ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 2008, p. 348–350 Vol. 52, No. 1
0066-4804/08/$08.00+0 doi:10.1128/AAC.00779-07

Increased Macrolide Resistance of *Mycoplasma pneumoniae* in Pediatric Patients with Community-Acquired Pneumonia▽

Miyuki Morozumi,[1] Satoshi Iwata,[2] Keiko Hasegawa,[1] Naoko Chiba,[1] Reiko Takayanagi,[3] Keita Matsubara,[2] Eiichi Nakayama,[1] Keisuke Sunakawa,[4] Kimiko Ubukata,[1]* and the Acute Respiratory Diseases Study Group

Laboratory of Molecular Epidemiology for Infectious Agents, Kitasato Institute for Life Sciences, Kitasato University, Tokyo, Japan[1]; National Hospital Organization Tokyo Medical Center, Tokyo, Japan[2]; Tohoku Rosai Hospital, Miyagi, Japan[3]; and Laboratory of Infectious Diseases, Kitasato Institute for Life Sciences, Kitasato University, Tokyo, Japan[4]

Received 18 June 2007/Returned for modification 10 August 2007/Accepted 12 October 2007

Among 380 *Mycoplasma pneumoniae* isolates from 3,678 pediatric patients with community-acquired pneumonia, 50 macrolide-resistant strains had an A2063G transition in domain V of the 23S rRNA, whereas 5 had an A2064G transition. These resistant strains increased rapidly from April 2002 to December 2006.

For *Mycoplasma pneumoniae*, a major etiologic agent of lower respiratory tract infections acquired in the community, 14-membered ring macrolides (ML) generally are recognized as first-choice agents. In Japan, ML-resistant (ML^r) *M. pneumoniae* possessing a 23S rRNA mutation first was isolated from pediatric patients with community-acquired pneumonia (CAP) and bronchitis as reported in 2001 by Okazaki et al. (4). Patient symptoms appeared to be prolonged when isolates showed ML resistance (5).

We subjected *M. pneumoniae* isolated from pediatric patients with CAP between 2002 and 2006 to susceptibility evaluation for eight agents, including ML. In strains showing ML resistance, the 23S rRNA gene was analyzed.

Between April 2002 and December 2006, 3,678 clinical samples were sent to our laboratory from pediatricians affiliated with 10 institutions participating in the Acute Respiratory Diseases Study Group. All samples originating from pediatric patients diagnosed with pneumonia according to clinical symptoms and chest X-ray images were collected after informed consent was given by the patients and/or their parents or guardians.

Immediately after receipt, the samples were suspended in 1.5 ml of pleuropneumonia-like organism (PPLO) broth (Difco, Detroit, MI). DNA then was extracted by using Extragen II (Tosoh, Tokyo, Japan) according to the manufacturer's protocol. Real-time PCR to detect *M. pneumoniae* was performed as described previously (2) using the extracted DNA. Culture of *M. pneumoniae* was carried out for PCR-positive samples using PPLO broth according to previously described methods (6).

The MICs of eight agents for *M. pneumoniae* isolates were determined with microdilution methods using PPLO broth. These agents were erythromycin, clarithromycin, azithromycin, josamycin, rokitamycin, telithromycin, minocycline, and levofloxacin. *M. pneumoniae* M129 strain was used as a control.

The full length of the 23S rRNA gene was sequenced by methods described previously (3) in 55 *M. pneumoniae* strains showing ML resistance.

For patients with adequate clinical information, clinical courses of CAP caused by ML^r *M. pneumoniae* (n = 53) were compared to those of CAP with ML-susceptible (ML^s) *M. pneumoniae* (n = 58). Variables compared included (i) the number of days from initiation of ML treatment until defervescence to 37°C and (ii) whether or not initial treatment with ML was changed later to another agent. Body temperature that exceeded 38°C at least once daily was defined as ongoing fever.

Table 1 shows the numbers of real-time PCR-positive samples for *M. pneumoniae* among samples tested from April 2002 to December 2006 (approximately 5 years). A total of 3,678 nasopharyngeal samples were collected from pediatric patients with CAP. Culture for *M. pneumoniae* using PPLO broth was performed in the 521 samples PCR positive for *M. pneumoniae*; 380 strains were isolated. In 2003 and 2006, *M. pneumoniae* infection was particularly prevalent in Japan, reflected by the occurrence of more PCR-positive cases and *M. pneumoniae* isolates than in other years. The percentages of culture positivity for *M. pneumoniae* in PCR-positive samples ranged from 66.7 to 85.7% during the 5-year period.

