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rpoB Mutations in *Streptococcus mitis* Clinical Isolates Resistant to Rifampin

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Activity of rifampin against 129 *Streptococcus mitis* isolates obtained from patients with hematologic cancer was investigated. One hundred twenty-five strains were susceptible to rifampin, and 4 were resistant (MIC = 32 to 64 μg/ml). Resistance to rifampin was related to mutations in the *rpoB* gene: $His_{526}Asn$ in three strains and $His_{526}Asp$ in one strain.

The oral streptococci are normal commensals of the human oropharynx mainly involved in endocarditis or infection in neutropenic patients. They play a role in resistance to colonization by other bacterial species such as staphylococci, but may also constitute a reservoir for resistance genes (4).

Rifampin is particularly active against gram-positive bacteria and mycobacteria. It has primarily been used as part of combination therapy for tuberculosis, and it has also been used as a therapeutic agent against (methicillin-resistant) staphylococci; for chemoprophylaxis for close contacts of patients with invasive infections due to *Neisseria meningitidis*, *Haemophilus influenzae* type b, or *Streptococcus pyogenes*; or for penicillin treatment failure in streptococcal infections (9). Increase in macrolide and β-lactam resistance in *S. mitis* can lead to the use of rifampin in infections due to this species.

Resistance to rifampin has been described in several bacterial species, such as *Mycobacterium tuberculosis* (11), *Escherichia coli* (13), *Staphylococcus aureus* (3), *N. meningitidis* (14), *S. pyogenes* (7), *Streptococcus pneumoniae* (9), and *Rhodococcus equi* (6).

Rifampin resistance is commonly due to mutations in the intracellular target of the drug, the β subunit of RNA polymerase, encoded by the *rpoB* gene. Resistance to rifampin has been linked to amino acid alterations found in three regions of *rpoB*, termed clusters I to III. These alterations arise mainly due to point mutations (9), but horizontal gene transfer may also play a role in the evolution of rifampin resistance in *S. pneumoniae* (5).

The mechanisms involved in rifampin resistance in oral streptococci have not been investigated. In this study, we tested the activity of rifampin against a large collection of *Streptococcus mitis* isolates from neutropenic patients and analyzed the *rpoB* gene in the four isolates that were found resistant to this antimicrobial.

One hundred twenty-nine nonrepetitive strains of *S. mitis* identified by API Rapid ID 32 Strep system (bioMérieux, La-Balme-les-Grottes, France) were isolated from various specimens (throat, 72%; sputum, 19%; nose, 3%; blood cultures, 2%; others, 4%) from 61 patients with hematologic cancer hospitalized in the Bone Marrow Transplant Center of Tunisia in 2002. When multiple isolates were obtained from a single patient, duplicates were eliminated on the basis of identical patterns of antimicrobial susceptibility. Determination of the MIC of rifampin (Gruppo Lepetit S.P.A., Milan, Italy) was done by an agar dilution method adapted from the recommendations of the National Committee for Clinical Laboratory Standards (10). Mueller-Hinton agar media supplemented with 5% defibrinated sheep blood were inoculated with 10^4 CFU/spot, and the MICs were read after 24 h of incubation at 37°C. Rifampin-resistant isolates were typed by pulsed-field gel electrophoresis after SmaI digestion (12).

The *rpoB* gene of isolates resistant to rifampin was amplified and sequenced. Analysis of the sequence of the *rpoB* gene of *S. mitis* NCTC 12261 (obtained from The Institute for Genomic Research website at http://www.tigr.org) allowed us to design six pairs of primers to amplify overlapping DNA fragments of the *rpoB* gene (Table 1). The gene was amplified nearly in its entirety from nucleotide 9 to nucleotide 3638 (*E. coli* coordinates), and the PCR products were sequenced on both strands. As controls, DNA fragments from six *S. mitis* strains susceptible to rifampin (MIC = 0.12 to 0.25 μg/ml) were amplified with primers RPOPN05 and RPOPN06 and sequenced.

TABLE 1. Primers used for amplification and sequencing of *rpoB*

Primer name	Primer sequence (5′ → 3′)[a]	Size of PCR product (bp)
RPOPN01	+GGC TTG GAA CTT ATT TAC AAA GG	751
RPOPN02	−TCT TTC AGG GCT TCG TCT GTA CG	
RPOPN03	+TTG GTT CGT GCC CTT GGT TTC TC	689
RPOPN04	−ACT TGG TTA GCA AGC AAT TCA CC	
RPOPN05	+TCC TTG CTG AAA TGA GCT ACT TC	681
RPOPN06	−CAA CAT TCG CTG GAT ACT CTT GG	
RPOPN07	+CTG AAT GAA GAT GGA ACA TTT GC	635
RPOPN08	−CGT GTT TCT GAT TCG TAT TCT TC	
RPOPN09	+GTT GCC TAC ATG ACT TGG GAA GG	713
RPOPN010	−TCA GAA CTT GCT CCG TCA AAG AC	
RPOPN011	+TCC AGT CGA TAT CAT GTT GAA CC	780
RPOPN012	−TGA ACT GCT TAT TCT TAT TCA GC	

[a] +, direct primer; −, reverse primer.

