Effect of Qnr on Plasmid Gyrase Toxins CcdB and ParE

Yee Gyung Kwak,^{a,b} George A. Jacoby,^c David C. Hooper^a

Division of Infectious Diseases, Massachusetts General Hospital, Boston, Massachusetts, USA^a; Inje University College of Medicine, Busan, South Korea^b; Lahey Hospital and Medical Center, Burlington, Massachusetts, USA^c

Plasmid toxins CcdB and ParE are part of addiction systems promoting plasmid maintenance. Both target host DNA gyrase, as do quinolones and plasmid-determined Qnr proteins that protect gyrase from quinolone inhibition. We cloned *qnrB4*, *qnrS1*, *ccdB*, *parE*, and the antitoxin-encoding genes *ccdA* and *parD* on compatible plasmids and tested them in combination. CcdB and ParE had no specific effect on quinolone susceptibility or Qnr protection, and Qnr did not act as a CcdB or ParE antitoxin.

Many low-copy-number plasmids ensure their stable maintenance by encoding a toxin-antitoxin pair. The antitoxin has a shorter half-life than the toxin, so that if both were no longer synthesized following plasmid loss, the cell would die, thus enforcing plasmid stability. Plasmid toxins have various targets, with CcdB encoded by IncF plasmids (1) and ParE encoded by IncP plasmids (2) known to poison host bacterial DNA gyrase. CcdB binds to the dimerization domain of the GyrA subunit at Arg462, where replacement with cysteine blocks binding and prevents gyrase inhibition (1, 3). Quinolones also interact with the GyrA subunit but at sites different from those of the plasmid toxins, with mutations at selected amino acid positions between 51 and 106 lowering susceptibility (4). Quinolone inhibition is blocked by plasmid-mediated Qnr, a pentapeptide repeat protein that binds to both gyrase subunits (5, 6). Whether Qnr interferes with CcdB or ParE toxicity is not known, nor is the effect of these toxins on quinolone susceptibility in the presence of Qnr (7). We investigated the interaction of quinolone and *ccdB*, *parE*, and *qnr* genes cloned into compatible vectors in *Escherichia coli*.

Toxin genes were cloned after PCR amplification with primers carrying terminal restriction sites, allowing ligation into EcoRI and KpnI sites of expression plasmid pBAD24 (Amp^r, 4.5 kb) (8). The *ccdB* gene was amplified from *E. coli* clinical isolate 6-74 (9), which is positive by PCR for both *qnrS1* and *ccdAB*, and the *parE* gene was amplified from plasmid RP4 (10). After ligation, plasmids were chemically transformed into XL1-Blue competent cells (Agilent) with selection on Luria-Bertani (LB; Difco) agar plates containing ampicillin (100 µg/ml) plus 0.2% glucose to repress expression of the toxin insert. Antitoxin genes *ccdA* (from strain 6-74) and *parD* (from plasmid RP4) were cloned by using BamHI and PstI into pACYCDuet-1 (Chl^r, 4.0 kb; Novagen), a vector compatible with pBAD24, by using selection on chloramphenicol at 34 µg/ml. *qnrB4* from plasmid pMG319 (11) and *qnrS1* from plasmid pMG306 (12) were cloned similarly. The accuracy of cloning was confirmed by sequencing. Strain DH10B (Life Technologies) with various plasmid combinations was constructed by transformation with these recombinant plasmids by using ampicillin, chloramphenicol, or both for selection.

MICs of ciprofloxacin (Sigma-Aldrich) were determined at least in duplicate by agar dilution in accordance with the guidelines of the Clinical and Laboratory Standards Institute (13), except that LB agar containing ampicillin (50 µg/ml) or ampicillin plus chloramphenicol (25 µg/ml) was used to prevent plasmid loss. The ciprofloxacin MIC for *recA1* mutant strain DH10B was

TABLE 1 Ciprofloxacin MICs

Strain	Ciprofloxacin MIC ^a (µg/ml) at arabinose concn (%) of:			
	0	0.00125	0.0025	0.005
DH10B	0.008	0.008	0.004	0.004
DH10B/pBAD24 <i>ccdB</i>	0.008	0.008	0.004	0.004
DH10B/pBAD24 <i>parE</i>	0.008	0.008	0.004	0.004
DH10B/pACYCDuet-1 <i>qnrS1</i>	0.032	0.032	0.032	0.016
DH10B/pBAD24 <i>ccdB</i> /pACYCDuet-1 <i>qnrS1</i>	0.016	0.016	0.016	0.016
DH10B/pBAD24 <i>parE</i> /pACYCDuet-1 <i>qnrS1</i>	0.016	0.016	0.016	0.016

^a Determined by agar dilution on LB agar plates containing ampicillin and/or chloramphenicol with 0.2% glucose and 0% arabinose or the arabinose concentration indicated and 0% glucose.

