Short Communication

The First Telithromycin-Resistant *Streptococcus pneumoniae* Isolate in Japan Associated with *erm*(B) and Mutations in 23S rRNA and Riboprotein L4

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**SUMMARY**: This report presents the case of a patient associated with a *Streptococcus pneumoniae* isolate that was resistant to a new ketolide antibiotic, telithromycin (minimum inhibitory concentration: 4 µg/ml). The patient, a 61-year-old female with bronchiectasis, was treated with 200-400 mg of clarithromycin daily for 6 years until the isolation of the resistant strain but without prior exposure to telithromycin. The strain was isolated from her sputum but not from the nasopharynx. This isolate carried *erm*(B) and had mutations in 23S rRNA and riboprotein L4. To our knowledge, this is the first case report concerning a telithromycin-resistant *S. pneumoniae* isolate in Japan by mutation in L4. Although the long-term clarithromycin administration may have contributed to the induction of resistance in this patient, this could not be confirmed, since *S. pneumoniae* was not isolated until the present episode.

*Streptococcus pneumoniae* is an important respiratory pathogen, and the predominant cause of community-acquired pneumonia and acute exacerbation of chronic lung diseases. The emergence of *S. pneumoniae* resistant to β-lactam, macrolide and fluoroquinolone antimicrobials has aroused global concern (1). Telithromycin (TEL) is the first ketolide macrolide and fluoroquinolone antimicrobials has aroused The emergence of pneumonia and acute exacerbation of chronic lung diseases. pathogen, and the predominant cause of community-acquired pneumoniae from community-acquired lower respiratory tract infections. The PROTEKT (Prospective Resistant Organism Tracking and Epidemiology for the Ketolide Telithromycin) study is an international, longitudinal antibacterial resistance surveillance study, which was commenced in 1999 to monitor the spread of resistance among bacterial pathogens isolated from community-acquired lower respiratory tract infections. Recently, it has been reported that a total of 10 TEL-resistant *S. pneumoniae* strains were isolated in the PROTEKT study between 1999 and 2003 (2). However, the clinical background of these patients from whom the TEL-resistant *S. pneumoniae* strains were isolated has not been reported. Here we describe a patient with bronchiectasis treated with long-term clarithromycin therapy from whom a TEL-resistant *S. pneumoniae* strain was isolated.

The patient was a 61-year-old female who was associated with bronchiectasis at the time the TEL-resistant *S. pneumoniae* was isolated. She had been administered clarithromycin 200-400 mg/day from the age of 55 years. She complained of purulent sputum (15-20 ml/day), occasional hemoptysis and exertional dyspnoea. She was a non-smoker, and at 2 years of age she had experienced severe measles associated with pneumonia, which was thought to be the cause of her bronchiectasis. Physical examination revealed no crackles on the auscultation of either lung field or finger clubbing. The presence of sinusitis was not found by plain X-ray, and absence of sinusitis was further confirmed on examination by an otorhinolaryngologist. A chest X-ray revealed mild overinflation, tram lines and diffuse small nodular shadows. Thoracic computed tomography showed cylindrical bronchiectasis and small nodular densities with calcification. The patient had no history of participation in a clinical trial of TEL.

Non-mucoid *Pseudomonas aeruginosa* had been obtained in sputum bacterial cultures since June 1997, then changed to mucoid type in November 2000. On the 25th of December 2001, a penicillin-resistant *S. pneumoniae* was isolated from her sputum at the outpatient service. Serial nasopharyngeal swabs were obtained and did not reveal *S. pneumoniae* in her upper respiratory tract. The patient had been living alone and did not have any significant contact with children. *S. pneumoniae* disappeared without administration of antibiotics active for the isolate. The patient has been cared at the outpatient service up to now.

The minimum inhibitory concentrations (MIC, µg/ml) of antibiotics tested against the *S. pneumoniae* isolate using the microdilution method in accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI) (3) and judged using the CLSI breakpoints (4) are summarized in Table 1. The MIC of TEL was confirmed in the reference laboratory of the PROTEKT study (2) and was re-confirmed at Nagasaki University Hospital, Nagasaki, Japan.

