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rpoB Mutation Conferring Rifampin Resistance in *Streptococcus pyogenes*

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Streptococcus pyogenes **BM4478 and** ***Staphylococcus aureus*** **BM4479 were isolated from a patient undergoing rifampin therapy. High-level resistance to rifampin was due to the following mutations in the** ***rpoB*** **gene: $Ser_{522}Leu$ in strain BM4478 and $His_{526}Asn$ and $Ser_{574}Leu$ in strain BM4479.**

Streptococcus pyogenes is responsible for high rates of morbidity due to an increase in invasive group A streptococcal infections and bacteremia worldwide, with the most commonly reported predisposing factor being skin lesions (R. C. George, A. Efstratiou, M. A. Monnickendam, M. B. Mcevoy, G. Hallas, A. P. Johnson, and A. Tanna, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 309, p. 658, 1999). *S. pyogenes* is also the leading cause of bacterial pharyngotonsillitis. An increase in macrolide resistance in this species (E. L. Kaplan, D. R. Johnson, and C. D. Rothermel, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 535, p. 714, 1999) and the reduced efficacy of oral penicillin V in its eradication from upper respiratory tracts in children have led to the proposal of penicillin being used in combination with rifampin (9).

The mechanisms involved in rifampin resistance in *S. pyogenes* have not been investigated. In gram-positive bacteria, resistance appears to be due to mutational alterations of the intracellular target of the drug, the RNA polymerase β subunit encoded by the *rpoB* gene (1, 3, 12). The mutations are generally clustered in a 702-bp fragment, from nucleotide positions 486 to 717 (*Escherichia coli* coordinates), corresponding to the rifampin resistance-determining (Rif) region in the center of the *rpoB* gene (1).

S. pyogenes BM4478 and *Staphylococcus aureus* BM4479, both resistant to high levels of rifampin (MIC, >256 μg/ml), were isolated in 1999 from a patient with a recurrent ulcer infection undergoing therapy with rifampin alone at the Hospital Henri Mondor, Créteil, France. The isolates remained susceptible to all antibiotics that are usually active against gram-positive cocci, except tetracycline for BM4478 and penicillin G and tetracycline for BM4479.

(An initial report of this work was presented at the 41st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Ill., 16 to 19 December 2001 [H. Aubry-Damon, G. Gerbaud, and P. Courvalin, Abstr. 41st Intersci. Conf. Antimicrob. Agents Chemother., abstract C1-159, p. 82, 2001].)

We amplified by PCR the entire *rpoB* gene (Table 1, primer pair A) from BM4478 and CIP5641T, an antibiotic-susceptible *S. pyogenes* type strain (rifampin MIC = 0.008 μg/ml), and a portion of the *rpoB* gene (Table 1, primer pair F) corresponding to the Rif mutated region in previously studied bacterial genera (1, 2, 4, 8) from *Staphylococcus aureus* BM4479 and plasmid-free *Staphylococcus aureus* RN4220, which is susceptible to antibiotics (rifampin MIC = 0.008 μg/ml). The amplification products were cloned in the pCRII vector (Invitrogen) and sequenced on both strands with an automated sequencer (CEQ 2000 DNA Analysis system; Beckman Coulter).

Relative to the susceptible strains CIP5641T and SF370 (ac-

TABLE 1. Oligonucleotides used to detect mutations in the *rpoB* genes

Primer[a]	Sequence (5′-3′)	Size of PCR product (bp)	Origin or reference
S. pyogenes			
Pair A			
+	GATAACTTAGTTGCGATTTGC	3,798	This study
−	TTTGATGACTTTACCAGTTCC		
Pair B			
+	CCTGCTGATATTTTGGC	520	This study
−	TCAACCTTACGGTAAGG		
Pair C			
+	ATCGTTATGGGTCGTCA	609	This study
−	GTATCACGCGTTTCAGA		
Pair D[b]			
+	CGTGAACGTATGTCTGT	370	This study
−	CGGTCAACCTTACGGTA		
Pair E[b]			
+	ATGCAACGTCAGGCTGT	210	This study
−	CCTGAGTTTGAACGACG		
Staphylococcus aureus, pair F			
+	AGTCTATCACACCTCAACAA	702	1
−	TAATAGCCGCACCAGAATCA		

[a] + and − indicate sense and antisense primers, respectively.
[b] The same primers were used for *S. pyogenes* and *S. pneumoniae*.

