J Antimicrob Chemother 2021; **76**: 1433–1440
doi:10.1093/jac/dkab045 Advance Access publication 20 February 2021

Journal of Antimicrobial Chemotherapy

Expression of the *qepA1* gene is induced under antibiotic exposure

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Received 26 November 2020; accepted 28 January 2021

Background: The *qepA1* gene encodes an efflux pump that reduces susceptibility to ciprofloxacin. Little is known about the regulation of *qepA1* expression.

Objectives: To assess the potential role of ciprofloxacin and other antibiotics in the regulation of *qepA1* gene expression. To identify the promoter that drives *qepA1* expression and other factors involved in expression regulation. To assess whether the identified features are universal among *qepA* alleles.

Methods: A translational *qepA1-yfp* fusion under the control of the *qepA1* upstream region was cloned into the *Escherichia coli* chromosome. Expression of the fusion protein was measured in the presence of various antibiotics. Deletions within the upstream region were introduced to identify regions involved in gene expression and regulation. The *qepA1* coding sequence and upstream region were compared with all available *qepA* sequences.

Results: Cellular stress caused by the presence of various antibiotics can induce *qepA1* expression. The *qepA1* gene is fused to a class I integron and gene expression is driven by the Pc promoter within the integrase gene. A segment within the integron belonging to a truncated *dfrB4* gene is essential for the regulation of *qepA1* expression. This genetic context is universal among all sequenced *qepA* alleles.

Conclusions: The fusion of the *qepA1* gene to a class I integron has created a novel regulatory unit that enables *qepA1* expression to be under the control of antibiotic exposure. This setup mitigates potential negative effects of QepA1 production on bacterial fitness by restricting high-level expression to environmental conditions in which QepA1 is beneficial.

Introduction

Fluoroquinolones, including ciprofloxacin, are a synthetic class of antimicrobial drugs with very good activity against Gram-negative and Gram-positive bacteria.[1–3] Ciprofloxacin binds to DNA gyrase and topoisomerase IV and inhibits re-ligation of cleaved DNA.[4–7] The accumulation of DNA breaks is thought to lead to cell death.[8] *Escherichia coli*, a major cause of urinary tract infections and invasive septicaemia, is commonly treated using ciprofloxacin.[9] However, many clinical isolates of *E. coli* have evolved resistance.[10,11] Evolution of resistance to ciprofloxacin in *E. coli* is a complex process that almost always includes multiple mutations in genes encoding DNA gyrase (*gyrA, gyrB*) and topoisomerase IV (*parC, parE*).[12,13] Additionally, mutations in genes encoding regulatory proteins of efflux pumps (*marR, acrR* and *soxR*) can lead to increased drug efflux[13] and mutations in transcription and translation-related genes (e.g. RNA polymerase and tRNA synthetase genes) can reduce susceptibility to ciprofloxacin by inducing global changes in bacterial protein synthesis.[14–16]

Horizontally acquired genes also contribute to the development of ciprofloxacin resistance. The *qnr* genes (*qnrA, qnrB, qnrC, qnrD, qnrS* and *qnrVC*) encode proteins that bind to the drug targets and protect the enzymes from ciprofloxacin.[17–22] A variant of an aminoglycoside acetyltransferase [encoded by *aac(6′)-Ib-cr*] acetylates ciprofloxacin, thus reducing its activity,[23] and two efflux pumps (encoded by *qepA* and *oqxAB*) reduce cellular ciprofloxacin concentrations.[24–27] Clinically observed plasmid-mediated ciprofloxacin resistance genes generally supplement the level of resistance caused by chromosomal mutations in drug target genes.[28] An exception is an *E. coli* isolate that acquired resistance by a combination of horizontally acquired genes (*qnrS* and *oqxAB*) and chromosomal mutations that increase drug efflux.[29] Additionally, it has been shown that overexpression of *qnrS* in *E. coli* can result in ciprofloxacin resistance levels above the clinical breakpoint.[30]

Since their discovery, 11 distinct *qepA* alleles have been identified (*qepA1–qepA11*). These alleles differ in one to five amino acids from *qepA1*.[31] The genetic context of the alleles *qepA1*,[24,27] *qepA2*,[32] *qepA3*,[33] *qepA4*[34] and *qepA7*[35] shows that the *qepA*

genes are linked to a truncated class I integron.[36] It is unclear whether the *qepA* genes are part of the truncated integron and whether the integron plays a role in expression of *qepA*. Here, we identify two novel *qepA* alleles, *qepA12* and *qepA13*, and show that 10 of the 13 *qepA* alleles share an identical upstream region consisting of a truncated integrase gene (*intI1*) and an ORF containing a partial *dfrB4* gene (upstream sequence is unavailable for *qepA6, qepA10* and *qepA11*). Using a translational fusion of the *qepA1* gene to a *yfp* gene [encoding yellow fluorescence protein (YFP)] we show that expression of *qepA1* is driven by the PcW_{TGN-10} promoter within the class I integron. Furthermore, expression of *qepA1* was induced by cellular stress caused by several antibiotics, including ciprofloxacin, and we were able to identify a region of around 250 nt that is essential for *qepA1* expression.

