NOTES

Increase in Quinolone Resistance in a *Haemophilus influenzae* Strain Isolated from a Patient with Recurrent Respiratory Infections Treated with Ofloxacin

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The increase in the level of quinolone resistance of *Haemophilus influenzae* clinical isolates during ofloxacin therapy of a patient with recurrent respiratory infections was investigated. The first isolate (MIC of ciprofloxacin of 2 µg/ml) and the second isolate (MIC of 32 µg/ml) belonged to the same clone, as shown by pulsed-field gel electrophoresis, and the increase in the resistance level was associated with a substitution in Ser-84 to Arg in the ParC protein. These results emphasize the potential risk of development of quinolone-resistant *H. influenzae* during fluoroquinolone therapy in patients with recurrent respiratory infection.

Haemophilus influenzae is a frequent cause of upper and lower respiratory tract infections (9). The new fluoroquinolones have been widely used as therapy for respiratory tract infections and have shown good activity against *H. influenzae* (3). Resistance to quinolones in *H. influenzae* is mainly due to chromosomal mutations in the *gyrA* and *parC* genes, which encode the A subunits of the DNA gyrase and topoisomerase IV, respectively (2, 7), similar to those found in other bacterial species (11, 12). Although ciprofloxacin-resistant *H. influenzae* strains have been isolated (2, 4), the development of quinolone resistance in *H. influenzae* from patients with chronic lung disease has been infrequently reported, and the mechanism of quinolone resistance acquisition has not been investigated (1).

We studied the increase in the level of quinolone resistance of an *H. influenzae* clinical isolate during ofloxacin therapy in a patient with recurrent respiratory infections. The patient was a 59-year-old female with severe bronchiectasis and recurrent respiratory infections repeatedly submitted to multiple courses of antibiotics (frequently including amoxicillin plus clavulanic acid or ciprofloxacin). In May 1997, she was admitted to the hospital for an episode of bronchial infection, respiratory failure, and severe hypoxemia. She was on ventilatory support and intravenous therapy. Sputum culture for noncapsulated *H. influenzae* (isolate 1) with susceptibility to ciprofloxacin (MIC, 2 µg/ml), ofloxacin (MIC, 4 µg/ml), and nalidixic acid (MIC, ≥256 µg/ml) was positive. The patient was treated with ofloxacin (200 mg/12 h orally) for 4 days and subsequently with amoxicillin plus clavulanic acid and was discharged after compensation. Four months later, she attended the outpatient clinic and a control sputum culture was positive, with two colonial morphotypes of *H. influenzae* being detected. One

(isolate 2) had the same resistance pattern as the one isolated in May (isolate 1), while the only difference with the other (isolate 3) was that the MIC of ciprofloxacin was 32 µg/ml (isolate 3). Finally, three months later, the patient was again admitted for respiratory deterioration, and two *H. influenzae* isolates with resistance patterns identical to those previously recovered were found in sputum. The epidemiological relationship of these isolates was investigated by pulsed-field gel electrophoresis (PFGE), showing that the three isolates belonged to the same clone (Fig. 1).

MICs were determined by a commercial microdilution test (Emiza 2E; Sensititre Ltd., Imberhorne, United Kingdom) and for nalidixic acid, by the E-test method (AB Biodisk, Dalvagen, Sweden) performed according to the manufacturers' instructions. In addition, for the strain with a MIC of ciprofloxacin higher than 2 µg/ml, the MIC was determined by the macrodilution broth method according to the National Committee for Clinical Laboratory Standards recommendations (10).

PCR amplification was used to amplify the quinolone resistance-determining region (QRDR) of the *gyrA* and *parC* genes, and the nucleotide sequences of these amplicons were determined. The oligonucleotide primers used to amplify the QRDR of the *gyrA* gene from nucleotides 17 to 816 (800 bp) (from codon 6 to 272) were 5' AATCATCTATCACCCCTG TC 3' and 5' TTTTGCTTTATTTACTTGGT 3', whereas for the amplification of the QRDR of the *parC* gene from nucleotides 95 to 471 (377 bp) (from codon 32 to 157) the oligonucleotide primers used were 5' ATCGTTCGTTGCCTTTTA TC 3' and 5' TTCAGCCAAGGTTCCATCAA 3'. The PCR program and the DNA sequencing methodology used were as described in reference 11.

Nucleotide sequencing of the 800- and 377-bp amplicons obtained from the QRDR of the *gyrA* and *parC* genes, respectively, revealed several mutations leading to the amino acid substitutions shown in Table 1.

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FIG. 1. Molecular typing of *H. influenzae* strains by PFGE. Lanes 1, 2, and 10 are molecular weight markers. Lanes 3, 4, and 5 are isolates 1, 2, and 3 of this study, respectively. Lanes 6, 7, 8, and 9 are different strains of *H. influenzae* chosen randomly.

The substitution at amino acid 84 (Ser-Leu) of the GyrA protein or its equivalent in other microorganisms is the most prevalent and has been found in *H. influenzae* (2, 7) and in other bacteria (11, 12). Georgiou et al. (7) found that strains with MICs of 2 µg/ml showed double mutations, one in the amino acid codon Asp-88 of the *gyrA* gene and another in the amino acid codon Ser-84 of the *parC* gene. Similarly, isolates 1 and 2 (MICs of ciprofloxacin of 2 µg/ml) of our study also present a double mutation, whereas the strain for which the MIC of ciprofloxacin was 32 µg/ml showed three main substi-

tutions, two in the GyrA protein (Ser-84 to Leu and Asp-88 to Ala) and one in the ParC protein (Ser-84 to Arg). These results are also in agreement with those found by Georgiou et al. (7). The mutation in the amino acid codon Asp-83, which generated a substitution to Asn, is apparently neutral despite the change in the charge.

Studying *H. influenzae* in sputum samples, Groeneveld et al. (8) found patients persistently infected with the same *H. influenzae* strain for up to 23 months. Similarly, the strain described herein persisted for at least 7 months despite the treatment with amoxicillin plus clavulanic acid.

These results emphasize the potential risk of development of quinolone-resistant *H. influenzae* during fluoroquinolone therapy in patients with recurrent respiratory infections and confirm the role of substitutions in positions Ser-84 and Asp-88 of the GyrA protein and Ser-84 of the ParC protein in the acquisition of quinolone resistance in this microorganism.

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TABLE 1. Mutations in the *gyrA* and *parC* genes of different isolates of *H. influenzae*

Strain	MIC of ciprofloxacin (µg/ml)	Amino acid change				
		GyrA			ParC	
Wild type ^a		Ser-84	Asp-88	Asp-83	Ser-84	Glu-88
Isolate 1	2	Leu	Ala	Asn		
Isolate 2	2	Leu	Ala	Asn		
Isolate 3	32	Leu	Ala	Asn	Arg	

^a The wild type was a quinolone-susceptible clinical strain isolated from a sputum sample from another patient.