Clinical Isolates of the Spain¹⁴-5 Clone of *Streptococcus pneumoniae* Carry a Recombinant *rpoB* Gene

A characterization of 61 rifampin (RIF)-resistant *Streptococcus pneumoniae* isolates revealed two isolates (Rif-15 and Rif-16) of the Spain¹⁴-5 clone with identical recombinant *rpoB* genes (5) that were collected at the Hospital Donostia (HD) from two human immunodeficiency virus-infected patients. Given that the Spain¹⁴-5 clone has been extensively studied in HD (10, 11), we have analyzed in this work 10 RIF-susceptible isolates representative of each *pbp2b* restriction fragment length polymorphism allelic profile among 93 isolates of the Spain¹⁴-5 clone from the HD (Table 1). The MICs, pulsed-field gel electrophoresis patterns, and *rpoB* sequences were determined as previously described (5). The two RIF-resistant strains carried, in addition to the H499N change responsible for RIF resistance (5), several residue changes common to five RIF-susceptible isolates (Fig. 1) and to the *Streptococcus mitis* NCTC 12261 type strain (5), changes that are therefore not involved in RIF resistance. Comparisons of the *rpoB* sequences of the 12 strains with that of *S. pneumoniae* R6 (Fig. 1) revealed three types of isolates: four isolates that were nonrecombinant (0.15% variation) whose sequences were identical to that of the type strain, seven recombinant isolates (R2 and R3; 4.1% variation) that had identical sequences (excluding the mutation responsible for the H499N change), and one recombinant isolate (R1; 1.2% variation) that featured two blocks of divergence, the first (L42 to T472) being identical to the sequence of *S. pneumoniae* R6 and the second (A473 to T700) being identical to that of strains R2 and R3.

Recombination events between *S. pneumoniae* and the viri-

dans streptococci of the mitis group (VSM), which have presumably occurred in the face of antibiotic selection pressure and have led to the acquisition of resistance, have been described for the genes encoding the targets of penicillin (7), fluoroquinolones (1, 4), and rifampin (5). Furthermore, recombination in genes not involved in antimicrobial resistance and therefore not subjected to selective pressure has also been detected (3, 8, 9). Given the great genetic diversity of neutral VSM genes (12), fluoroquinolone target genes (1, 6), and *rpoB* (M. J. Ferrández et al., unpublished), the most plausible explanation for the identity of the sequences of the *rpoB* recombinant isolates is that they derive from an ancestral isolate that underwent recombination with an RIF-susceptible VSM at, or before, 1987. Isolate Rif-16 would have acquired RIF resistance by point mutation after recombination in the presence of RIF selection (this patient had a *Mycobacterium avium*-disseminated infection and was treated with RIF). Transmission among patients infected with Rif-16 and Rif-15 could have occurred, since there is no evidence of RIF treatment in the patient infected with Rif-15 and both patients were admitted in the same unit of HD. However, in other cases, RIF resistance could be acquired directly by recombination (5), given that, under the ideal laboratory conditions, the frequency of transformation is several orders of magnitude greater than that of spontaneous mutation, being 5×10^{-9} (2; our unpublished results), and the frequencies of transformation are 1×10^{-2} (5) and 2×10^{-4} for chromosomal DNAs from *S. pneumoniae* and VSM, respectively (data not shown). The frequency of

TABLE 1. Characteristics of *S. pneumoniae* isolates of the Spain¹⁴-5 clone from the Hospital Donostia

Isolate ^a	Yr	Origin	Multilocus sequence type	Resistance pattern ^b	RIF MIC (μg/ml)	<i>rpoB</i> sequence ^c
ATCC 700902			18	P T C SxT	≤0.03	
C-12950	1981	Pharynx	17	P E Cl TC	≤0.03	NR
C-54576	1990	Ear	18	P T C SxT	≤0.03	NR
H-9232-J	1995	Blood	18	P T C SxT	≤0.03	NR
E-232472	1999	Sputum	18	P T C SxT CIP	≤0.03	NR
E-234662	2002	Sputum	17	P E Cl T C SxT	≤0.03	R1
C-38500	1987	Ear	17	P E Cl T C SxT	≤0.03	R2
E-87-C	1991	Sputum	17	P E Cl T C SxT	≤0.03	R2
H-2728-H	1992	Blood	17	P E Cl T C SxT	≤0.03	R2
H-2950-L	1996	Blood	17	P E Cl T C SxT CIP	≤0.03	R2
E-232014	2001	Sputum	17	P E Cl T C SxT	≤0.03	R2
Rif-15	1993	Sputum	17	P E Cl T C SxT CIP R	16	R3
Rif-16	1993	Sputum	17	P E Cl T C SxT CIP R	16	R3

^a ATCC 700902 is the strain representative of the Spain¹⁴-5 clone.

^b P, resistant to penicillin (MICs, ≥2 μg/ml); T, resistant to tetracycline (MICs, ≥4 μg/ml); C, resistant to chloramphenicol (MICs, ≥8 μg/ml); E, resistant to erythromycin (MICs, ≥0.5 μg/ml); Cl, resistant to clindamycin (MICs, ≥0.5 μg/ml); SxT, resistant to trimethoprim-sulfamethoxazole (MICs, ≥4 and 76 μg/ml, respectively); CIP, resistant to ciprofloxacin (MICs, ≥4 μg/ml); R, resistant to RIF (MICs, ≥4 μg/ml).

^c NR, nonrecombinant; R1, R2 and R3, recombinant.

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