Materials and methods

Bacterial strains and growth conditions

All strains were derived from *E. coli* K12 MG1655 (Table S1, available as Supplementary data at *JAC* Online). Bacteria were grown at 37°C in LB broth (10 g/L yeast extract, 5 g/L tryptone, 10 g/L NaCl) or on LA plates (LB solidified with 1.5% agar, Oxoid). Strains harbouring temperature-sensitive plasmids were grown at 30°C. Antibiotics were from Sigma–Aldrich (Stockholm, Sweden) and added to induction assays as described. When added to growth medium, concentrations were: chloramphenicol, 25 mg/L; ciprofloxacin, 0.1 mg/L; kanamycin, 50 mg/L; and tetracycline, 15 mg/L. Sucrose counterselection was performed on salt-free LA supplemented with 5% sucrose.

Strain constructions

A 1.1 kb segment containing the first 600 nt of the *qepA1* gene and 546 nt of the upstream region were amplified from a clinical *E. coli* isolate (CH459)[37] and inserted into the *galK* gene of WT *E. coli* MG1655 (CH1464) using lambda-red recombineering[38,39] and sucrose counterselection.[40] The translational fusion of *yfp* to the *qepA1* coding sequence was constructed by inserting a *yfp* gene that is transcriptionally fused to a kanamycin resistance gene. Segmental deletions were constructed by inserting a *cat-sacB* selectable/counterselectable cassette upstream of *qepA1* followed by lambda-red recombineering using ssDNA oligonucleotides[41] to delete various segments. All oligonucleotides are listed in Table S2. The *gyrA* S83L D87N and $lexA^{Ind-}$ alleles were moved into strains using P1 *virA*-mediated transduction.[42]

PCR and local sequencing

DNA fragments used for recombineering were amplified using Phusion High Fidelity 2× PCR Master Mix (New England Biolabs, Ipswich, USA) and all other DNA amplifications were performed using 2× PCR Mastermix (Thermo Scientific, Waltham, USA) according to the protocols of the manufacturers. Amplification products were purified using SureClean Plus (Bioline, Germany) and sequencing of purified PCR products was performed by Macrogen (Amsterdam, The Netherlands). Sequences were analysed using CLC Main Workbench 20.0.4 (CLCbio, QIAGEN, Denmark).

Induction assay

One millilitre cultures in LB and LB containing subMIC antibiotics were initiated with ~10^6 cfu from overnight cultures and incubated for ~18 h at 37°C with 200 rpm shaking. Grown cultures were diluted 200-fold in PBS and *qepA* expression measured with a MACSQuant VYB (Miltenyi Biotec) as the average YFP fluorescence level of 30 000 cells. Each strain was grown in at least three biological replicates per antibiotic concentration. Mean fluorescence levels and 95% CIs for each strain were determined using a *t*-test comparing fluorescence of biological replicates that carry the *qepA1-yfp* fusion with the fluorescence level of biological replicates lacking the *qepA1-yfp* fusion and grown in identical conditions.

Identification and comparison of qepA alleles

qepA alleles were identified with a BLAST[43] search of the *qepA1* nucleotide sequence against the NCBI nucleotide collection database (nr/nt). Coding sequences and upstream regions of all sequences with >90% nucleotide identity were compared using the alignment tool of CLC Main Workbench 20.0.4 (CLCbio, QIAGEN).

qepA origin analysis

The protein sequence of *qepA* (WP_012372821.1) was aligned to three protein databases at NCBI (nr, refseq_protein and refseq_select_prot; alignment performed on 19 November 2020) using blastp.[43] After inspection of the taxonomic distribution of hits, the results of the alignment to refseq_protein was selected. Records with less than 50% identity or less than 80% query coverage were discarded, resulting in a dataset of 418 proteins. These sequences and the 22 sequences in Figure 4 of Yamane *et al.*[24] were aligned with MAFFT v7.471,[44] using the L-INS-i algorithm. Upon inspection of the alignment with SeaView v5.0.4,[45] six partial sequences were discarded, resulting in an alignment comprising 434 sequences. The alignment was trimmed with trimAl v1.4.rev22,[46] discarding sites with >50% gaps. The trimmed alignment was used to infer a maximum-likelihood tree with IQ-TREE v2.1.2,[47] drawing 1000 ultrafast bootstraps with the UFBoot2 algorithm[48] with the bnni option to reduce the risk of overestimating branch supports. The integrated ModelFinder[49] determined that the best fitting model was LG + F + R10, thus using the LG substitution matrix, empirical state frequency as observed from the data, and determining rate heterogeneity with the FreeRate model with 10 categories. The resulting tree was visualized with FigTree v1.4.4.[50]

Identification of functional sequence element

Potential transcription factor binding sites (TFBSs) were identified using the FIMO tool[51] from the MEME suite v5.3.0,[52] using default settings, including a threshold of 10^{-5}. We used the latest version available for download of PRODORIC[53] (release 8.9) as a TFBS database. The presence of rho-independent terminators was tested using the web tool ARNold,[54] screening both strands. We also used two tools of the RNAstructure package v6.0.1[55] to predict the presence of other secondary structures in the 247 nt segment: FOLD[56] and MaxExpect.[57] All algorithms were run with default settings, activating the DNA option.

