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# Clinical Isolates of the Spain[14]-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 Spain[14]-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 Spain[14]-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 Spain[14]-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 viridans 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genetic diversity of neutral VSM genes (12), fluoroquinolone target genes (1, 6), and *rpoB* (M. J. Ferrándiz et al., unpublished), the most plausible explanation for the identity of the sequences of the *rpoB* recombinant isolates is that they derive from an ancestral isolate that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 $5 \times 10^{-9}$ (2; our unpublished results), and the frequencies of transformation are $1 \times 10^{-2}$ (5) and $2 \times 10^{-4}$ for chromosomal DNAs from *S. pneumoniae* and VSM, respectively (data not shown). The frequency of

TABLE 1. Characteristics of *S. pneumoniae* isolates of the Spain[14]-5 clone from the Hospital Donostia

| Isolate[a] | Yr | Origin | Multilocus sequence type | Resistance pattern[b] | RIF MIC (μg/ml) | *rpoB* 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 ClP | ≤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 ClP | ≤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 ClP R | 16 | R3 |
| Rif-16 | 1993 | Sputum | 17 | P E Cl T C SxT ClP R | 16 | R3 |

[a] ATCC 700902 is the strain representative of the Spain[14]-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 trimethropin-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.

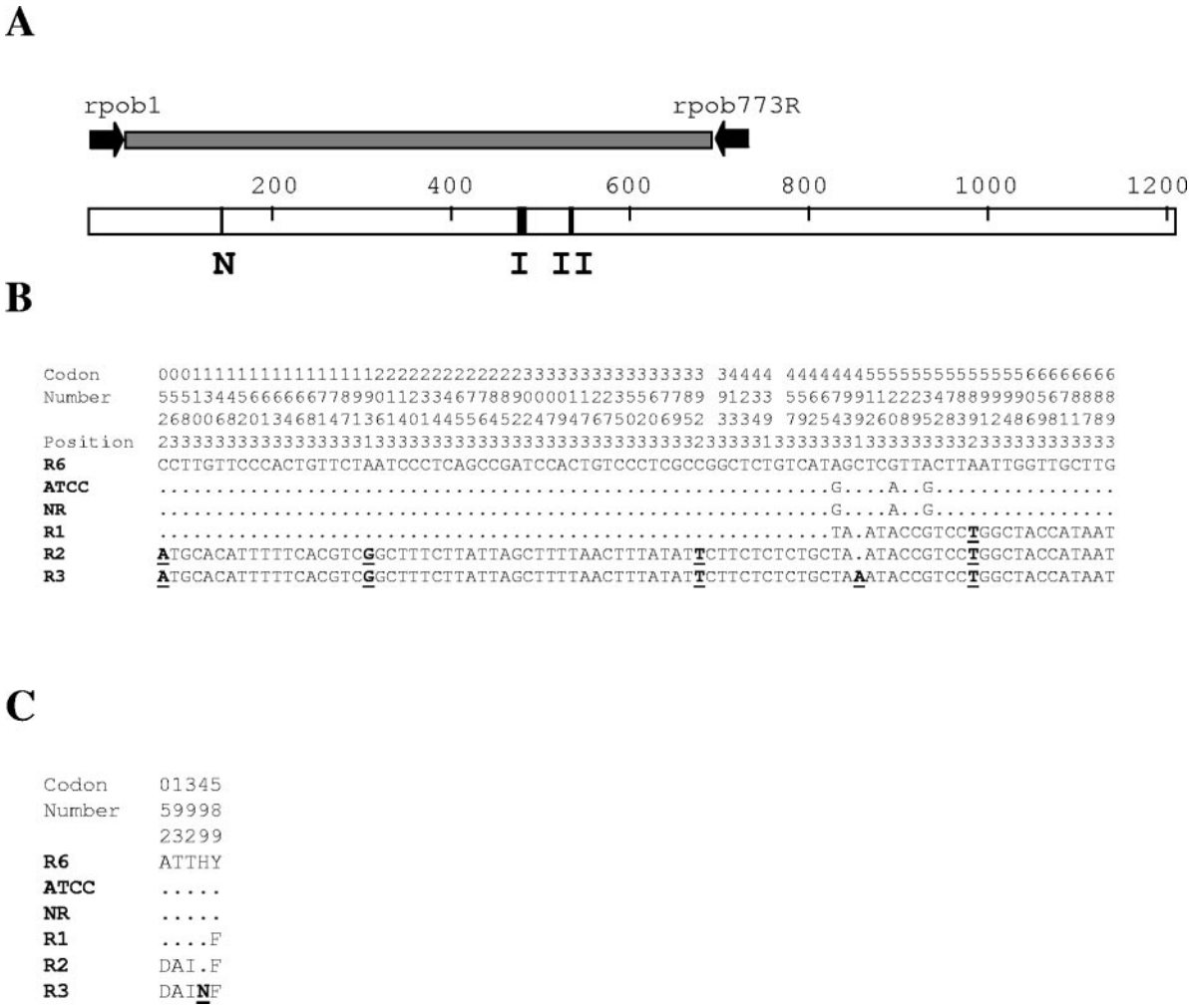


FIG. 1. Genetic structure of the *rpoB* genes of isolates of the Spain[14]-5 clone. Schematic representation of RpoB showing clusters N, I, and II (A). The PCR fragments used for sequencing (gray box) and primers (black arrows) are indicated above the schematic. Nucleotide (B) and amino acid (C) sequence variations in the RpoB L42-to-T700 region of Spain[14]-5 isolates are also shown. The nucleotides and amino acids present at each polymorphic site are shown in full for strain R6, but for the other isolates, only sites that differ from those of R6 are shown. Codon numbers are indicated vertically above the sequences. Positions 1, 2, and 3 in the fourth row refer to the first, second, and third nucleotides in the codon, respectively. Sense mutations and amino acid changes involved in RIF resistance are shown in bold and underlined.

transformation in the natural environment is presumably much less than under laboratory conditions, given the low proportion of recombinants among RIF-resistant isolates (5).

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