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Genetic Characterization of Fluoroquinolone-Resistant *Streptococcus pneumoniae* Strains Isolated During Ciprofloxacin Therapy from a Patient with Bronchiectasis

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ABSTRACT

Five Spain^{9V}-3 *Streptococcus pneumoniae* were isolated from a patient with bronchiectasis who had received long-term ciprofloxacin therapy: one ciprofloxacin-susceptible strain was isolated before treatment and four ciprofloxacin-resistant strains were isolated during treatment. Resistant strains derived from the susceptible either by *parC* mutation (low-level resistance) or by *parC* plus *gyrA* mutation (high-level resistance). This study shows that ciprofloxacin therapy in a patient colonized with susceptible *S. pneumoniae* may select fluoroquinolone-resistant mutants.

Streptococcus pneumoniae remains a major etiological agent of community-acquired pneumonia, meningitis and acute otitis media. The emergence of resistance to antibiotics commonly used for the treatment of pneumococcal infections (13, 23) has highlighted the importance of the new fluoroquinolones that have been recommended for the treatment of respiratory tract infections (5). Although the prevalence of ciprofloxacin (Cip) resistance in *S. pneumoniae* is still low in Spain (3-7%) (1, 16, 27) and Canada (2%) (7), prior fluoroquinolone administration is a risk factor for resistant strains selection, as observed for infections caused by ciprofloxacin-resistant (Cip^R) (28) and levofloxacin-resistant (8, 34) *S. pneumoniae*. Likewise, resistance has been reported in blood isolates of viridans streptococci from neutropenic cancer patients who received fluoroquinolone prophylaxis (11, 35).

The targets of the fluoroquinolones are DNA gyrase (gyrase, $GyrA_2GyrB_2$) and DNA topoisomerase IV (topo IV, $ParC_2ParE_2$) enzymes (9). The pneumococcal *parC* and *parE* genes are homologous to *gyrA* and *gyrB*, respectively (3, 19, 26). Biochemical studies have established that Cip inhibits preferentially the pneumococcal topo IV that gyrase enzymes (10, 18, 24). Genetic studies have identified fluoroquinolone-resistance mutations in a discrete region of ParC, ParE, and GyrA termed the quinolone resistance-determining region (QRDR). Low-level (LL) Cip^R strains had mutations altering the QRDRs of one of the two subunits of topo IV: S79 or D83 of ParC (12, 15, 19, 26, 32), D435 of ParE (29). High-level (HL) Cip^R strains had changes affecting both QRDRs of ParC and GyrA (S81, E85)(12, 15, 19, 26, 32) or ParE and GyrA (29). Direct biological evidence showing that those mutations are involved in resistance has been obtained by transformation experiments. Single *parC* mutations confer low-level (LL) Cip-resistance (14, 19, 32), and, once the cells have acquired this LL-Cip^R phenotype, it is possible to transform to a higher level of resistance using DNA containing the *gyrA* QRDR from the HL-Cip^R strains (14, 19).

We describe herein the *in vivo* emergence of fluoroquinolone resistance in *S. pneumoniae* strains isolated from a patient that received multiple courses of Cip therapy for the treatment of a persistent *Pseudomonas aeruginosa*-infected bronchiectasis.

Patient history. A 64-year-old man was first seen in September-1996 with a longstanding history of chronic cough productive of purulent sputum. In his first clinical evaluation, a high-resolution thoracic scanner demonstrated the presence of bilateral bronchiectasis, and a ciprofloxacin-sensitive (Cip^S) *S. pneumoniae* (3983) was isolated from the sputum. In April-1997 he was first admitted to the hospital with a severe hypercapnic respiratory failure, and a Cip^S *P. aeruginosa* was isolated. Subsequently the patient received Cip (500 mg/ 12 h for 10 days) for the exacerbations. In October-1997, a HL-Cip^R *S. pneumoniae* (4371) was isolated. The patient remained under control and free of exacerbations until March-1998, when a Cip^S *S. pneumoniae* (4579) was isolated. In September 1998, due to the isolation of a Cip^S *Haemophilus influenzae* and Cip^S *Moraxella catharralis*, the patient was reintroduced into Cip, and one month later a LL-Cip^R *S. pneumoniae* (4837) was isolated. In December-1998 the patient had a hypercapnic respiratory failure and a HL-Cip^R *S. pneumoniae* (4866) and a Cip^S *P. aeruginosa* were isolated. In May-1999 he was readmitted to the hospital with a new infectious episode, a Cip^S *S. pneumoniae* (5181) and a Cip^S *P. aeruginosa* strain were isolated from sputum. From September-1999 to April-2000, the patient was treated regularly with Cip. On the visit at the Respiratory out-patient clinic (April-2000) the sputum yielded a Cip^R *P. aeruginosa* and a HL-Cip^R *S. pneumoniae* (5558). The patient died two weeks later due to an irreversible hypercapnic respiratory failure.

