qnrR* is irrelevant to the β-lactam resistance. Since Sm*qnr* has been known to be a determinant for the quinolone resistance in *S. maltophilia*,[7-8] it is reasonably speculated that the increased tetracycline resistance observed in KJΔQnrR can result from the overexpression of Sm*tcrA* gene.

134 To further elucidate the roles of Sm*qnr* and Sm*tcrA* in the antimicrobial
135 resistance, ΔSm*qnr* and ΔSm*tcrA* were, respectively, introduced into KJΔQnrR,
136 resulting in KJΔQnrRΔQnr and KJΔQnrRΔTcrA. A 206-bp PstI-fragment and a
137 395-bp SphI-fragment were deleted from pRKmQnr and pRKmTcrA, respectively,
138 and the resultant ΔSm*qnr* and ΔSm*tcrA* fragments were subcloned into pEX18Tc to
139 obtain mutagenic plasmids pΔQnr and pΔTcrA (Table 1, Fig. 1) for mutant
140 construction. Thereafter, mutants KJΔQnrRΔQnr and KJΔQnrRΔTcrA were
141 complemented with plasmids pRKmQnr and pRKmTcrA, respectively. The
142 susceptibility test for strains KJ, KJΔQnrR, KJΔQnrRΔQnr, KJΔQnrRΔTcrA,
143 KJΔQnrRΔQnr(pRKmQnr), and KJΔQnrRΔTcrA(pRKmTcrA) was performed. The
144 significant changes observed included: (i) The MICs of strains KJ, KJΔQnrR,
145 KJΔQnrRΔQnr, and KJΔQnrRΔQnr(pRKmQnr) toward nalidixic acid were 8, 16, 8,
146 and 16 mg/L and toward moxifloxacin were 0.094, 0.19, 0.094, and 0.125 mg/L,
147 respectively. These findings indicate that SmQnrR negatively regulates Sm*qnr*
148 expression and overexpression of SmQnr slightly contributes to the quinolone
149 resistance. (ii) The tetracycline MICs for strains KJ, KJΔQnrR, KJΔQnrRΔTcrA, and
150 KJΔQnrRΔTcrA(pRKmTcrA) were 16, 128, 16, and 64 mg/L, respectively. Therefore,
151 Sm*tcrA* is responsible for the acquired tetracycline resistance observed in KJΔQnrR.
152 To assess the contribution of Sm*qnr* and Sm*tcrA* genes to the intrinsic antibiotic

153 resistance of *S. maltophilia*, the MICs of strains KJ, KJΔQnr, and KJΔTcrA were
154 measured. Strains KJ, KJΔQnr, and KJΔTcrA exhibited the same MICs in all
155 antimicrobials tested, indicating that Sm*qnr* and Sm*tcrA* genes hardly contribute to
156 the intrinsic resistance in *S. maltophilia.*

157 The evidence of a short intergenic region (IG) of 11 bp between Sm*qnrR* and
158 Sm*tcrA* genes highly suggests that the two genes may form an operon. To verify the
159 possibility, a polar mutant KJQnrRΩ was constructed. A transcriptional terminator, Ω
160 cassette, retrieved from pX1918GT[11] was inserted into the SphI site of pΔQnrR,
161 resulting in the mutagenic plasmid pQnrRΩ for construction of mutant KJQnrRΩ (Fig.
162 1). The Sm*tcrA* transcript of KJQnrRΩ, determined by qRT-PCR, was as low as that
163 of the wild-type KJ. In addition, reverse transcription-PCR (RT-PCR) was performed
164 to amplify the joint between Sm*qnrR* and Sm*tcrA* genes using the primers set
165 QnrRC-F/QnrRC-R. A 489-bp PCR amplicon was observed in KJΔQnrR, but absent
166 in KJQnrRΩ. Accordingly, Sm*qnrR* and Sm*tcrA* genes form an operon.