0.008 µg/ml and was unchanged by the addition of pBAD24*ccdB* or pBAD24*parE* with glucose present in the medium to minimize toxin expression. With toxin expression stimulated by 0.0025 or 0.005% arabinose, MICs decreased by 1 dilution but a comparable change was seen with toxin-free strain DH10B, indicating that the slight effect was not toxin specific (Table 1). QnrS1 increased the ciprofloxacin MIC 4-fold compared to that for plasmid-free DH10B. The MIC elevation was 1 dilution less in DH10B with *ccdB* or *parE* present as well as *qnrS1* but was unchanged with arabinose stimulation, suggesting that the toxins had little, if any, effect on the ability of QnrS1 to protect gyrase from quinolone inhibition.

DH10B containing pBAD24*ccdB* or pBAD24*parE* could grow in LB broth with 0.01 or 0.02% arabinose but not higher concentrations, unless the CcdA or ParD antitoxin was supplied via plasmid pACYCDuet-1 derivatives (Table 2). Growth of strain CSH501/pBAD24*ccdB* in arabinose at up to 0.8% occurred since

Received 3 March 2015 Returned for modification 28 March 2015

Accepted 1 June 2015

Accepted manuscript posted online 8 June 2015

Citation Kwak YG, Jacoby GA, Hooper DC. 2015. Effect of Qnr on plasmid gyrase toxins CcdB and ParE. Antimicrob Agents Chemother 59:5078–5079. doi:10.1128/AAC.00524-15.

Address correspondence to George Jacoby, george.a.jacoby@lahey.org.

Copyright © 2015, American Society for Microbiology. All Rights Reserved.

doi:10.1128/AAC.00524-15

TABLE 2 Viability of CcdB and ParE toxin-containing clones

Strain	Growth at arabinose concn (%) of:						
	0.01	0.02	0.05	0.1	0.2	0.4	0.8
DH10B/pBAD24ccdB	+	+	—	—	—	—	—
DH10B/pBAD24ccdB/pACYCDuet-1qnrS1	+	+	—	—	—	—	—
DH10B/pBAD24ccdB/pACYCDuet-1qnrB4	+	+	—	—	—	—	—
DH10B/pBAD24ccdB/pACYCDuet-1ccdA	+	+	+	+	—	—	—
CSH501 pBAD24ccdB	+	+	+	+	+	+	+
DH10B/pBAD24parE	+	+	—	—	—	—	—
DH10B/pBAD24parE/pACYCDuet-1qnrS1	+	+	—	—	—	—	—
DH10B/pBAD24parE/pACYCDuet-1qnrB4	+	+	—	—	—	—	—
DH10B/pBAD24parE/pACYCDuet-1parD	+	+	+	+	+	—	—

^a +, growth; —, no growth (in LB broth containing the concentration of arabinose indicated).

CSH501 (14) has a *gyrA462* mutation blocking *ccdB* toxicity. Neither pACYCDuet-1*qnrS1* nor pACYCDuet-1*qnrB4* could substitute for *ccdA* or *parD*, indicating that the *qnr* genes lack an anti-toxin effect. The same result was obtained by measuring growth rates by colony counting. The growth rate of DH10B with pBAD24ccdB or pBAD24parE was the same whether pACYCDuet-1*qnrS1* was present in the strain or not and the medium was LB plus 0.2% glucose or LB with 0.005% arabinose, where the growth rate of the toxin-expressing strains was reduced 60%. We also found no plasmid instability in strains like clinical isolate 6-74 containing both *qnrS1* and *ccdB*.

Qnr-encoding genes are widely distributed on bacterial chromosomes, as well as plasmids, and predated the therapeutic use of quinolones. Their native role is not known, but a function as antidotes to plasmid maintenance systems mediated by gyrase toxins CcdB and ParE can be eliminated.