Characterization of *S. pneumoniae* isolates. The antibiotic resistance patterns of the strains, serotypes, MICs of selected fluoroquinolones (determined as previously described, 20) and QRDRs mutations are shown in Table 1. PCR products containing *gyrA*, *gyrB*, *parC*, and *parE* QRDRs were obtained as previously described (11), separated in agarose gels (30),

purified and sequenced on both strands. The HL-Cip^R (MIC ≥ 64 μg/ml) strains showed cross-resistance to other fluoroquinolones. Given the fluoroquinolone MICs for the LL-Cip^R 4837 strain, this strain could be considered as susceptible according to the NCCLS breakpoint criteria (21). However this strain has mutations that would favor the appearance of HL-Cip^R strains and maybe those breakpoints should be revised in accordance. The LL-Cip^R strain 4837 (MIC of 8 μg/ml) had a *parC* mutation and the HL-Cip^R strains had *parC* plus *gyrA* mutations.

Strains 3983, 4371 and 4837 share the same serotype (9V) and PFGE patterns (determined as described previously, 31), belonging to the Spain^{9V}-3 clone (17). The two last 9V serotype HL-Cip^R isolates (4866 and 5558) share an identical PFGE pattern that differs from that of the Cip^S 3983 and ATCC 700671 strains by three-band difference and are considered to be Spain^{9V}-3 subtypes (33). Despite those PFGE pattern differences that could be a consequence of genome rearrangements that are common among *S. pneumoniae* (6), all Spain^{9V}-3 strains showed identical polymorphisms on their QRDRs with respect to the sequence of the R6 strain: K137N change in ParC, I460V change in ParE, and a change in the Y74 codon of GyrA (TAT instead TAC) (Table 1). A genealogy of the strains was derived. The HL-Cip^R strain 4371 could derive from the Cip^S 3983 strain by acquisition of two changes: ParC S79Y and GyrA S81F. Although the LL-Cip^R strain from which strain 4371 (October, 1997) has been derived has not been identified in this work, because sputum cultures were not performed between April-September 1997, that strain could be present in the respiratory tract of the patient during this period. Likewise, the LL-Cip^R 4837 strain could also derive from the Cip^S 3983 strain by acquisition of a S79F ParC change. The two last HL-Cip^R isolates (strain 4866 and 16 months later, strain 5558) showed S79F ParC and S91F GyrA changes.

The analysis of the *S. pneumoniae* strains sequentially isolated from this patient clearly shows that resistance develops during treatment by mutation in the primary (topo IV) and

secondary (gyrase) targets. Initially, the patient was infected-colonized by a Cip^S $Spain^{9V}$-3 strain (3983) and it was undergoing serial mutagenesis when he was receiving Cip therapy, yielding deferent degrees of Cip resistance (Fig. 1). This *in vivo* acquisition of resistance is consistent with genetic transformation experiments (15, 19) and with generation of Cip^R mutants (25) under laboratory conditions. The emergence of Cip^R *S. pneumoniae* occurred concurrently with Cip treatments and could be favored for the low serum concentrations yielded with this compound (1.5 – 3 μg/ml), which is close to the MIC value (0.5 – 1 μg/ml) for Cip^S strains. On the other hand two Cip^S strains (4579 and 5181), with different serotypes, PFGE types and gene polymorphisms appeared after periods without treatment, showing that without antibiotic pressure there was no selection of resistant mutants.

