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# *parC* Mutation Conferring Ciprofloxacin Resistance in *Streptococcus pyogenes* BM4513

*Streptococcus pyogenes* is responsible for high rates of morbidity due to an increase in invasive group A streptococcal infections and bacteremia worldwide during the last decade (2, 3). The goal of this study was to elucidate the mechanism of fluoroquinolone resistance in *S. pyogenes* BM4513 isolated in 2000 from the pharynx of a patient at the Hospital de Basurto, Bilbao, Spain.

In gram-positive cocci, fluoroquinolone resistance has been associated with mutational alterations in both targets, DNA gyrase and topoisomerase IV, or with active efflux of the drugs (4). Low-level fluoroquinolone resistance usually results from mutations in the quinolone resistance-determining regions of either the ParC subunit of topoisomerase IV or the GyrA subunit of the DNA gyrase, depending on the fluoroquinolone used as selector. For example, in *Streptococcus pneumoniae*, ciprofloxacin, levofloxacin, norfloxacin, pefloxacin, and trovafloxacin have ParC or ParE as the primary target, whereas GyrA is the preferred target of clinafloxacin, gatifloxacin, gemifloxacin, moxifloxacin, and sparfloxacin (5, 7, 8, 10).

The MICs of antibiotics were determined by the microdilution method according to the NCCLS guidelines (Table 1). The ciprofloxacin MICs remained unchanged in the presence of reserpine (10 μg/ml).

A 614-bp PCR fragment internal to *gyrA* and a 520-bp amplified fragment of *parC* obtained with specific primers (11) and total DNA of BM4513 as a template were sequenced directly on both strands with an automated sequencer (CEQ 2000 DNA Analysis System; Beckman Coulter).

As compared to the susceptible strains CIP5641T and ATCC 700294 (GenBank accession number AF220946), *S. pyogenes* BM4513 had a base pair change (TCC/GCC) in the *parC* gene at position 366 that resulted in amino acid substitution Ser79Ala (*S. pyogenes* coordinates). No mutations in the quinolone resistance-determining region of *gyrA* were detected.

To determine the contribution of the mutation to resistance in *S. pyogenes* BM4513, amplified DNA (1 μg) containing the mutation was transformed into *S. pneumoniae* CP1000 (6) with selection on ciprofloxacin (2 μg/ml). The TCC/GCC (Ser79Ala) mutation was amplified as part of a 792-bp PCR product obtained with the forward primer (11) and the reverse primer 5′-GTTAACTTCATAAGGAATCTCAGT-3′ (nucleotides 769 to 792, *S. pyogenes* coordinates). The corresponding PCR product was also amplified from DNA of susceptible strain CIP5641T. Only the transformation using amplified DNA from BM4513 yielded resistant colonies with a frequency of $1.1 \times 10^{-6}$ versus $<10^{-9}$ for the control. The DNA of two transformants amplified with the same primers was sequenced and found to contain the GCC mutation at codon 79. The MICs of fluoroquinolones for one of the transformants, BM4514, were determined and found to be identical to those against *S. pyogenes* BM4513 (Table 1). The corresponding mutation in the *parC* gene has been shown to confer low-level fluoroquinolone resistance (MICs, 4 to 8 μg/ml) in *S. pyogenes* mutants obtained in vitro (9) and in *S. pneumoniae* (1).

This study indicates that, in *S. pyogenes* BM4513, ciprofloxacin resistance was due to the Ser79Ala substitution in the ParC subunit of topoisomerase IV and that massive use of fluoroquinolones can select resistant mutants in nontarget bacterial species.

TABLE 1. Susceptibility of streptococci to selected fluoroquinolones and substitution in the ParC subunit

| Strain | MIC (μg/ml) of[a]: | | | | | | Mutation[b] |
|---|---|---|---|---|---|---|---|
| | CIP | CIP + R | LVX | MXF | OFX | SPX | |
| *S. pneumoniae* CP1000 | 0.5 | 0.5 | 0.5 | 0.125 | 1 | 0.25 | — |
| *S. pyogenes* CIP5641T | 0.5 | 0.5 | 0.5 | 0.125 | 1 | 0.25 | — |
| *S. pyogenes* BM4513 | 4 | 4 | 2 | 0.25 | 4 | 0.5 | Ser79Ala |
| *S. pneumoniae* BM4514 | 4 | 4 | 2 | 0.25 | 4 | 0.5 | Ser79Ala |

[a] CIP, ciprofloxacin; LVX, levofloxacin; MXF, moxifloxacin; OFX, ofloxacin; SPX, sparfloxacin; and R, reserpine (10 μg/mL).
[b] Position of substitution is according to the coordinates of *S. pneumoniae* (GenBank accession number X95717). —, no change.

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