Statistical analysis

Statistical analysis was performed using R, v3.5.0.[58]

Results and discussion

Expression of qepA1 is induced under cellular stress conditions

We previously identified a *qepA1* gene within an *E. coli* isolated from a urinary tract infection.[37] To study expression levels of *qepA1* in the absence of other resistance factors present within the clinical isolate, we cloned a 1.1 kb segment containing the first 600 nt of the *qepA1* gene and 546 nt of the upstream region into the chromosome of WT *E. coli* MG1655. Subsequently, a *yfp* gene (encoding YFP) was translationally fused to the *qepA1* coding sequence. This setup enables the use of YFP fluorescence

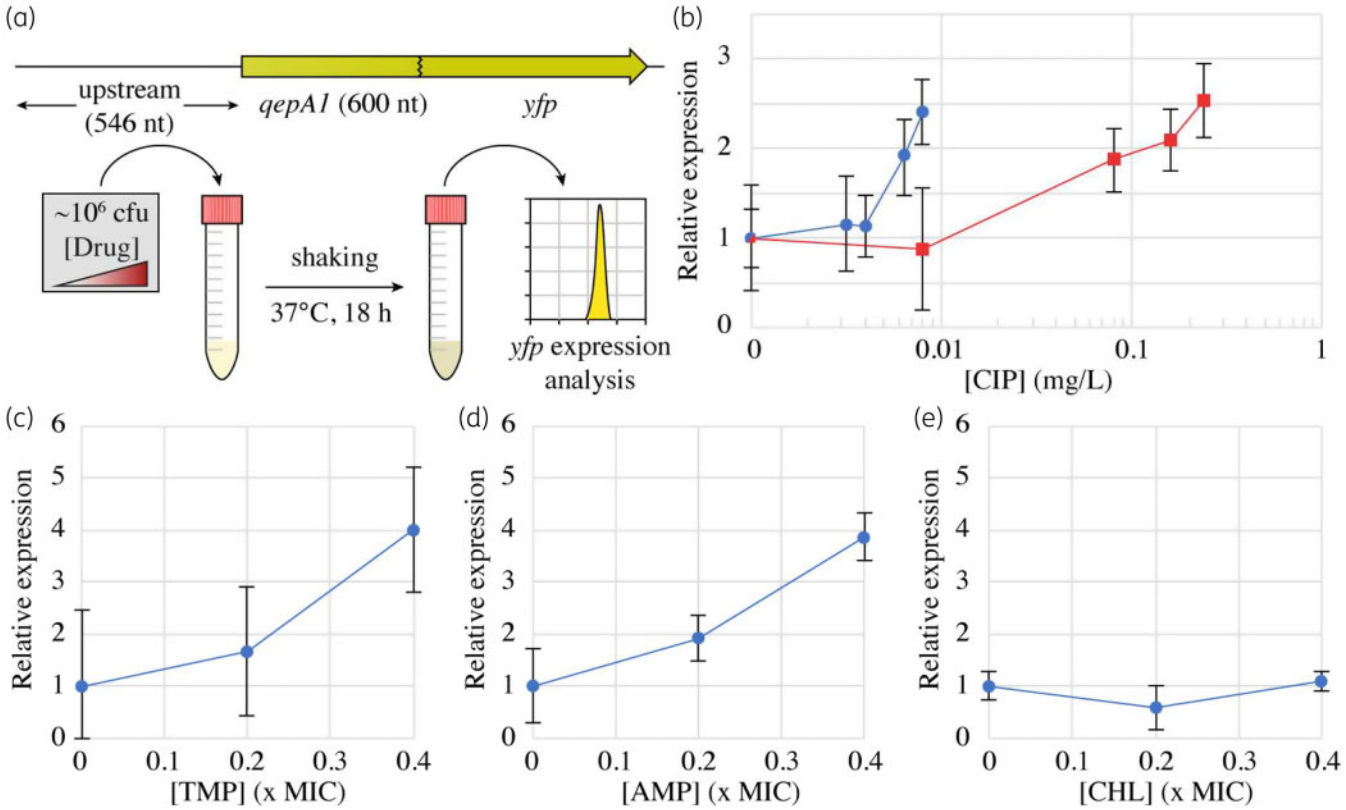


Figure 1. Induction analysis. (a) A translational fused *qepA1-yfp* gene under the control of the *qepA1* upstream region was cloned into the *E. coli* chromosome. Cultures were grown overnight in the presence of various antibiotic concentrations followed by YFP fluorescence level measurements. (b) YFP expression as a function of ciprofloxacin concentration in a WT (blue) and *gyrA* S83L D87N (red) background. (c–e) YFP expression in the presence of increasing concentrations of trimethoprim (TMP; c), ampicillin (AMP; d) and chloramphenicol (CHL; e). The MICs of the antibiotics were 0.016 mg/L ciprofloxacin, 0.5 mg/L trimethoprim, 16 mg/L ampicillin and 8 mg/L chloramphenicol. Each point is the average of at least three biological repeats. Error bars indicate 95% CIs.

measurements to indirectly measure *qepA1* expression levels (Figure 1a). We initially tested whether *qepA1* expression is induced by ciprofloxacin since *qepA1* is a quinolone resistance determinant. For this, cultures were grown in the presence of various subMIC concentrations of ciprofloxacin and fluorescence levels were measured. Fluorescence levels increased as a function of ciprofloxacin concentration indicating that *qepA1* expression is induced in the presence of ciprofloxacin with a 2.4-fold increase of expression in the presence of 0.008 mg/L ciprofloxacin, corresponding to 0.5× MIC_{CIP} of the WT (Figure 1b, blue line). We next tested whether *qepA1* induction is the direct effect