167 The intergenic region (IG) between Sm*qnrR* and Sm*qnr* is as short as 145 bp.
168 DeoR-type regulator generally works as a repressor and binds onto the upstream of
169 the regulated gene. Therefore, it is likely that the binding site of SmQnrR overlaps
170 with the promoter of Sm*qnrR* gene. To determine whether the Sm*qnrR* gene has the
171 phenomenon of autoregulation, a promoter-*xylE* transcriptional fusion construct,

pRK145R$_{xylE}$, was obtained. The cloned 312-bp region contained the partial 5' terminus of Sm*qnr* gene, intact 145-bp intergenic region of Sm*qnr* and Sm*qnrR* genes, and partial 5' terminus of Sm*qnrR* gene. A *xylE* cassette was inserted behind the Sm*qnrR* gene to give rise to the Sm*qnrR-xylE* fusion construct pRK145R$_{xylE}$ (Fig. 1). The orientation of the *xylE* gene was opposite to that of $P_{lacZ}$ of the pRK415 vector. The catechol 2,3-dioxygenase (C23O) activities[10] of strains KJ(pRK145R$_{xylE}$) and KJΔQnrR(pRK145R$_{xylE}$) were measured. The C23O activities were seven times higher in KJΔQnrR(p145R$_{xylE}$) (50 ± 0.8 Uc/OD$_{450nm}$) than in the parental strain KJ(p145R$_{xylE}$) (7 ± 0.1 Uc/OD$_{450nm}$). Therefore, SmQnrR negatively regulates the expression of Sm*qnrR*-Sm*TcA* operon. In the absence of SmQnrR, overexpression of the Sm*qnrR*-Sm*TcA* operon will confer to the acquired tetracycline resistance in *S. maltophilia*.

*Nucleotide sequence accession number*

The nucleotide sequences of the *S. maltophilia* KJ Sm*tcrA*-Sm*qnrR*-Sm*qnr* regulon have been deposited in GenBank under accession no. HQ259608.

**Funding**

This work was supported by the National Science Council (NSC 98-2320-B-039-011-MY3) and the China Medical University (CMU99-S-42).

**Transparency declarations**

None to declare.

## References


**1.** Ruiz J. Mechanisms of resistance to quinolones: target alterations, decreased accumulation and DNA gyrase protection. *J Antimicrob Chemother* 2003; **51:** 1109-17.

**2.** Robicsek A, Strahilevitz J, Jacoby GA *et al*. Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. *Nat Med* 2006; **12:** 83–8.

**3.** Yamane K, Wachino J, Suzuki S, *et al.* New plasmid-mediated fluoroquinolone efflux pump, QepA, found in an *Escherichia coli* clinical isolate. *Antimicrob Agents Chemother* 2007; **51:** 3354–60.

**4.** Cattoir V, Nordmann P. Plasmid-mediated quinolone resistance in gram-negative bacterial species: an update. *Curr Med Chem* 2009; **16(8):** 1028-46.

**5.** Cattoir V, Poirel L, Mazel D *et al*. *Vibrio splendidus* as the source of plasmid-mediated QnrS-like quinolone resistance determinants. *Antimicrob Agents Chemother* 2007; **51:** 2650-1.

**6.** Shimizu K, Kikuchi K, Sasaki T *et al*. Sm*qnr*, a new chromosomal-carried quinolone resistance gene in *Stenotrophomonas maltophilia*. *Antimicrob Agnet Chemother* 2008; **52:** 3823-5.

**7.** Sanchez MB, Martinez JL. SmQnr contributes to the intrinsic resistance to

quinolones of *Stenotrophomonas maltophilia*. *Antimicrob Agnet Chemother* 2009; **54:** 580-1.

**8.** Gordon NC, Wareham DW. Novel variants of the Sm*qnr* family of quinolone resistance genes in clinical isolates of *Stenotrophomonas maltophilia*. *J. Antimicrob Chemther* 2010; **65:** 483-9.

**9.** Crossman LC, Gould VC, Dow JM *et al*. The complete genome, comparative and functional analysis of *Stenotrophomonas maltophilia* reveals an organism heavily shielded by drug resistance determinants. *Gen Biol* 2008; **9:** R74.

**10.** Hu RM, Huang KJ, Wu LT *et al*. Induction of L1 and L2 β-lactamases of *Stenotrophomonas maltophilia*. *Antimicrob Agents Chemother* 2008; **52:** 1198-200.

**11.** Huang YW, Lin CW, Hu RM *et al*. AmpN-AmpG operon is essential for expression of L1 and L2 β-lactamases in *Stenotrophomonas maltophilia.* *Antimicrob Agents Chemother* 2010; **54:** 2583-9.

