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The JONES Group/JMI Laboratories
North Liberty, IA, USA; www.jmilabs.com
319.665.3370, fax 319.665.3371
ronald-jones@jmilabs.com

Initial Description of the L22 Mutation Responsible for Quinupristin/Dalfopristin Resistance in *Streptococcus pneumoniae*: Case Reports from the SENTRY Antimicrobial Surveillance Program

RN Jones, DJ Farrell, I Morrissey, The SENTRY Program Participants Group.
The JONES Group/JMI Laboratories, North Liberty, Iowa, USA (www.jmilabs.com); and GR Micro, Ltd., London, UK

ABSTRACT

Background: Although resistance (R) to macrolides and clindamycin remains common and escalating, quinupristin/dalfopristin (Synercid)-R among *S. pneumoniae* strains is extremely rare. Recently Malbruny et al. described an L22 ribosomal protein alteration responsible for Synercid-R in *Staphylococcus aureus*. A similar mutational insertion in L22 was observed in pneumococcal isolates from the SENTRY Program (2001-2002).

Methods: Among *S. pneumoniae* isolates from community-acquired respiratory tract infections in the SENTRY Program (North America, 2001), 158 erythromycin (ER) -R strains were screened for *erm*(B) and *mef*(A) by multiplex rapid cycle PCR/microwell-format probe hybridization. Nine ER-R pneumococci remained negative (USA 6 strains; Canada 3 strains) after PCR screen and were investigated for 23S, L4 and L22 mutations by sequencing. An isolate from pleural fluid in a 36 year old female living in New York (17-3167B) had the following MICs (μg/ml): Synercid 4, quinupristin 8, dalfopristin 128, ER 1, azithromycin 0.5, clarithromycin 1, rokitamycin 2, roxithromycin 16, and clindamycin ≤ 0.12. A second strain (107-6120) with an identical MLS_B resistance pattern was observed in a patient from Kentucky (March, 2002).

Results: A 5 amino acid (AA) insert at the C terminus of the L22 riboprotein was found in both strains. The insert was a duplication of AA 104-107 of L22 (RTAHI) into AA position 108-112. This insert overlaps with the insert (also a duplication) described as the mechanism of Synercid-R previously noted in *S. aureus*. An A2059G mutation in 1 of 4 alleles of the 23S rRNA gene was also detected in strain 17-3167B.

Conclusions: This is the first report of Synercid-R *S. pneumoniae* produced by a 5 AA insertion in L22, a mechanism only recently described in *S. aureus* (AAC 46:2200-7, 2002). Since all non-*erm*(B), non-*mef*(A) MLS_B-R isolates occurred in the North American sample, R surveillance in this region should routinely include expanded molecular characterization.

INTRODUCTION

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 antimicrobial resistances among *S. pneumoniae* specifically for the penicillins, some cephalosporins, macrolides, tetracyclines and trimethoprim/sulfamethoxazole. Most disturbing has been the escalating rates of high-level resistance to penicillin and resistance to agents in the macrolide-lincosamide-streptogramin B class (MLS_B). The latter group of antimicrobials has two principal mechanisms of resistance, target modification and efflux pump activation; but streptogramin combinations such as quinupristin/dalfopristin (Synercid®) generally remain active. Recent reports show Synercid® resistance ranging from zero to 1.1% for unconfirmed cases. The mechanisms of resistance in these *S. pneumoniae* strains remain unstudied.

In 2002, Malbruny et al. reported resistance (MIC, 4 μg/ml) to Synercid® mediated by a L22 ribosomal protein gene (*rplV*) mutation in a clinical isolate of *Staphylococcus aureus* (Strain 740-1). Sequencing of *rplV* revealed a 21-bp duplication encoding a seven amino acid (SAINKRT; see Figure 1) insertion starting at position 101. After discovering two clinical strains of *S. pneumoniae* with resistant Synercid® 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.

MATERIALS AND METHODS

During the respiratory tract disease seasons of 1997-2001, 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 using reference broth microdilution methods of the National Committee for Clinical Laboratory Standards, and those strains with non-susceptible MIC results were further tested for molecular mechanisms of resistance. The initial molecular screens utilized a novel rapid cycle multiplex PCR/probe detection format described earlier by Farrell et al. Among 332 *S. pneumoniae* 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 *mef*(A). One of these organisms was also resistant to Synercid® (MIC, 4 μg/ml; strain 17-3167B) for an overall prevalence rate of 0.01% over the five-year interval. This strain and a second similar pneumococcus (107-612B) identified in 2002, were further characterized by: 1) gene sequencing for all alleles of the 23S rRNA, L4 and L22 ribosomal protein genes to detect mutations; 2) antibiogram analysis; 3) pulsed-field gel electrophoresis (PFGE) and automated ribotyping; and 4) serotyping.

For gene sequencing of the L22 ribosomal protein, a 176 bp segment was amplified by PCR. The PCR primers used were:

DF-L22-F GAACTCAGCTGTAGCTAACGC

DF-L22-R TTCTGCAACAGCTACAGTGATG

The cycling (Perkin-Elmer 9700 cycler, Applied Biosystems, Warrington, UK) parameters were as follows: 94ºC for two minutes followed by 35 cycles of 94ºC 30 sec., 52ºC 30 sec., 72ºC 40 sec.; followed by a product extension cycle of 72ºC for 7 minutes. The sequences were determined with the ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems). Sequence analysis was performed using the DNASTAR analysis program (DNASTAR, Madison, Wisconsin).

Figure 1: *Staphylococcus aureus* L22 riboprotein amino acids (AA) sequences for positions 91-106 [Mulbruny et al., 2002]. Duplication of AA 93-99 indicated by shading for strain 740-1. Region corresponding to region of tandem duplication found in *Streptococcus pneumoniae* strains described here indicated by shading.