of ciprofloxacin (e.g. by binding to the promoter) or an indirect effect (e.g. due to DNA damage). To test this, we moved a *gyrA* S83L D87N allele into the strain containing the translational *qepA1-yfp* fusion and measured expression in various ciprofloxacin concentrations. The *gyrA* allele reduces susceptibility to ciprofloxacin (MIC_{CIP} WT: 0.016 mg/L; *gyrA* S83L D87N: 0.38 mg/L) without affecting the intracellular ciprofloxacin concentration. If expression of *qepA1* is a direct effect of the presence of ciprofloxacin then the *gyrA* S83L D87N allele should not affect ciprofloxacin-dependent *qepA1* expression. Any indirect effects of ciprofloxacin such as DNA damage should be reduced by the *gyrA* S83L D87N allele so that higher ciprofloxacin concentrations would be required for induction of *qepA1* expression. The results of the induction assay showed no increase in *qepA1* expression in the presence of 0.008 mg/L ciprofloxacin compared with the 2.4-fold increase in the strain without the *gyrA* S83L D87N allele. Further increases in ciprofloxacin concentration resulted in increased expression with a 2.5-fold increase in the presence of 0.24 mg/L ciprofloxacin (Figure 1b, red line). This indicates that ciprofloxacin indirectly induces *qepA1* expression. To test whether expression of *qepA1* is exclusively induced by quinolones we measured expression levels in the presence of subMIC trimethoprim, ampicillin and chloramphenicol. Trimethoprim and ampicillin each caused ~4-fold induction of *qepA1* expression whereas chloramphenicol did not increase expression (Figure 1c–e). Taken together, the expression analysis indicates that cellular stress caused by the presence of various, but not all, antibiotics can induce *qepA1* expression.

qepA1 is fused to an inactive class I integron

The *qepA1* gene used in this study is preceded by an ORF that is a fusion of a truncated *dfrB4* gene (45 nt) and a segment of unknown origin (144 nt). Both genes (*orf* and *qepA1*) are located downstream of the attachment site (*attI1*) of a truncated integrase gene (*intI1*). The integron contains a PcW promoter with a 'TGN' extended −10 motif (PcW_{TGN-10})[59] that can drive expression