**12.** Sanchez MB, Hernandez A, Rodriguez-Martinez JM *et al*. Predictive analysis of transmissible quinolone resistance indicates *Stenotrophomonas maltophilia* as a potential source of a novel family of Qnr determinants. *BMC Microbiol* 2008; **8:** 148.

**Figure Legends**

FIG. 1. A schematic representation of the Sm*tcrA*-Sm*qnrR*-Sm*qnr* region in *S. maltophilia* KJ and the structure of recombinant plasmids pRKmTcrA, pRKmQnrR, pRKmQnr, pΔQnrR, pQnrRΩ, pΔQnr, pΔTcrA, and pRK415$R_{xylE}$. The filled straight arrows represent ORFs and transcription direction. The solid lines represent the PCR amplicons and the dashed lines represent the deleted region for each plasmid construct. The symbol Ω indicates the inserted transcriptional terminator. The crosshatched arrows represent the *xylE* cassette.

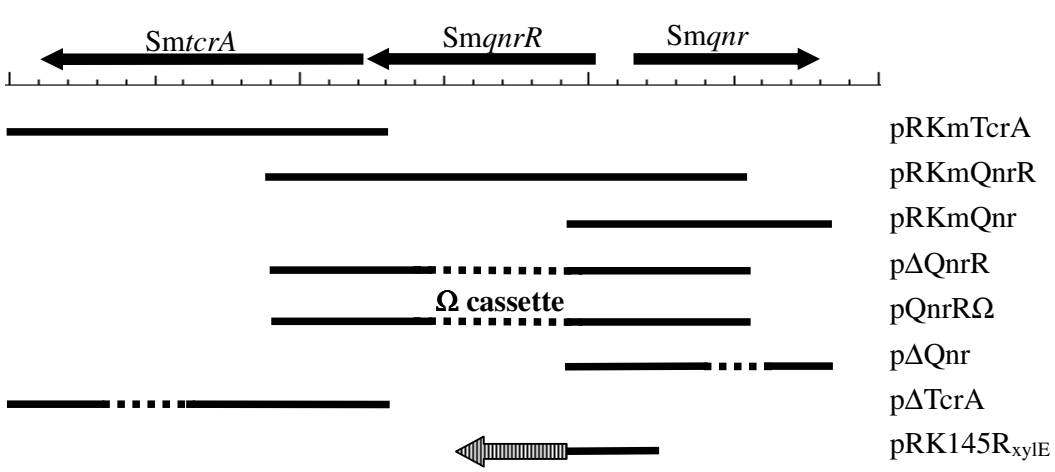
267 **TABLE 1. Bacterial strains, plasmids, and primers used in this study**

268

| Strain, plasmid or primer | Genotype or properties | Reference |
|---|---|---|
| ***S. maltophilia*** | | |
| KJ | Wild type, a clinical isolate from Taiwan | 10 |
| KJΔL1ΔL2 | *S. maltophilia* KJ *L1* and *L2* double mutant | 10 |
| KJΔL1ΔL2ΔQnrR | *S. maltophilia* KJ L1, L2 and Sm*qnrR* triple mutant | This study |
| KJΔQnrR | *S. maltophilia* KJ Sm*qnrR* isogenic mutant; deletion of 599-bp internal DNA fragment of Sm*qnrR* gene | This study |
| KJΔQnr | *S. maltophilia* KJ Sm*qnr* isogenic mutant; deletion of 207-bp internal DNA fragment of Sm*qnr* gene | This study |
| KJΔQnrRΔQnr | *S. maltophilia* KJ double mutant of Sm*qnrR* and Sm*qnr* genes | This study |
| KJΔTcrA | *S. maltophilia* KJ Sm*tcrA* isogenic mutant; deletion of 396-bp internal DNA fragment of Sm*tcrA* gene | This study |
| KJΔQnrRΔTcrA | *S. maltophilia* KJ double mutant of Sm*qnrR* and Sm*tcrA* genes | This study |
| KJQnrRΩ | *S. maltophilia* KJ Sm*qnrR* polar mutant; replacing the 599-bp internal DNA fragment of Sm*qnrR* gene with a transcriptional terminator Ω cassette | This study |
| *Escherichia coli* | | |
| DH5α | F- ϕ80d*lacZ*Δ*M15* Δ(*lacZYA-argF*)*U169 deoR recA1 endA1 hsdR17* ($r_k^-$ $m_k^+$) *phoA supE44*$\lambda^-$ *thi-1 gyrA96 relA1* | Invitrogen |
| S17-1 | λ pir + mating strain | 11 |
| **Plasmids** | | |
| pEX18Tc | *sacB oriT*, $Tc^r$ | 11 |
| pRK415 | Mobilizable broad-host-range plasmid cloning vector, RK2 origin; $Tc^r$ | 11 |
| pRKm415 | Derived from pRK415, replacing the tetracycline resistance gene with kanamycin resistance gene; $Km^r$ | This study |
| pX1918GT | Plasmid containing the *xylE*-gentamicin resistance cassette; $Amp^r$, $Gm^r$ | 11 |
| pRKmTcrA | pRKm415 vector with a 1419-bp DNA fragment | This study |