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TABLE 2. Origin, rifampin MICs, and RpoB amino acid substitutions for the rifampin-resistant *S. mitis* strains tested

Strain no.	Date of isolation (day/mo/yr)	Origin	Department	Rifampin MIC (μg/ml)	Resistance phenotype[a]	Amino acid change[b]
185b	15/01/02	Throat	Hematology	32	Amx^r Ery^r Tet^r Cip^r	$His_{526}Asp$
933c	12/03/02	Throat	Hematology	32	Ery^r Cm^r Tet^r Sxt^r	$His_{526}Asn$
983b	16/03/02	Biopsy	Graft	64	Ery^r Cm^r Fos^r Cip^r	$His_{526}Asn$
3929b	16/09/02	Throat	Hematology	32	Ery^r Lin^r Tet^r Fos^r	$His_{526}Asn$

[a] Resistance phenotype: Amx, amoxicillin; Ery, erythromycin; Lin, lincomycin; Tet, tetracycline; Cip, ciprofloxacin; Fos, fosfomycin; Cm, chloramphenicol; Sxt, trimethoprim-sulfamethoxazole.
[b] All alterations were in cluster I.

Susceptibility of clinical isolates to rifampin. Of the 129 isolates, 125 were susceptible to rifampin, with MICs ranging from <0.125 μg/ml to 0.25 μg/ml and MICs at which 50 and 90% of the isolates tested were inhibited of <0.125 μg/ml. Four strains (3%) showed high-level rifampin resistance: MICs were equal to 32 μg/ml for three strains (185b, 3929b, and 983b) and 64 μg/ml for 1 strain (933c). These strains had different antibiotypes (Table 2) and were distinct by their pulsed-field gel electrophoresis patterns (data not shown). Few data concerning the rates of resistance to rifampin in oral streptococci are available. In other species of streptococci, rifampin resistance seems to be rare. In *S. pneumoniae*, rifampin resistance was described in South Africa in the late 1970s in the context of extensive use of this antimicrobial for the treatment of tuberculosis in children (8). The majority of young children have colonization with pneumococci in their nasopharynx, a fact that results in the bacteria being directly exposed to the selective pressure of the antimicrobial agent (12). Reported rates of resistance vary between 0.4 and 1.5% in *S. pneumoniae*, and the rate is estimated to be approximately 0.3% among *S. pyogenes* isolates from Spain (7).

Amino acid sequence analysis. The deduced amino acid sequences of the RpoB proteins of the four mutants were compared to those of *S. mitis* NCTC 12261 and *S. pneumoniae* R6. Several mutations were found in the N and C termini of the protein, which are highly variable according to the bacterial species. A single mutation in the conserved cluster I at position 526 (*E. coli* numbering) was found in four isolates: $His_{526}Asn$ in three isolates (933c, 3929b, and 983b) and $His_{526}Asp$ in one isolate (185b) (Table 2). No mutations were found in the conserved clusters II and III. Mutations in cluster I have been reported previously for *S. pneumoniae* (5), *N. meningitidis* (14), *S. pyogenes* (2), *S. aureus* (3), *E. coli* (13), *M. tuberculosis* (11), *Bacillus anthracis* (15), *Bacillus cereus* (15), and *Rhodococcus equi* (1, 6). Indeed, it has been shown in *E. coli* that residues 516 to 540 are part of the target of rifampin and participate with residues 1065 and 1237 in the formation of the initiation site when the β subunit is assembled in the β RNA polymerase complex (13). It seems that, as in other bacterial species, cluster I in the crucial rifampin resistance-determining region is the primary target for rifampin in *S. mitis*.

In our strains, $His_{526}Asn$ and $His_{526}Asp$ mutations conferred high-level rifampin resistance. Generally, the resistance levels are dependent on both the location and the nature of the substitution. The $His_{526}Asn$ mutation, when present alone, confers low-level resistance to rifampin (MIC = 2 to 4 μg/ml) in *S. aureus* (16, 17), *S. pneumoniae* (5), and *Rhodococcus equi* (6). However, this substitution accounted for high-level resistance to rifampin in an *S. pyogenes* strain (MIC = 32 μg/ml) (7). The $His_{526}Asp$ mutation was found to be predominantly associated with high-level rifampin resistance in *R. equi* strains (6).

Other types of substitutions at position 526 have been reported that confer generally moderate or high levels of resistance to rifampin, such as $His_{526}Tyr$ mutation in *S. aureus* and in *R. equi* (3, 6), $His_{526}Arg$ substitution in *R. equi* (1, 6), and $His_{526}Leu$ in *M. tuberculosis* (11). In *B. anthracis* and *B. cereus*, changes of His at position 526 for Tyr, Leu, or Pro (15) led also to high-level resistance. In conclusion, the mechanisms behind rifampin resistance in *S. mitis* are similar to mechanisms found in other species, confirming that the regions implicated in the interaction with rifampin are conserved among prokaryotes.

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