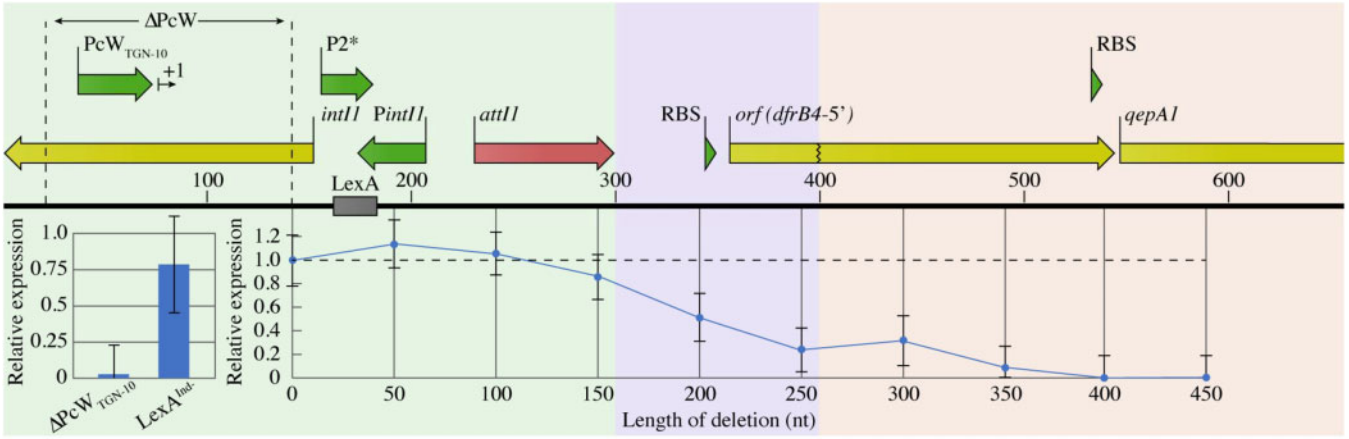


Figure 2. Analysis of the *qepA1* upstream region. (Top) Protein coding sequences are indicated in yellow, promoter sequences and ribosomal binding sites (RBSs) in green, PcW transcription start site indicated as +1, the attachment site of the integron in red and the LexA binding site in grey. (Bottom left) Relative *qepA1-yfp* expression in strains carrying a deletion of the PcW_{TGN-10} promoter (deletion is indicated above) or the non-inducible $LexA^{Ind-}$ allele in the presence of 0.008 mg/L ciprofloxacin (0.5× MIC_{CIP}). (Bottom right) Relative *qepA1-yfp* expression in strains carrying increasing deletions of the region between the PcW_{TGN-10} promoter and the *qepA1* coding sequence. The starting point of all deletions is indicated by the dotted line above the *y*-axis and the endpoint of each deletion is indicated by the corresponding grey line. Each expression value is the average of at least three biological repeats. Error bars indicate 95% CIs.

of the integrated genes and an inactive P2 promoter (P2*)[60] (Figure 2). A BLAST analysis of the 400 nt that contain the integrase gene and truncated segment of the *dfrB4* gene show that it is 99.5% identical to the corresponding segment of an integron found on the *Aeromonas caviae* pAeca2 plasmid (accession number CP039628). The integron found in *A. caviae* contains five resistance genes (*dfrB4, catB3, aadA, qacEΔ1* and *sul1*), which are expressed by a PcH1 promoter. The PcH1 promoter in the *A. caviae* integron differs in two nucleotides from the PcW_{TGN-10} promoter of the *qepA1* integron. These two nucleotide differences are the only differences within the segments containing the integrase and truncated *dfrB4* gene. This sequence comparison indicates that the *qepA1* region investigated in this study can be divided into three distinct regions: (i) an inactive class I integrase containing the segment from the truncated *intI1* gene to the *attI1* site (Figure 2, green background); (ii) the 5′ segment of a *dfrB4* gene including a 56 nt upstream region that was originally integrated into the class I integron (Figure 2, purple background); and (iii) the *qepA1* gene including 146 nt upstream sequence, of which 144 nt form a fused ORF with the truncated *dfrB4* gene (Figure 2, red background). This general structure agrees with previously described *qepA* alleles.[24,27,32–35] That *dfrB4* is truncated indicates that *qepA1* was not inserted by integrase but is rather the result of an integrase-independent recombination event as previously proposed.[32]

The qepA gene was probably acquired from Betaproteobacteria

Since the genetic context indicates an unusual fusion of *qepA1* to an inactive integron, we decided to determine the evolutionary origin of QepA. The similarity between QepA and MFS-type efflux pumps has previously been described.[24] To extend the evolutionary analysis of QepA, we searched the refseq_protein database of NCBI for sequences similar to QepA. We found 418 proteins at least 50% similar to QepA and covering at least 80% of its sequence. A phylogenetic analysis of these and 22 sequences from Yamane *et al.*[24] reveals that all QepA variants cluster in a well-supported clade (Figures 3 and S1). The phylogenetic analyses also show that MFS homologues form clusters with genomes from the same taxon, suggesting these proteins spread mostly by vertical inheritance, or horizontal gene transfer between related species. A notable exception is QepA, exclusively found in Enterobacterales, which branches inside a large clade constituted of Betaproteobacteria, mostly from Burkholderiales, but also from Nitrosomonadales. The clade most closely related to the QepA proteins contains proteins belonging to six species of Comamonadaceae (one *Variovorax* and five *Pseudorhodoferax*) and to a *Pseudomonas asiatica* strain MY680.[61] *Pseudorhodoferax* is a recently described genus of the Comamonadaceae.[62] The genomes in which the MFS transporter is found were isolated from soil (*Pseudorhodoferax soli*),[62] groundwater (*Pseudorhodoferax aquiterrae*)[63] and *Arabidopsis thaliana* leaves (other *Pseudorhodoferax* strains). These sequences represent all the available genomes of *Pseudorhodoferax*, suggesting that the last common ancestor of the genus already harboured the MFS transporter. The phylogenetic placement of the QepA clade as sister to the clade containing all *Pseudorhodoferax*, within a clade containing mostly Comamonadaceae, is consistent with a scenario where the MFS transporter was horizontally transferred from an ancestor of the *Pseudorhodoferax* genus to Enterobacterales.