| | | |
|---|---|---|
| | of *S. maltophilia* KJ, containing an intact Sm*tcrA* gene; $Km^r$ | |
| pRKmQnrR | pRKm415 vector with a 1683-bp DNA fragment of *S. maltophilia* KJ, containing an intact Sm*qnrR* gene; $Km^r$ | This study |
| pRKmQnr | pRKm415 vector with a 826-bp DNA fragment of *S. maltophilia* KJ, containing an intact Sm*qnr* gene; $Km^r$ | This study |
| pΔQnrR | pEX18Tc vector with a 1359-bp DNA fragment of *S. maltophilia* KJ, containing the partial N-terminus of Sm*qnr* and Sm*qnrR* genes, partial C-terminus of Sm*qnrR* gene, and partial N-terminus of Sm*tcrA* gene; $Tc^r$ | This study |
| pΔQnr | pEX18Tc vector with a 640-bp DNA fragment of *S. maltophilia* KJ, containing the Sm*qnr* gene with a internal 207-bp deletion; $Tc^r$ | This study |
| pΔTcrA | pEX18Tc vector with a 1023-bp DNA fragment of *S. maltophilia* KJ, containing the Sm*tcrA* gene with a internal 396-bp deletion; $Tc^r$ | This study |
| pΔQnrRΩ | Derived from pΔQnrR, inserting a transcriptional terminator Ω cassette into the SphI site of pΔQnrR; $Tc^r$ | This study |
| $pRK145R_{xylE}$ | pRK415 with a 226-bp DNA fragment containing the 145-bp Sm*qnrR*-Sm*qnr* intergenic region and a Sm*qnrR*::*xylE* transcriptional fusion | This study |
| **Primer** | | |
| QnrB-F | 5'- GCAAAAGCTTGCATTGGTCGGG-3' | This study |
| QnrB-R | 5'- GTTCTAGACAGGCTTCAGCTTC-3' | This study |
| TcrA-F | 5'- GTAAGCTTCGCCGCCACCACTG -3' | This study |
| TcrA-R | 5'- GGAGCTCCTGCTGCTGAGCCTG -3 | This study |
| QnrRN-F | 5'- GCGCATGCCGCAAGTCTGCACGTTC -3' | This study |
| QnrRN-R | 5'- TTCCCAAAGCTTGCACTTTTCC -3' | This study |
| QnrRC-F | 5'- GAACACGAATTCCACCACGCCC -3' | This study |
| QnrRC-R | 5'- CGTCGCATGCACCACTGA AAAG -3' | This study |
| 16rDNAQ-F | 5'- GACCTTGCGCGATTGAATG -3' | 11 |
| 16rDNAQ-R | 5'- CGGATCGTCGCCTTGGT -3' | 11 |
| QnrBQ-F | 5'- TTCTACGATGCCGACAGCC -3' | This study |
| QnrBQ-R | 5'- CCACAGCTCGCACTTTTCC -3' | This study |
| TcrAQ-F | 5'- CGAGCAGAGCACCGAATAC -3' | This study |
| TcrAQ-R | 5'- ACCTACTGGCACCACAGCGAA -3' | This study |
| 415-F | 5'- CGACGACACCCGAAAAAAG -3' | 11 |
| 415-R | 5'- CATTAGCAACATTATCGCACAG -3' | 11 |

Fig. 1


Sm*tcrA*
Sm*qnrR*
Sm*qnr*
pRKmTcrA
pRKmQnrR
pRKmQnr
pΔQnrR
**Ω cassette**
pQnrRΩ
pΔQnr
pΔTcrA
pRK145R$_{xylE}$