Table 2 shows the MIC range, MIC_{50}, and MIC_{90} for eight agents according to the presence or absence of a mutation of the 23S rRNA gene in the 380 *M. pneumoniae* isolates; 50 strains had an A2063G transition in domain V, and 5 strains had an A2064G transition. ML^r strains showed high resistance to erythromycin, clarithromycin, azithromycin, telithromycin, and josamycin. Among 16-membered ring ML, only rokitamycin had a effective MIC_{90} (0.25 μg/ml) for strains with the A2063G transition. However, strains with the A2064G transition showed intermediate resistance to rokitamycin. The

* Corresponding author. Mailing address: Laboratory of Molecular Epidemiology for Infectious Agents, Kitasato Institute for Life Sciences, Kitasato University, 5-9-1 Shirokane, Minatoku, Tokyo 108-8641, Japan. Phone: 81-3-5791-6385. Fax: 81-3-5791-6386. E-mail: ubukatak@lisci.kitasato-u.ac.jp.

▽ Published ahead of print on 22 October 2007.

TABLE 1. PCR and culture results for *M. pneumoniae* from 2002 to 2006

PCR and culture	No. of strains (%)[a] in:					Total samples (n = 3,678)
	2002 (n = 522)	2003 (n = 699)	2004 (n = 533)	2005 (n = 749)	2006 (n = 1,175)	
PCR positive	70 (13.4)	140 (20.0)	60 (11.3)	75 (10.0)	176 (15.0)	521 (14.2)
Culture positive	47 (67.1)	120 (85.7)	40 (66.7)	52 (69.3)	121 (68.8)	380 (72.9)

[a] In the first row, the percentage of all samples tested is given in parentheses; in the second row, the percentage of PCR-positive samples is given in parentheses. *n*, Number of samples tested.

TABLE 2. In vitro antimicrobial activity of eight oral agents against *M. pneumoniae* strains (n = 380)

Antimicrobial agent and ML^r class	MIC (μg/ml)[a]		
	50%	90%	Range
Erythromycin			
Susceptible	0.0078	0.0156	0.00195–0.0313
Resistant			
A2063G	64	>64	32–>64
A2064G	>64	>64	64–>64
Clarithromycin			
Susceptible	0.0039	0.0078	0.00049–0.0313
Resistant			
A2063G	64	>64	32–>64
A2064G	64	64	16–>64
Azithromycin			
Susceptible	0.00049	0.00098	0.00024–0.00195
Resistant			
A2063G	32	64	16–>64
A2064G	32	64	16–64
Telithromycin			
Susceptible	0.00098	0.00195	0.00024–0.0039
Resistant			
A2063G	32	64	16–>64
A2064G	4	16	1–16
Josamycin			
Susceptible	0.0313	0.0625	0.0156–0.0625
Resistant			
A2063G	8	16	0.0625–64
A2064G	64	>64	64–>64
Rokitamycin			
Susceptible	0.0156	0.0156	0.0039–0.0313
Resistant			
A2063G	0.125	0.25	0.0156–16
A2064G	16	16	8–16
Minocycline			
Susceptible	0.5	1	0.0313–2
Resistant			
A2063G	0.5	1	0.0625–1
A2064G	0.5	1	0.0313–1
Levofloxacin			
Susceptible	0.5	1	0.125–1
Resistant			
A2063G	1	1	0.5–1
A2064G	1	1	0.5–1

[a] MICs were determined by microdilution methods using PPLO broth. Samples (10 μl) from these cultures estimated to contain 10^5 CFU/ml were inoculated into 96-well microplates filled with 90 μl of PPLO broth containing serially diluted antibiotics. These were incubated aerobically from 10 to 14 days at 37°C until a color change was confirmed in an antibiotic-free growth control. The MIC of each agent was defined as the lowest concentration of each antibiotic preventing the color change.

MIC_{90}s of minocycline and levofloxacin for ML^r strains were equal to the MIC_{90}s for susceptible strains.

Figure 1 shows year-by-year changes in ML^r *M. pneumoniae* from April 2002 to December 2006. Resistant strains increased rapidly each year: 0% (0/47) in 2002, 5.0% (6/120) in 2003, 12.5% (5/40) in 2004, 13.5% (7/52) in 2005, and 30.6% (37/121) in 2006. In parallel with the overall increased prevalence of *M. pneumoniae* infections in 2006, the prevalence of ML^r strains isolated from widespread regions of Japan clearly also increased.

