Macrolide Resistance in *Streptococcus pneumoniae* Strains Collected in the Far East of Russia from 2000 to 2002

The last decade was noted for the emergence and prevalence of antimicrobial agent-resistant pneumococci. Strains resistant to macrolides, chloramphenicol, and tetracycline have been registered in a number of countries (1). In some regions, resistance to macrolides prevails over the resistance to penicillin. Macrolide resistance has been reported to be high among pneumococci in Asian countries (4), but the distribution of these macrolide resistance determinants is not known.

The incidence of erythromycin-resistant strains among *Streptococcus pneumoniae* isolates in such a large territory as the Far East of Russia was approximately 5% until the early 1990s, but nowadays erythromycin-resistant strains have been greatly increasing (5). Because macrolides are frequently used as first-line treatment for infections of lower respiratory tract in the Far East of Russia and the use of these agents has been associated with an increase in resistance rates, it was extremely important to investigate macrolide resistance rate in our area.

The main aim of our investigation was to estimate the macrolide-resistance rate in isolates of *S. pneumoniae*. In our investigation, we had studied macrolide resistance of *S. pneumoniae* strains derived from young patients with community-acquired pneumonia at the Far East of Russia. A total of 134 nonduplicate isolates of *S. pneumoniae* collected from 2000 to 2002 in the Far East were evaluated with the common NCCLS-recommended standards (2).

MICs of penicillin, erythromycin, clarithromycin, and clindamycin were determined by the agar dilution method (2). Inocula of 10^6 CFU/spot were incubated at 35°C for 18 h on Mueller-Hinton agar supplemented with 5% defibrinated horse blood. According to NCCLS criteria, strains were considered erythromycin resistant if an inhibition zone of <15 mm was found using a 15-µg erythromycin disk (bioMérieux, Marcy l’Étoile, France). *S. pneumoniae* ATCC 49619 was included as controls. MIC induction tests were performed by determining the MICs of macrolide antibiotics without and with preexposure to 0.05 µg of erythromycin per ml (6). Conserved primer sets were chosen to amplify a 640-bp fragment of the erm methylase genes as follows: *ermA*, 5’-TCTAAAGACATGT AAAAGAA-3’ and 5’-CTTCGATGTATTATATATTGTAT-3’; *ermB*, 5’-GAAAAGGTACTCAACCAATA-3’ and 5’-GTAACGTTATTTATATTGTAT-3’. A 1.2-kb fragment of the *mef* E gene in *S. pneumoniae* was amplified using primer pair 5’-GAAAATACCAATGGAAGAAC-3’ and 5’-AAATCTTAATTTCTCAACCTCA-3’. PCRs were performed on the OmniGene DNA thermal cycler (Hybaid) using an initial denaturation at 94°C for 5 min followed by 35 cycles of amplification at 94°C for 30 s, 50°C for 30 s, and 72°C for 90 s. A final elongation step was performed at 72°C for a further 7 min (3).

A total of 35.82% (48 of 134 strains) of the *S. pneumoniae* strains were resistant to erythromycin with a MIC of ≥1.0 µg/ml. Of these, 31.25% (15 of 48) showed an MLSB phenotype with erythromycin and clindamycin 50% MICs (MIC<sub>50</sub>) and MIC<sub>90</sub> of >64 µg/ml; 66.6% (32 of 48) showed resistance to erythromycin alone (M phenotype), with a MIC<sub>50</sub> and MIC<sub>90</sub> of 8.0 µg/ml. One isolate was positive with both *ermB* and *mef*E primers.

Of the isolates expressing the MLS<sub>B</sub> phenotype, only the *ermB* gene was detected in 86.63% (13 strains of 15) of the isolates by PCR. Two isolates were repeatedly negative on testing for *ermB* but were positive for *ermA* gene. All the isolates expressing the M phenotype were positive for the *mef*E gene by PCR. The majority of the M-phenotype strains (84.37%, or 27 of 32) had constitutive resistance (cML phenotype): only 15.625% of these strains had inducible resistance (iML phenotype).

Before 2000, it was recorded (5) that among the erythromycin-resistant *S. pneumoniae* isolates, the majority (78%) had an ML phenotype and 22% had an M phenotype. All *S. pneumoniae* isolates exhibiting a cML or iML phenotype harbored the *ermB* gene.

Thus, the study indicated a high percentage of erythromycin resistance among clinical isolates of *S. pneumoniae* in the Far East of Russia; this fact requires a more careful approach to diagnostics of macrolide resistance in pneumococci in the clinical microbiology laboratory, particularly in areas with high rates of macrolide resistance.

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REFERENCES


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