Expression of qepA1 is driven by the PcW_{TGN-10} promoter

We next focused on the analysis of the region upstream of *qepA1*. A potential promoter outside the integron has previously been described for the *qepA2* allele.[32] We noticed that the potential −10 promoter sequence of the *qepA2* gene (TGTCGT) differed from the corresponding sequence of the *qepA1* gene used in our

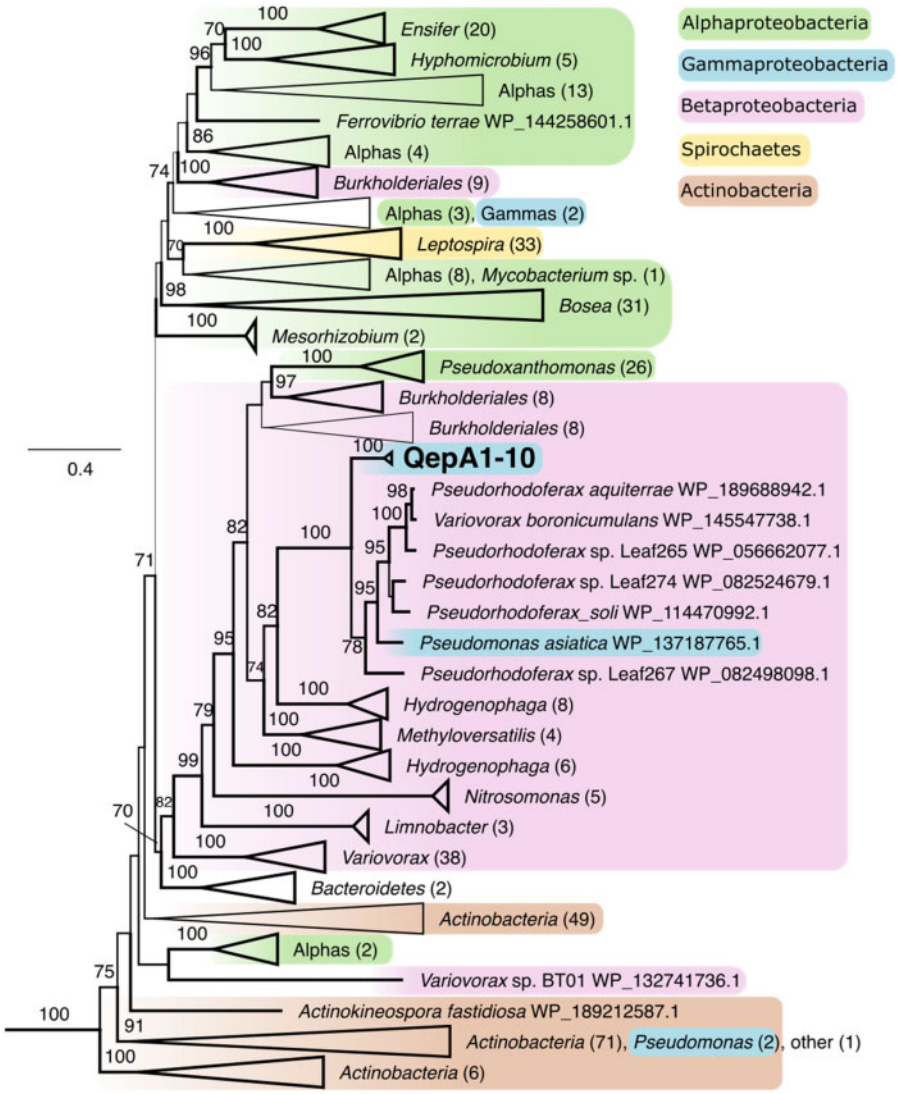
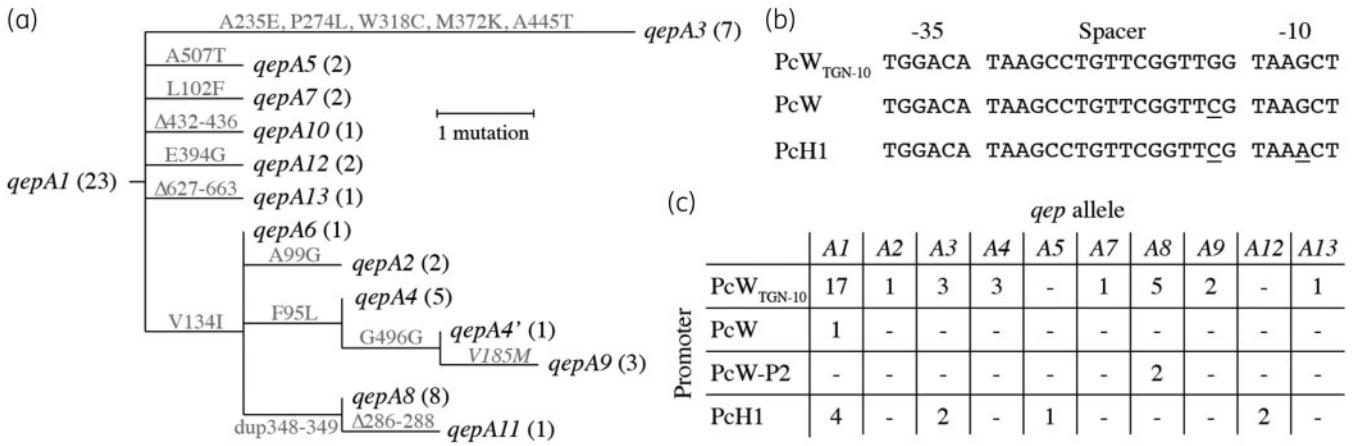


