Quinupristin-Dalfopristin Resistance in *Streptococcus pneumoniae*: Novel L22 Ribosomal Protein Mutation in Two Clinical Isolates from the SENTRY Antimicrobial Surveillance Program

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Received 17 January 2003/Returned for modification 27 March 2003/Accepted 24 May 2003

Resistance to quinupristin-dalfopristin (Q/D) among gram-positive cocci has been very uncommon. Two clinical isolates among 8,837 (0.02%) *Streptococcus pneumoniae* isolates were discovered in 2001 to 2002 with Q/D MICs of 4 μg/ml. Each had a 5-amino-acid tandem duplication (RTAHI) in the L22 ribosomal protein gene (*rplV*) preventing synergistic ribosomal binding of the streptogramin combination. Similar gene duplication has been reported in Q/D-resistant *Staphylococcus aureus*.

*Streptococcus pneumoniae* is one of the most commonly isolated pathogens from patients diagnosed at any age with community-acquired respiratory tract infections. Recent reports have chronicled the steady increase and global dissemination of several forms of antimicrobial resistance among *S. pneumoniae* specifically for the penicillins, some cephalosporins, macrolides, tetracyclines, and trimethoprim-sulfamethoxazole (1–3, 5–7; R. N. Jones, D. Biedenbach, M. Beach, and D. Farrell, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. C2-1978, 2002). Most disturbing has been the escalating rates of high-level resistance to penicillin and resistance to agents in the macrolide-lincosamide-streptogramin B class (1, 3, 5, 8; Jones et al., 42nd ICAAC). The latter group of antimicrobials has two principal mechanisms of resistance, target modification and efflux pump acquisition, but streptogramin combinations such as quinupristin-dalfopristin (Q/D) generally remain active (2, 6). Recent reports show Q/D resistance ranging from 0 to 1.1% (2, 6) for unconfirmed cases. The mechanisms of resistance in these *S. pneumoniae* strains remain unstudied.

In 2002, Malbruny et al. (9) reported resistance (MIC, 4 μg/ml) to Q/D mediated by a L22 ribosomal protein gene (*rplV*) mutation in a clinical isolate of *S. aureus* (strain 740-1). Sequencing of *rplV* revealed a 21-bp duplication encoding a 7-amino-acid (SAINKRT [Fig. 1]) insertion starting at position 101 (9). Also in 2002, Musher and colleagues noted a 6-amino-acid tandem duplication in a pneumococcal isolate associated with macrolide therapy for which the Q/D MIC was elevated (Fig. 2) (10). After discovering two clinical strains of *S. pneumoniae* with resistant Q/D MIC results (4 μg/ml) in the SENTRY Antimicrobial Surveillance Program, we explored the mechanism of resistance with an emphasis on possible mutations of *rplV* that could negate the synergistic ribosomal binding of streptogramins A and B (9).

During the respiratory tract disease seasons of 1997 to 2001, a total of 8,837 community-acquired isolates of *S. pneumoniae* were monitored for emerging resistance patterns to >30 antimicrobial agents. In 2001, isolates forwarded to the SENTRY Program monitor (JMI Laboratories, North Liberty, Iowa) were also screened for macrolide resistance by using broth microdilution methods of the National Committee for Clinical Laboratory Standards, and those strains with nonsusceptible MIC results were further tested for molecular mechanisms of resistance (11, 12). The initial molecular screens utilized a novel rapid-cycle multiplex PCR–probe detection format described earlier by Farrell et al. (4). Among 332 *S. pneumoniae* strains tested from Europe (120 strains from 18 centers), Canada (31 strains from five centers), Latin America (23 strains from six centers), and the United States (158 strains from 22 centers), nine strains were noted to possess macrolide resistance but were negative by PCR screens for *erm*(B) and *erm*(A) (Jones et al., 42nd ICAAC). One of these organisms was also resistant to Q/D (MIC, 4 μg/ml; strain 17-3167B) for an overall prevalence rate of 0.01% over the 5-year interval. This strain and a second similar pneumococcus (107-612B) identified in 2002 were further characterized by (i) gene sequencing for all four alleles of the 23S rRNA and the L4 and L22 ribosomal protein genes to detect mutations; (ii) antibiogram analysis; (iii) pulsed-field gel electrophoresis and automated ribotyping; and (iv) serotyping.

For gene sequencing of the L22 ribosomal protein, a 176-bp segment was amplified by PCR. The PCR primers used were derived from strain R6 (GenBank accession numbers AE008402 and AF126059): DF-L22-F (GAATCGAGCCTGTA GCTAACGC) and DF-L22-R (TTCTGCAACAGCTACAGT GATG).

