ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 2009, p. 2158–2159 Vol. 53, No. 5
0066-4804/09/$08.00+0 doi:10.1128/AAC.01563-08

Molecular Mechanisms of Macrolide Resistance in Clinical Isolates of *Mycoplasma pneumoniae* from China▽

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Received 24 November 2008/Returned for modification 22 December 2008/Accepted 22 February 2009

Fifty clinical *Mycoplasma pneumoniae* strains were isolated from 370 children with respiratory tract infections. Four strains were susceptible to macrolides, while the other 46 (92%) were macrolide resistant. The molecular mechanism of resistance was shown to be associated with point mutations in 23S rRNA at positions 2063 and 2064.

Mycoplasma pneumoniae is a common pathogen found in respiratory tract infections of children and teenagers and is commonly treated with macrolides. In recent years, strains which are resistant to common drugs have been isolated from patients (1, 3–8). In order to evaluate the prevalence of macrolide resistance, we collected clinical samples during 2003 to 2006, cultured *M. pneumoniae* isolates, and screened for macrolide drug resistance. We investigated the mechanism of resistance by examining the erythromycin target site in the 23S rRNA gene of these strains.

Throat swab specimens were collected from 300 inpatient and 70 outpatient children with respiratory tract infection at the Pediatric Department of Beijing Friendship Hospital, affiliated with Capital Medical University, during June 2003 to June 2006. Modified Hayflick medium was used for the isolation and growth of *M. pneumoniae*. Nested PCR was carried out to verify the identity of *M. pneumoniae*, using primers which amplify part of the 16S rRNA gene as described previously (2). The MICs of erythromycin, azithromycin, and josamycin required to inhibit *M. pneumoniae* growth were determined by the microdilution method (1). A reference strain, FH, was used as a drug-sensitive control. Erythromycin resistance was defined as having a MIC of ≥32 μg/ml in accordance with the 2006 standards recommended by the CLSI (formerly NCCLS). To examine the molecular mechanisms of drug resistance, the 23S rRNA gene was amplified by nested PCR and the product was sequenced as described previously (8). The DNA sequences were compared with the sequence of *M. pneumoniae* M129 (GenBank accession no. X68422).

Fifty clinical *M. pneumoniae* strains (44 of them from inpatients) were isolated from the 370 specimens collected. Four strains were susceptible to macrolides, and the other 46 (92%) strains were macrolide resistant. MICs of resistant strains to erythromycin, azithromycin, and josamycin were higher than that of the reference strain and higher than the CLSI guidelines (especially in the case of erythromycin and azithromycin). Table 1 shows the MIC range, MIC_{50}, and MIC_{90} of clinical isolate strains and the *M. pneumoniae* reference strain.

The 23S rRNA gene sequences of four susceptible strains and the reference strain FH were identical to that of the *M. pneumoniae* gene in GenBank (accession no. X68422). All 46 resistant strains displayed a point mutation in the 23S rRNA: 40 strains had an A-to-G transition at position 2063, 1 had an A-to-C transition at position 2063, and the other 5 showed an A-to-G transition at position 2064.

M. pneumoniae is a common pathogen associated with respiratory tract infection in children and teenagers, and macrolides are the first choice for treatment. Since their first introduction, increasing use of macrolides has given rise to increasing resistance among bacteria, but the sensitivity of *M. pneumoniae* to these drugs was unclear. In recent years, drug-resistant *M. pneumoniae* strains have been isolated from patients in a number of locations. In Japan, macrolide-resistant strains have been reported by Morozumi (3) and Matsuoka (1). In March 2005, D. Xin reported four of five *M. pneumoniae* strains were resistant to erythromycin in China (7, 8). Pereyre reported two macrolide-resistant strains in France (5), and Wolff reported five macrolide-resistant strains isolated in America (6). In these and our own studies, mutations at position 2063 or 2064 in domain V in the 23S rRNA gene were shown to be the predominant mutations associated with resistance.

The 44 inpatients who produced macrolide-resistant samples

TABLE 1. MIC range, MIC_{50}, and MIC_{90} of clinical isolate strains and the *M. pneumoniae* reference strain

Antimicrobial	MIC (μg/ml) for[a]:			
	Clinical isolates			Reference strain FH
	Range	50%	90%	
Erythromycin	0.01–512	128	512	0.01
Azithromycin	0.001–256	128	256	0.001
Josamycin	0.01–64	32	64	0.01

[a] 50% and 90%, MIC_{50} and MIC_{90}, respectively.

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▽ Published ahead of print on 9 March 2009.

showed positive *M. pneumoniae* immunoglobulin M antibody tests around the 8th to 14th day of the illness and were diagnosed clinically as being infected with *M. pneumoniae* (including four cases which were complicated with pleural effusion). All of the patients had had prior treatment with macrolides before hospitalization. In this study, most of the isolates cultured were shown to be macrolide resistant in vitro and it was not possible to determine whether macrolides would have been effective in vivo. Furthermore, there were no patients infected with susceptible strains to act as a control. Further research will be needed to determine whether macrolides retain any clinical efficacy in patients shown to be harboring strains of *M. pneumoniae* which may be defined in vitro as macrolide resistant.

This work was supported in part by three grants, one each from the National Natural Science Foundation of China, the Beijing Natural Sciences Fund, and the Beijing Chinese Medicine Bureau.

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