Figure 3. Maximum-likelihood phylogeny of the QepA protein and its close homologues. The phylogeny is based on an alignment of 418 proteins found through a blastp[43] search for proteins at least 50% similar to QepA and covering at least 80% of its sequence, using the refseq_protein as a database, and of 22 proteins from Yamane *et al.*[24] The tree is rooted with the 17 more distantly related sequences found to be homologous to QepA by Yamane *et al.*[24] In the labels, Alphaproteobacteria and Gammaproteobacteria are shortened to Alphas and Gammas, respectively. The position of the QepA variants (A1–A10) is indicated with a larger, bold font. Numbers between parentheses in the labels indicate the number of sequences in the collapsed node. The highlighted areas indicate sequences belonging to larger taxonomic groups (see legend on the figure). Numbers over branches represent the percentage of bootstrap support of that branch. Bootstrap supports <70% are not represented. The scale represents the average number of substitutions per site. A complete, non-collapsed tree is available in Figure S1.

study, which contains an additional C nucleotide (TCGTCGT). We asked which of these two potential promoter sequences is more common among *qepA* variants. A BLAST of *qepA1* nucleotide sequences revealed a total of 59 *qepA* sequences available in the NCBI nucleotide database (Table S3). Interestingly, we identified two *qepA* alleles not previously described, hereafter referred to as *qepA12* (as *qepA1* with E394G) and *qepA13* (as *qepA1* with Δ627–663) (Table S4). The most frequent *qepA* allele was *qepA1* (39%, 23/59) and most other *qepA* alleles (9/12) were only represented by 1–3 sequences (Figure 4a). We also identified a synonymous mutation (G496G, GGG to GGA) within *qepA9* that was also present in a single *qepA4* allele (hereafter named *qepA4'*). Fourteen of the 59 *qepA* sequences contained only the *qepA* coding sequence with no or little (100 nt) of the upstream sequence (Table S3). The upstream regions of the other 45 *qepA* sequences were compared with the sequence of the *qepA1* gene used in our study. We found that 67% (30/45) of the sequences were identical to the upstream region of the *qepA1* gene used in our study. Almost all of the remaining sequences differed only within the PcW_{TGN-10} promoter sequences (10/15) or in the PcW_{TGN-10} and P2* promoter sequences (2/15) (Table S3). The nucleotide variants found within PcW_{TGN-10} change the sequence to the PcW or PcH1 promoter (Figure 4b, Table S3). The changes identified within the P2* promoter increase the spacer length, potentially activating it but at



Promoter	A1	A2	A3	A4	A5	A7	A8	A9	A12	A13
PcW_{TGN-10}	17	1	3	3	-	1	5	2	-	1
PcW	1	-	-	-	-	-	-	-	-	-
PcW-P2	-	-	-	-	-	-	2	-	-	-
PcH1	4	-	2	-	1	-	-	-	2	-

Figure 4. Analysis of the *qepA* coding and associated Pc promoter sequences available within the NCBI nucleotide sequence database. (a) Phylogenetic relationship of all available *qepA* allele sequences. Differences relative to the *qepA1* sequence are indicated above the branches. The number of individual sequences available for each *qepA* allele is indicated in parentheses. (b) Sequences of the three Pc promoters identified upstream of *qepA* alleles within the database. Nucleotides that differ relative to the PcW_{TGN-10} promoter sequence are underlined. (c) Distribution of promoter sequences associated with the *qepA* alleles available within the NCBI database.

the same time altering the −10 sequence so that it is not possible to determine from the sequence whether the promoter is active or not (Table S3).[60] The upstream region of *qepA2* was the only sequence that contained the previously identified potential −10 promoter sequence.[32] This sequence comparison shows that the *qepA1* segment used in this study contains the most commonly found *qepA* variant (23/59 available sequences) with the most commonly found upstream region (30/45 available sequences). The lack of the −10 sequence found in the potential promoter of *qepA2*, coupled with the finding that almost all nucleotide variations found in the various *qepA* upstream sequences were located in the PcW_{TGN-10} promoter sequence, strongly suggests that the Pc promoter within the integron is important for *qepA* expression. We tested this by deleting the PcW_{TGN-10} promoter in our *qepA1-yfp* fusion construct. Expression analysis showed that deletion of the PcW_{TGN-10} promoter abolished gene expression (Figure 2). These data indicate that expression of most *qepA* alleles is driven by the Pc and potentially the P2 promoters within the integrase gene (Figure 4c).