Wild-type	R A S A I N K R T - - - - - - - S H I T I V V
740-1	R A S A I N K R T S A I N K R T S H I T I V V

RESULTS

- These two, Synercid®-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 (strain MIC in μg/ml for 17-3167B/107-612B) were: Synercid® (4/4); erythromycin (1/1); clindamycin (≤ 0.06/≤0.06); penicillin (≤ 0.015/≤ 0.015); amoxicillin/clavulanic acid (≤ 0.06/≤0.06); cefepime (≤ 0.06/≤ 0.06); ceftriaxone (0.03/0.015); cefuroxime (≤ 0.06/≤ 0.06); ciprofloxacin (4/1); garenoxacin (0.03/0.03); gatifloxacin (0.25/0.25); levofloxacin (1/1); chloramphenicol (≤ 2/≤ 2); doxycycline (≤ 0.5/≤ 0.5); rifampin (≤ 0.5/≤ 0.5); trimethoprim/sulfamethoxazole (≤ 0.5/≤ 0.5); linezolid (1/0.5); and vancomycin (0.25/0.25). The MIC results for the components of Synercid® were: quinupristin at 8 μg/ml; and dalfopristin at 128 μg/ml. MIC values for other macrolides (azithromycin, clarithromycin, rokitamycin, roxithromycin) ranged from 0.5 μg/ml for azithromycin to 16 μg/ml for roxithromycin, each MIC indicating reduced susceptibility.

- 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 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 five amino acid tandem duplication (RTAHI) at positions 103-107 that was present in both Synercid®-resistant *S. pneumoniae* strains. Although having an identical resistance mechanism and similar antibiograms, these isolates were not the same by PFGE, riboprints or serotype (e.g. not clonal emergence and dissemination).

Figure 2: *Streptococcus pneumoniae* L22 riboprotein amino acid (AA) sequences for positions 96-113. Duplication of AA 103-107 (RTAHI) indicated by shaded area for both described clinical isolates.

R6	TCAGCTTCACCAATCAACAAACGTACAGCTCACATC- - - - - - - - - - - - - - ACTGTAGCTGTTGCAGAA
	S A S P I N K R T A H I T V A V A E
017-3167B	TCAGCTTCACCAATCAACAAACGTACAGCTCACATCCGTACAGCTCACATCACTGTAGCTGTTGCAGAA
107-612B	TCAGCTTCACCAATCAACAAACGTACAGCTCACATCCGTACAGCTCACATCACTGTAGCTGTTGCAGAA
	S A S P I N K R T A H I R T A H I T V A V A E

CONCLUSIONS

- The mechanism of low-level Synercid® resistance in *S. pneumoniae* appears to be secondary to a tandem duplication of five amino acids (RTAHI; see Figure 2) in the L22 ribosomal protein, a finding similar to the duplication phenomenon responsible for resistance to streptogramins in *S. aureus*.

- These L22 mutations did not effect the susceptibility to clindamycin, but did slightly compromise the potency of the ketolides.

- Since the use of macrolides has been implicated in the increased resistance to MLS_B agents and penicillin, continued surveillance appears necessary to detect the wider selection of Synercid®-resistant streptococci where these agents have been applied clinically.

- The tandem duplications of ribosomal target proteins found in this study and elsewhere may be more wide-spread and needs further investigation in other Gram-positive species such as *Enterococcus faecium*, coagulase-negative staphylococci and as an intrinsic resistance trait among *E. faecalis* strains.

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SELECTED REFERENCES

Bronzwaer SLAM, Cars O, Buchholz, Molstad S, Goettsch W, Veldhuijzen IK, Kool JL, Sprenger MJW, Degher JE, Participants in EARSS. A European study on the relationship between antimicrobial use and antimicrobial resistance. *Emerging Infectious Disease* 2002; 8:278-282.

Farrell DJ, Morissey I, Bakker S, Felmingham D. Detection of macrolide resistance mechanisms in *S. pneumoniae* and *S. pyogenes* using a multiplex rapid cycle PCR with microwell-format probe hybridization. *Journal of Antimicrobial Chemotherapy* 2001; 48:541-544.

Hoban DJ, Doern GV, Fluit AC, Roussel-Delvallez M, Jones RN. Worldwide prevalence of antimicrobial resistance in *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* in the SENTRY Antimicrobial Surveillance Program, 1997-1999. *Clinical Infectious Disease* 2001; 32(Suppl 2):S81-S93.

Jones RN, Biedenbach DJ, Farrell D. Evaluation of the macrolide-lincosamide-streptogramin susceptibility patterns among community-acquired *S. pneumoniae* isolates: A five-year report from the SENTRY Antimicrobial Surveillance Program, abstr. #C2-1978. *In: Program and Abstracts of the Interscience Congress of Antimicrobial Agents and Chemotherapy*, San Diego, CA, 2002.

Leclercq R. Mechanisms of resistance to macrolides and lincosamides: Nature of the resistance elements and their clinical implications. *Clinical Infectious Disease* 2002; 34:482-492.

Malbruny B, Canu A, Bozdogan B, Fantin B, Zarrouok I, Dutka-Malen S, Feger C, Leclercq R. Resistance to quinupristin/dalfopristin due to mutation of L22 ribosomal protein in *S. aureus*. *Antimicrobial Agents and Chemotherapy* 2002; 46:2200-2207.

National Committee for Clinical Laboratory Standards. (2000). *Methods for dilution antimicrobial tests for bacteria that grows aerobically. Approved standard M7-A5*. Wayne, PA:NCCLS.

National Committee for Clinical Laboratory Standards. (2003). *Performance standards for antimicrobial susceptibility testing. Supplemental tables, M100-S13*. Wayne, PA:NCCLS.