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Characterization of a Mutation in the *parE* Gene That Confers Fluoroquinolone Resistance in *Streptococcus pneumoniae*

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We report a mutation in the *parE* genes of two in vitro mutants of *Streptococcus pneumoniae* responsible for low-level resistance to fluoroquinolones. Sequential acquisition of mutations in *parE* and *gyrA* leads to higher levels of resistance. This confirms that topoisomerase IV is the primary target of fluoroquinolones in *S. pneumoniae*.

Several new fluoroquinolones, such as sparfloxacin, exhibit improved activity against pneumococci, but resistance is likely to increase rapidly since sparfloxacin-resistant clinical isolates have already been detected (1, 5).

DNA gyrase and topoisomerase IV (topo IV) are known to be the intracellular targets of fluoroquinolones. The gyrase is composed of two A (GyrA) and two B (GyrB) subunits encoded, respectively, by the *gyrA* and *gyrB* genes, whereas topo IV includes two C (ParC) and two E (ParE) subunits encoded by the *parC* and *parE* genes, respectively.

In a previous study (13), we showed that a mutation in the *parC* gene was responsible for resistance in four of seven mutants examined that were resistant to low levels of fluoroquinolone (MICs of ciprofloxacin, 8 to 16 μg/ml; MICs of sparfloxacin, 0.5 to 1 μg/ml). Thus, as for *Staphylococcus aureus* (3, 4), topo IV appears to be the primary target of fluoroquinolones in *Streptococcus pneumoniae* (7, 9–11). However, another mutant studied, BM4205-R3, was resistant to higher levels of the drugs and carried a mutation in *gyrA* but not in *parC* (Table 1).

In the present study, we investigated if mutations in the *parE* gene could be associated with resistance in BM4205-R3 and in the three strains with low levels of resistance (BM4203-R3, BM4205-R1, and BM4205-R2) that are devoid of mutations in *gyrA*, *gyrB*, and *parC*. A 357-bp PCR fragment of *parE*, from position 2511 to 2867 (11), was obtained with specific primers SPPARE7 (5′CCAATCTAAGAATCCTG3′) and SPPARE8 (5′GCAATATAGACATGACC3′) under the following conditions, 30 s at 94°C, 45 s at 54°C, and 45 s at 72°C (30 cycles), and was sequenced directly on both strands from codons 408 to 476. A mutation, Asp to Asn at position 435 (Asp435→Asn [GAC→AAC]), encoded by *parE* was found in two of the three mutants with low levels of resistance examined and in BM4205-R3 (Table 1). Thus, the latter strain was in fact a *parE-gyrA* double mutant. Another, yet unidentified, mutation causing low-level resistance could be present in BM4205-R2 since a twofold difference between the MICs of ciprofloxacin and sparfloxacin for BM4205-R1 and those for BM4205-R2 was found.

In the transformation experiments that had been performed with total DNA from BM4205-R3, two phenotypic classes of transformants were obtained (13). In the present study, two transformants of each class were examined for the presence of mutations identical to those in BM4205-R3. Transformants of the first class, obtained on 2 μg of ciprofloxacin per ml (MIC of ciprofloxacin, 16 μg/ml; MIC of sparfloxacin, 1 μg/ml), carried only the mutation in *parE*, whereas those of the second class, obtained on 1 μg of sparfloxacin per ml (MIC of ciprofloxacin, 64 μg/ml; MIC of sparfloxacin, 4 μg/ml), had a mutation in both *parE* and *gyrA*. It thus appears that in BM4205-R3 the mutation in *parE*, responsible for resistance to low levels of fluoroquinolones, occurred prior to the mutation in *gyrA*.

It has been recently demonstrated that a mutation in *parE* plays a role in quinolone resistance in *Escherichia coli* (2). We report here the first mutation in *parE* implicated in fluoroquinolone resistance in gram-positive bacteria. In addition, our results provide further support for the notion that topo IV is the primary target of fluoroquinolones in *S. pneumoniae* (7, 9–11, 13). However, this may not hold true for sparfloxacin since the level of susceptibility to this drug is slightly affected or not affected by a mutation in either *parC* or *parE* (Table 1).

The Asp435→Asn substitution in ParE is located in a highly conserved portion of the protein which is homologous to the region of GyrB that corresponds to the quinolone resistance-determining region (QRDR) of *gyrB*. Mutations in *gyrB* leading to a similar Asp→Asn change have been described for several species (8, 10, 12, 14). Other mutations in *gyrB* (6, 8, 14), as well as the *parE* mutation found in *E. coli*, are also located in this highly conserved region. Thus, our data and

TABLE 1. Susceptibility of *S. pneumoniae* strains to selected fluoroquinolones and mutations in the *gyrA* and *parE* genes

Strain	MIC (μg/ml)[a] of:			Amino acid substitution (mutation)[b] in:	
	CIP	PEF	SPA	GyrA[c]	ParE
BM4203 (wild)	2	8	0.5		
BM4203-R3	8	16	0.5		
BM4205 (wild)	2	8	0.5		
BM4205-R1	8	32	0.5		Asp435→Asn (GAC→AAC)
BM4205-R2	16	32	1		Asp435→Asn (GAC→AAC)
BM4205-R3	64	128	4	Ser84→Phe (TCC→TCT)	Asp435→Asn (GAC→AAC)

[a] CIP, ciprofloxacin; PEF, pefloxacin; SPA, sparfloxacin.
[b] Updated from Tankovic et al. (13).
[c] Position of substitution is according to the *S. aureus* coordinates.