It has been previously reported that this strain was serotype...
We investigated these genes in our isolate, and found two rrl mutations in 23S rRNA (Biosystems, Foster City, Calif., USA) with a BigDye Termin- were determined by using a 3130 Genetic Analyzer (Applied the nucleotide sequences of TEL-resistance in the isolate, the primers for amplifying and 6B and was found to carry the erm(B) gene, and the sequence number, AB233351) and one in L4 (TIGR4). No mutation was found in L22 (number, AB233352). These mutations were not found in TEL- resistance to TEL itself shows very low potency for induction of MLSB resistance. More recently, Rantala et al. (11) have reported that some ermB-positive isolates showed heterogenous resistance to TEL, which can change to stable, homogenous and high-level resistance to TEL.

6B and was found to carry the erm(B) gene, and the sequence type was 902n with MLST (multilocus sequence typing) numbers 2-13-2-1-6-121-121 for aroE, gdh, gk, recP, spi, xpt, and ddl, respectively (2). In order to clarify the mechanism of TEL-resistance in the isolate, the primers for amplifying the genes of 23S rRNA (rrl), L4 (rplD) and L22 (rplV) were used as described previously (5). The nucleotide sequences were determined by using a 3130 Genetic Analyzer (Applied Biosystems, Foster City, Calif., USA) with a BigDye Terminator v.1.1 (Applied Biosystems). Laboratory mutants with reduced susceptibility to TEL were reported to have mutations in 23S rRNA (rrl), L4 (rplD) and/or L22 (rplV) (6). We investigated these genes in our isolate, and found two mutations in 23S rRNA (rrl) (A138G and C724T; accession number, AB233351) and one in L4 (rplD) (G59A; accession number, AB233352). These mutations were not found in TEL-sensitive Streptococcus pneumoniae strains ATCC 49619, DP-1, R6 or TIGR4. No mutation was found in L22 (rplV).

Canu et al. (7) reported that a laboratory-generated mutant strain selected by TEL (for which the MIC increased from 0.008 to 0.25 μg/ml) contained a combination of three mutations in the L22 gene. Another mutant strain selected by clarithromycin and resistant to all macrolides tested (MIC, >32 μg/ml) and TEL (MIC, 4 μg/ml) had a single base deletion in domain II. Walsh et al. (8) also reported that the Lys94 to Gln94 change in L22 is associated with TEL resistance in laboratory-generated mutants. Hisanaga et al. have shown that mutation(s) in domains II and V of 23S rRNA affect the macrolide and ketolide susceptibilities of Streptococcus pneumoniae (6). Compared to Escherichia coli 23S rRNA, the mutations in our isolate are located in domains I and II, respectively. No mutation was found in domain VII in our isolate. Though the relationship between mutations in domain I and macrolide/ketolide resistance in Streptococcus pneumoniae remains obscure, the clinical isolate NZR 796, having both an A138G mutation in the 23S rRNA domain I and an S20N amino acid change in L4, was reported to have 8 μg/ml and 0.03 μg/ ml MICs of erythromycin and TEL, respectively (9). This clinical isolate does not have erm(B) or mef(A/E). In the presence of erm(B) and the A138G mutation of 23S rRNA, another clinical isolate NZR 462 exhibited 0.125 μg/ml MIC of TEL (9). This isolate, however, does not have any mutation in L4. It is an attractive hypothesis that the mutations in 23S RNA and in L4 in conjunction with erm(B) would lead to elevation of TEL MIC in our isolate. To elucidate the relationship between 23S rRNA (rrl), L4 (rplD), L22 (rplV) and erm(B) in ketolide resistance, further genetic studies will be required.

In the case reported in this article, the patient was administered clarithromycin for 6 years until the isolation of a TEL-resistant Streptococcus pneumoniae strain. Thus, long-term clarithromycin therapy may have been one of the causes of the induction of resistance in the Streptococcus pneumoniae isolate from this patient. However, we could not confirm this, since S. pneumoniae had not been isolated at all until the above episode. The report by Davies et al. (10) in which a TEL-resistant mutant (MIC, 4 μg/ml) was obtained from an erythromycin-sensitive strain by serial passage in vitro in the presence of a subinhibitory concentration of clarithromycin may support this hypothesis. More recently, Rantala et al. (11) have reported that some ermB-positive isolates showed heterogenous resistance to TEL, which can change to stable, homogenous and high-level resistance to TEL.

Since low-dose and long-term erythromycin therapy have been found to carry the erm(B) gene, and the sequence the prevalence of antimicrobial resistance among isolates of Streptococcus pneumoniae from the PROTEKT surveillance study, and comparative in vitro activity of the ketolide, telithromycin. J. Antimicrob. Chemother., 50 (Suppl. 1), 25-37.