The dfrB4 fragment is essential for qepA1 expression

Previous studies have shown that the bacterial SOS response is triggered by exposure to fluoroquinolones, β-lactams and trimethoprim but not by chloramphenicol.[64–66] We found that the *qepA1* gene was induced by the three antibiotics that trigger the SOS response but not by chloramphenicol (Figure 1). This suggests that *qepA1* might be under the regulation of the SOS response as shown for the ciprofloxacin resistance gene *qnrB2*.[67] The promoter of the *intI1* gene contains a LexA binding site and expression of the integrase gene is induced by the SOS response.[68,69] However, mutational alteration of the LexA binding site to prevent inhibition of the integrase promoter has shown that this specific LexA binding site has no effect on the transcription from any of the Pc promoter variants.[70] To test whether LexA binding to an alternative, so-far-unknown, binding site is involved in regulation of *qepA1* expression, we introduced a $lexA^{Ind-}$ allele into a strain that contains the *qepA1-yfp* fusion. The $lexA^{Ind-}$ allele carries a G80D mutation that prevents cleavage of the resulting LexA protein thus inhibiting induction by the SOS response.[71] If LexA is part of the *qepA1* regulation then a strain carrying the $lexA^{Ind-}$ allele should not exhibit *qepA1* induction in the presence of ciprofloxacin. We measured expression of the *qepA1-yfp* fusion protein in the presence of 0.008 mg/L ciprofloxacin (0.5× MIC_{CIP}) and found that the $lexA^{Ind-}$ allele did not influence induction of *qepA1* relative to the WT *lexA* allele ($P = 0.18$, *t*-test). This indicates that ciprofloxacin-dependent induction of the *qepA1* gene is not dependent on the SOS response (Figure 2).

We next asked whether the region between the PcW_{TGN-10} promoter and the *qepA1* gene is involved in the regulation of gene expression. For this, we removed increasingly larger segments of the region downstream of the PcW_{TGN-10} promoter and measured ciprofloxacin-dependent induction (Figure 2). We found that removal of the segment belonging to the integrase (including P2* and P*intI1* promoters, LexA binding site and *attI1* site) had no effect on the induction of the *qepA1* gene (Figure 2, green background). However, any deletion that included sequence from the truncated *dfrB4* segment significantly inhibited *qepA1* induction (Figure 2, purple background). We searched the region upstream of *qepA1* (247 nt, from the end of the *attIl* site, corresponding to position 300 on Figure 2) for the presence of functional elements such as TFBSs and possible hairpin structures, including transcription terminators. No TFBS or rho-independent transcription terminator could be identified in that region. The DNA secondary structure analysis revealed a complex structure comprising eight stems and six loops (Figures S2 and S3). However, the probabilities associated with the stems were, for the most part, lower than 80%. These data indicated that induction of the *qepA1* gene is independent of LexA and at least partly dependent on the sequence that was not originally part of the genetic context of the *qepA1* gene but we were so far not able to identify a functional element within this sequence (Figure 2, red background).

Conclusions

Here, we analysed the regulation of *qepA1* gene expression. We found that the *qepA1* gene is fused to a class I integron and that gene expression is driven by the Pc promoter within the integrase gene. Cellular stress caused by the presence of several, but not all, antibiotics can induce *qepA1* expression, and the segment within the integron belonging to a truncated *dfrB4* gene is essential for expression regulation. This genetic context is universal among all *qepA* alleles with available sequence data. Expression of efflux pumps is often tightly regulated since overexpression can be associated with large fitness costs to bacteria.[72–74] The acquisition of the *qepA* gene by horizontal gene transfer from Betaproteobacteria and fusion to an inactive class I integron has created a novel regulatory unit that enables *qepA* expression to be under the control of ciprofloxacin exposure. This setup mitigates potential negative effects of QepA production on bacterial fitness by restricting high-level expression to environmental conditions in which QepA is beneficial.

Funding

This work was supported by grants to Diarmaid Hughes from Vetenskapsrådet (the Swedish Research Council) (grant number 2017-03593) and from the Scandinavian Society for Antimicrobial Chemotherapy (grant numbers SLS-693211, SLS-876451). The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

Transparency declarations

None to declare.

Supplementary data

Figures S1 to S3 and Tables S1 to S4 are available as Supplementary data at *JAC* Online.

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