In our patient, previous chronic use of fluoroquinolones for a persistent bronchial infection was a risk factor in the development of antibiotic resistance, not only in the microorganisms considered causative of infectious exacerbations, such as *P. aeruginosa*, but also for those colonizing or co-infecting bronchiectasis. Results from our group demonstrated that prior fluoroquinolone use, purulent bronchitis and prior hospitalization, are risk factors for developing respiratory tract infections caused by Cip^R pneumococci (J. Liñares, F. Tubau, R. Pallarés, M. J. Ferrándiz, M. A. Domínguez, F. Manresa, A. G. de la Campa, and R. Martín. Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2106, 2000)

Since infectious episodes are frequent and recurrent in chronic obstructive pulmonary disease (COPD) and bronchiectasis, antibiotics are generally prescribed in an empirical way, without bacteriological studies. Should the clinician reuse a fluoroquinolone in a patient with bronchiectasis once it has already been used? According to our own experience, and previous published data (8), a high risk of fluoroquinolone resistance development of *S. pneumoniae* in patients with recent fluoroquinolone therapy may exist, and it must be considered before the introduction of an empirical antibiotic. In our experience, previous use of fluoroquinolones

may develop cross-resistance to levofloxacin and other newer fluoroquinolones. Thus the empiric and systematic use of levafloxacin in the treatment of exacerbations of COPD or bronchiectasis has to be questioned, and a modification of the ATS (2) and IDSA (4) guidelines could be necessary.

Today, restriction of the use of fluoroquinolones and performance of susceptibility studies for monitoring the prevalence of fluoroquinolone-resistant pneumococci is recommended. It is important to keep in mind that most patients infected with invasive multiresistant pneumococci may still be treated with an appropriate betalactam such as amoxycillin or ceftriaxone (22, 23).

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FIG. 1. Time course of ciprofloxacin treatments and emergence of *S. pneumoniae* strains. Only mutations involved in fluoroquinolone resistance are indicated. NT, not typeable.

TABLE 1. Susceptibilities to fluoroquinolones and mutations in the topoisomerases QRDR of *S. pneumoniae* clinical isolates [a]

Strain	Type	PFGE [a]	Resistance pattern [b]	MIC (μg/ml) of [c]:				Mutation(s) in the QRDR of: [d]		
				CIP	LVX	GAT	MXF	ParC	GyrA[b]	ParE[b]
R6	NT		S	0.5	0.5	0.25	0.12	None	None	None
ATCC 49619	19F		PEN	1	1	0.25	0.12	None	Y74 (TAT)	None
ATCC 700671	9V	Spain^{9V} -3	PENSXT	1	0.5	0.25	0.12	K137N	Y74 (TAT)	I460V
3983	9V	Spain^{9V} -3	PENSXT	1	0.5	0.25	0.12	K137N	Y74 (TAT)	I460V
4371	9V	Spain^{9V} -3	PENSXT	128	32	8	4	S79Y, K137N	S81F, Y74 (TAT)	I460V
4579	10	B	S	1	0.5	0.25	0.12	K137N	—	—
4837	9V	Spain^{9V} -3	PENSXT	8	2	0.5	0.25	S79F, K137N	Y74 (TAT)	I460V
4866	9V	Spain^{9V} -3*	PENSXT	64	16	4	4	S79F, K137N	S81F, Y74 (TAT)	I460V
5181	NT	D	S	0.5	0.5	0.25	0.12	K137N	—	—
5558	9V	Spain^{9V} -3*	PENSXT	64	16	4	4	S79F, K137N	S81F, Y74 (TAT)	I460V

[a] The asterisk indicates that those strains are Spain^{9V}-3 clone subtypes.

[b] S, susceptible to all antibiotics tested; PEN, resistant to penicillin (MICs of 2-4 μg/ml, except for ATCC 49619 that was 0.25 μg/ml); SXT; resistant to trimethropim-sulfamethoxazole (MICs of 4/76 μg/ml); .

[c] CIP, ciprofloxacin; LVX, levofloxacin; GAT, gatifloxacin; MXF, moxifloxacin.

[d] Residue changes involved in fluoroquinolones resistance are underlined. Mutations (by reference to the R6 DNA sequence) were as follows: ParC, S79F (TCT→TTT), K137N (AAG→AAT); GyrA S81F (TCC→TTC); ParE I460V (ATC→GTC). The strains indicated harbored a silent mutation (TAC→TAT) at codon 74 of the *gyrA* sequence. No changes in the QRDR of GyrB were found. —, not determined.