ACKNOWLEDGMENTS

We thank R. Varadarajan (Indian Institute Science, Bangalore) for supplying strain CSH501.

This work was supported by the Inje Research and Scholarship Foundation in 2011 (Y.G.K.) from the Inje University College of Medicine and by grant R01 AI057576 (to D.C.H. and G.A.J.) from the National Institutes of Health, U.S. Public Health Service.

REFERENCES

- Bernard P, Couturier M. 1992. Cell killing by the F plasmid CcdB protein involves poisoning of DNA-topoisomerase II complexes. *J Mol Biol* 226: 735–745. [http://dx.doi.org/10.1016/0022-2836\(92\)90629-X](http://dx.doi.org/10.1016/0022-2836(92)90629-X).
- Jiang Y, Pogliano J, Helinski DR, Konieczny I. 2002. ParE toxin encoded by the broad-host-range plasmid RK2 is an inhibitor of *Escherichia coli* gyrase. *Mol Microbiol* 44:971–979. <http://dx.doi.org/10.1046/j.1365-2958.2002.02921.x>.
- Dao-Thi MH, Van Melderden L, De Genst E, Afif H, Buts L, Wyns L, Loris R. 2005. Molecular basis of gyrase poisoning by the addition toxin CcdB. *J Mol Biol* 348:1091–1102. <http://dx.doi.org/10.1016/j.jmb.2005.03.049>.
- Hooper DC. 2003. Mechanisms of quinolone resistance, p 41–67. In Hooper DC, Rubinstein E (ed), *Quinolone antimicrobial agents*, 3rd ed. ASM Press, Washington, DC.
- Tran JH, Jacoby GA. 2002. Mechanism of plasmid-mediated quinolone resistance. *Proc Natl Acad Sci U S A* 99:5638–5642. <http://dx.doi.org/10.1073/pnas.082092899>.
- Tran JH, Jacoby GA, Hooper DC. 2005. Interaction of the plasmid-encoded quinolone resistance protein Qnr with *Escherichia coli* DNA gyrase. *Antimicrob Agents Chemother* 49:118–125. <http://dx.doi.org/10.1128/AAC.49.1.118-125.2005>.
- Ellington MJ, Woodford N. 2006. Fluoroquinolone resistance and plasmid addition systems: self-imposed selection pressure? *J Antimicrob Chemother* 57:1026–1029. <http://dx.doi.org/10.1093/jac/dkl110>.
- Guzman LM, Belin D, Carson MJ, Beckwith J. 1995. Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. *J Bacteriol* 177:4121–4130.
- Kim HB, Park CH, Kim CJ, Kim EC, Jacoby GA, Hooper DC. 2009. Prevalence of plasmid-mediated quinolone resistance determinants over a 9-year period. *Antimicrob Agents Chemother* 53:639–645. <http://dx.doi.org/10.1128/AAC.01051-08>.
- Datta N, Hedges RW, Shaw EJ, Sykes RB, Richmond MH. 1971. Properties of an R factor from *Pseudomonas aeruginosa*. *J Bacteriol* 108: 1244–1249.
- Robicsek A, Strahilevitz J, Sahm DF, Jacoby GA, Hooper DC. 2006. *qnr* prevalence in ceftazidime-resistant *Enterobacteriaceae* isolates from the United States. *Antimicrob Agents Chemother* 50:2872–2874. <http://dx.doi.org/10.1128/AAC.01647-05>.
- Gay K, Robicsek A, Strahilevitz J, Park CH, Jacoby G, Barrett TJ, Medalla F, Chiller TM, Hooper DC. 2006. Plasmid-mediated quinolone resistance in non-Typhi serotypes of *Salmonella enterica*. *Clin Infect Dis* 43:297–304. <http://dx.doi.org/10.1086/505397>.
- CLSI. 2009. Performance standards for antimicrobial susceptibility testing; Nineteenth informational supplement. CLSI document M100-S19. Clinical and Laboratory Standards Institute, Wayne, PA.
- Bajaj K, Chakrabarti P, Varadarajan R. 2005. Mutagenesis-based definitions and probes of residue burial in proteins. *Proc Natl Acad Sci U S A* 102:16221–16226. <http://dx.doi.org/10.1073/pnas.0505089102>.