The cycling (Perkin-Elmer 9700 cycler; Applied Biosystems, Warrington, United Kingdom) parameters were as follows: 94°C for 2 min followed by 35 cycles of 94°C for 30 s, 52°C for 30 s, and 72°C for 40 s, followed by a product extension cycle of 72°C for 7 min. The sequences were determined with the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems).
Sequence analysis was performed by using the DNASTAR analysis program (DNASTAR, Madison, Wis.).

These two, Q/D-resistant community-acquired strains of *S. pneumoniae* were isolated in June 2001 (17-3167B) and March 2002 (107-612B) in New York and Kentucky, respectively. Both strains were isolated from an invasive tissue site (pleural or shoulder joint fluid) in female patients aged 36 and 79 years, respectively. The antibiogram (MIC for the strain in micrograms per milliliter) for 17-3167B and 107-612B were Q/D (4 and 4); erythromycin (1 and 1); telithromycin (0.5 and 0.25); clindamycin (0.06 and 0.06); penicillin (0.015 and 0.015); amoxicillin-clavulanic acid (0.06 and 0.06); cefepime (0.06 and 0.06); ceftriaxone (0.03 and 0.03); ciprofloxacin (4 and 1); garenoxacin (0.03 and 0.03); gatifloxacin (0.25 and 0.25); levofloxacin (1 and 1); chloramphenicol (2 and 2); doxycycline (0.5 and 0.5); rifampin (0.5 and 0.5); trimethoprim-sulfamethoxazole (0.5 and 0.5); linezolid (1 and 0.5); and vancomycin (0.25 and 0.25). The MICs for the components of the streptogramin combination were as follows: quinupristin at 8 g/ml and dalfopristin at 128 g/ml. MICs for other macrolides (azithromycin, clarithromycin, rokitamycin, and roxithromycin) ranged from 0.5 g/ml for azithromycin to 16 g/ml for roxithromycin, each MIC indicating reduced susceptibility (11).

Among the nine PCR screen-negative *S. pneumoniae* isolates observed in 2001 (all in North America), mechanisms of resistance were determined for all strains that included 23S rRNA mutations at A2059G (six strains), A2058G (two strains), and A2059C (one strain) and the L22 mutation P84T (two strains). Strain 17-316B had an A2059G (one allele) mutation and an additional unique change in the L22 ribosomal protein, shared with the year-2002 isolate (107-612B). Figure 2 illustrates the 5-amino-acid duplication (RTAHIT) at positions 103 to 107 that was present in both Q/D-resistant *S. pneumoniae* strains presented here. Figure 2 also shows the 6-amino-acid duplication noted earlier in another *S. pneumoniae* isolate (10). Although having an identical resistance mechanism and similar antibiograms, our described isolates were not the same by pulsed-field gel electrophoresis patterns, riboprints, or serotyping, e.g., not clonal emergence and dissemination.

The mechanism of low-level Q/D resistance in *S. pneumoniae* appears to be secondary to a tandem duplication of 5 or 6 amino acids (RTAH or RTAHIT [Fig. 2]) in the L22 ribosomal protein, a finding similar to that duplication phenomenon responsible for resistance to the streptogramin combination in *S. aureus* (9) and in other pneumococci reported by Musher et al. (10). These L22 mutations did not effect the susceptibility to clindamycin but did compromise the potency of the ketolides in *S. aureus* (9). Since the use of macrolides has been implicated in the increased resistance to macrolide-lincosamide-streptogramin B agents and penicillin (3), continued surveillance appears necessary to detect the wider selection of Q/D-resistant streptococci where these agents have been applied clinically. The tandem duplications of ribosomal target proteins found in this study and elsewhere (9, 10) may be more widespread and need further investigation in other gram-positive species such as *Enterococcus faecium* and coagulase-negative staphylococci and as an intrinsic resistance trait among *Enterococcus faecalis* strains.

**FIG. 1.** *S. aureus* L22 riboprotein amino acids (AA) sequences for positions 91 to 106 (9). Duplication of amino acids 93 to 99 is indicated by shading for strain 740-1. Regions corresponding to the tandem duplications found in *S. pneumoniae* strains described here and by Musher et al. (10) appear with shading.

**FIG. 2.** *S. pneumoniae* L22 riboprotein amino acids (AA) sequences for positions 96 to 113. Duplication of amino acids 103 to 108 (RTAH) and of amino acids 103 to 108 is indicated by shaded area for both described clinical isolates.
This study was funded by an unrestricted research grant from The JONES Group. The SENTRY Program was sponsored via an educational research grant from Bristol-Myers Squibb.

We also acknowledge Sarah Bakker for DNA sequencing.

REFERENCES