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those of Breines et al. (2) are consistent with the hypothesis that similar regions in the GyrB subunit of DNA gyrase and the ParE subunit of topo IV are implicated in the interaction with quinolones.

One of the in vitro mutants analyzed, BM4203-R3, was devoid of mutations in the QRDRs of *gyrA*, *gyrB*, *parC* (13), and *parE*. Other portions of these genes could be implicated in fluoroquinolone resistance. A more likely explanation for this finding, however, is that another resistance mechanism is involved, possibly active efflux of the drugs.

REFERENCES

1. **Bernard, L., J.-C. Nguyen Van, and J.-L. Mainardi.** 1995. In vivo selection of *Streptococcus pneumoniae* resistant to quinolones, including sparfloxacin. Clin. Microbiol. Infect. **1:**60–61.
2. **Breines, D. M., S. Ouabdesselam, E. Y. Ng, J. Tankovic, S. Shah, C. J. Soussy, and D. C. Hooper.** 1997. Quinolone resistance locus *nfxD* of *Escherichia coli* is a mutant allele of the *parE* gene encoding a subunit of topoisomerase IV. Antimicrob. Agents Chemother. **41:**175–179.
3. **Ferrero, L., B. Cameron, and J. Crouzet.** 1995. Analysis of *gyrA* and *grlA* mutations in stepwise-selected ciprofloxacin-resistant mutants of *Staphylococcus aureus*. Antimicrob. Agents Chemother. **39:**1554–1558.
4. **Ferrero, L., B. Cameron, B. Manse, D. Lagneaux, J. Crouzet, A. Famechon, and F. Blanche.** 1994. Cloning and primary structure of *Staphylococcus aureus* DNA topoisomerase IV: a primary target of fluoroquinolones. Mol. Microbiol. **13:**641–653.
5. **Fremaux, A., G. Sissia, C. Spicq, S. Aberrane, and P. Geslin.** 1993. Abstr. 367. *In* Program and abstracts of the 13th Interdisciplinary Meeting on Anti-Infection Chemotherapy. Société Française de Microbiologie, Paris, France.
6. **Gensberg, K., Y. F. Jin, and L. Piddock.** 1995. A novel *gyrB* mutation in a fluoroquinolone-resistant clinical isolate of *Salmonella typhimurium*. FEMS Microbiol. Lett. **132:**57–60.
7. **Gootz, T. D., R. Zaniewski, S. Haskell, B. Schmieder, J. Tankovic, D. Girard, P. Courvalin, and R. J. Polzer.** 1996. Activity of the new fluoroquinolone trovafloxacin (CP-99,219) against DNA gyrase and topoisomerase IV mutants of *Streptococcus pneumoniae* selected in vitro. Antimicrob. Agents Chemother. **40:**2691–2697.
8. **Ito, H., H. Yoshida, M. Bogaki-Shonai, T. Niga, H. Hattori, and S. Nakamura.** 1994. Quinolone resistance mutations in the DNA gyrase *gyrA* and *gyrB* genes of *Staphylococcus aureus*. Antimicrob. Agents Chemother. **38:** 2014–2023.
9. **Muñoz, R., and A. G. De La Campa.** 1996. ParC subunit of DNA topoisomerase IV of *Streptococcus pneumoniae* is a primary target of fluoroquinolones and cooperates with DNA gyrase A subunit in forming resistance phenotype. Antimicrob. Agents Chemother. **40:**2252–2257.
10. **Pan, X. S., J. Ambler, S. Mehtar, and L. M. Fisher.** 1996. Involvement of topoisomerase IV and DNA gyrase as ciprofloxacin targets in *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **40:**2321–2326.
11. **Pan, X. S., and L. M. Fisher.** 1996. Cloning and characterization of the *parC* and *parE* genes of *Streptococcus pneumoniae* encoding DNA topoisomerase IV: role in fluoroquinolone resistance. J. Bacteriol. **178:**4060–4069.
12. **Stein, D. C., R. J. Danaher, and T. M. Cook.** 1991. Characterization of a *gyrB* mutation responsible for low-level nalidixic acid resistance in *Neisseria gonorrhoeae*. Antimicrob. Agents Chemother. **35:**622–626.
13. **Tankovic, J., B. Périchon, J. Duval, and P. Courvalin.** 1996. Contribution of mutations in *gyrA* and *parC* genes to fluoroquinolone resistance of mutants of *Streptococcus pneumoniae* obtained in vivo and in vitro. Antimicrob. Agents Chemother. **40:**2505–2510.
14. **Yoshida, H., M. Bogaki, M. Nakamura, L. M. Yamanaka, and S. Nakamura.** 1991. Quinolone resistance-determining region in the DNA gyrase *gyrB* gene of *Escherichia coli*. Antimicrob. Agents Chemother. **35:**1647–1650.