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FIG. 1. Amino acid sequence comparison of the Rif region of the *rpoB* gene of *E. coli*, *Staphylococcus aureus*, *S. pneumoniae*, and *S. pyogenes*. Clusters I, II, and III are indicated by a double line above the alignment. Dots indicate gaps introduced to optimize the alignment. Residues where substitutions are known to be involved in rifampin resistance are in bold. Mutations leading to rifampin resistance in *S. pyogenes* BM4478 and in *Staphylococcus aureus* BM4479 are indicated by double and single upward-pointing arrows, respectively.

cession number AE006480), *S. pyogenes* BM4478 had two base pair changes in the *rpoB* gene that resulted in amino acid substitutions (Fig. 1) as follows: in cluster I at position 522 ($Ser_{522}Leu$) and outside of the Rif region at position 722 ($Gln_{722}His$) (*E. coli* coordinates). The two mutational changes, $His_{526}Asn$ in cluster I and $Ser_{574}Leu$ in cluster II (*E. coli* coordinates), found in *Staphylococcus aureus* BM4479 have already been shown to confer high-level resistance and cross-resistance to the rifamycins (12). In *Streptococcus pneumoniae* and in *Staphylococcus aureus*, the $His_{526}Asn$ mutation, when present alone, confers low-level resistance (3, 12). Few $Ser_{574}Leu$ mutations were found in cluster II of rifampin-resistant *E. coli* (6). However, the level of resistance conferred was not investigated.

To determine the role of the two alterations in *S. pyogenes* BM4478, purified PCR products (1 μg), each containing a single mutation, were added to rifampin-susceptible competent cells (5) of *S. pneumoniae* CP1000 (rifampin MIC = 0.023 μg/ml), and transformants were selected on rifampin at 10 μg/ml. The TCA/TTA ($Ser_{522}Leu$) mutation was amplified as part of a 520-bp PCR product obtained with primer pair B and mutation CAA/CAC ($Gln_{722}His$) was amplified as part of a 609-bp PCR product obtained with primer pair C (Table 1). The corresponding PCR products were also amplified from DNA of susceptible strain CIP5641T. The DNA of two transformants from each experiment was amplified and sequenced using primer pairs D and E (Table 1) to screen positions 522 and 722, respectively. Only transformation with the 520-bp PCR product containing the TTA mutation yielded resistant colonies at a frequency (Table 2) compatible with monogenic transformation (10). In the remaining experiments resistant derivatives of the recipient strain with mutations outside of the 370- and 210-bp portions sequenced were obtained at a frequency of ca. 10^{-6}. These data indicate that in *S. pyogenes* BM4478 rifampin resistance was due to the $Ser_{522}Leu$ substitution located in cluster I. Mutations at this position also lead to high levels of resistance in *Staphylococcus aureus* (1), *E. coli* (4), *Neisseria meningitidis* (2), and *Mycobacterium tuberculosis* (8).

TABLE 2. Transformation of *S. pneumoniae* CP1000 with PCR-amplified DNA from BM4478 and CIP5641T

Sequence in donor DNA (amino acid)[a]	Frequency of resistant recipient on rifampin at 10 μg/ml	Sequence in resistant recipient DNA (amino acid)
520-bp product		
No DNA	1.2×10^{-6}	TCA (Ser_{522})
TCA (Ser_{522})	1.3×10^{-6}	TCA (Ser_{522})
TTA (Leu_{522})	3.1×10^{-3}	TTA (Leu_{522})
609-bp product		
No DNA	1.1×10^{-6}	CAA (Gln_{722})
CAA (Gln_{722})	1.2×10^{-6}	CAA (Gln_{722})
CAC (His_{722})	1.1×10^{-6}	CAA (Gln_{722})

[a] Amino acid positions are from *E. coli* coordinates.

Interestingly, the substitutions in *S. pyogenes* and *Staphylococcus aureus* clinical isolates were different. *Staphylococcus aureus* BM4479 probably acquired high-level rifampin resistance in two steps. Rifampin resistance emerges easily in *Staphylococcus aureus*, in particular in methicillin-resistant strains (1). The occurrence of multiple mutations may be explained by epidemic dissemination of strains and their frequent exposure to rifampin. The mutations in BM4478 and BM4479 were clustered from nucleotide positions 522 to 526 (*E. coli* coordinates), suggesting that substitution of the corresponding amino acids in cluster I of *S. pyogenes*, like in *E. coli*, prevents the binding of rifampin to RNA polymerase (11). 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 RNA polymerase complex (7).

This study suggests that, as in *Staphylococcus aureus*, cluster I in the central Rif region is the primary target for rifampin in *S. pyogenes*.

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