Treatment was changed from ML to minocycline or levofloxacin in 6.9% (4/58) of patients infected with ML^s *M. pneumoniae* and in 35.8% (19/53) of patients infected with ML^r *M. pneumoniae*, representing a significant difference between groups (P = 0.0023). Only one patient had a change in treatment to levofloxacin, which was done to address coinfection with β-lactamase-nonproducing and ampicillin-resistant *Haemophilus influenzae*. The mean times from the initiation of macrolide use to defervescence were 1.6 ± 0.8 days for ML^s *M. pneumoniae* and 4.1 ± 2.3 days for ML^r *M. pneumoniae*, a significant difference (P = 0.0020).

In our laboratory, ML^r *M. pneumoniae* first was isolated from a patient with acute bronchitis in 2002 (3) and then began to be isolated from CAP cases in 2003. In parallel with the prevalence of *M. pneumoniae* infection, the prevalence of ML^r isolates have increased rapidly, attaining a 30.6% prevalence in 2006.

A question might be posed as to whether ML^r *M. pneumoniae* represents a serious clinical therapeutic issue, since *M. pneumoniae* infection typically produces mild symptoms that spontaneously diminish, with ultimate recovery. However, in CAP patients infected with ML^r strains, ML frequently was changed to minocycline or levofloxacin because of persistent fever and cough or nonresolution or worsening of chest X-ray abnormalities. Levofloxacin and minocycline are not ordinarily recommended for children. Only in cases where other antibiotics cannot be used or are ineffective against organisms is the use of these antibiotics approved by the Japanese Ministry of Health, Labor, and Welfare. If ML are ineffective against *M. pneumoniae* infection, pediatricians have little choice but to use minocycline. In the future, improvement or augmentation of treatment for patients with ML^r *M. pneumoniae* infections should be considered promptly, including symptomatic measures such as steroid therapy.

In addition to affecting children, *M. pneumoniae* is a common pathogen among young adults with CAP (1, 6). No ML^r *M. pneumoniae* was observed among 30 isolates from adult patients with CAP. Although the absence of resistant isolates is unexplained, the wide use of new quinolone agents for adult patients with acute respiratory tract infections may have con-

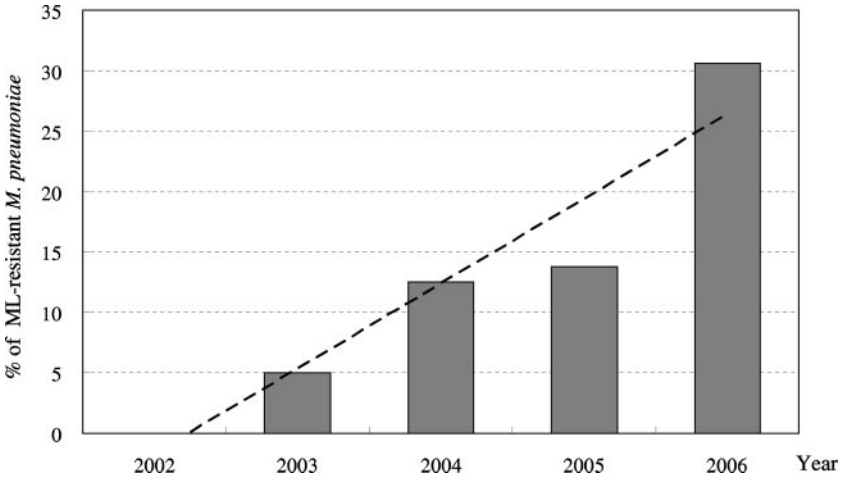

FIG. 1. Year-by-year increases in the frequency of ML^r *M. pneumoniae* cases. The dotted line can be expressed by the equation, $y = 7x - 8.62$ ($r = 0.9510$).

tributed to this finding. Nonetheless, oral ML treatment still is a common choice for adult patients, amounting to 25% or more compared to ca. 24% for quinolones. In this population, ML^r *M. pneumoniae* strains may also ultimately emerge and become more prevalent.

Based upon our results, we recommend increased worldwide surveillance for ML^r *M. pneumoniae* and stress the need to establish the most appropriate chemotherapy against those infections.

We thank the members of the Acute Respiratory Diseases Study Group: Shinobu Ishizawa, Mika Numata, and Michiyo Kojima (Tohoku Rosai Hospital), Takeshi Tajima (Hakujikai Memorial Hospital), Haruo Kuroki (Sotobo Kodomo Clinic), Kouichi Shimizu (Saiseikai Ibaraki Hospital), Naohisa Kawamura (Osaka Rosai Hospital), Ritsuko Sakai (Sakai Clinic), Masaaki Kobayashi (Kobayashi Pediatric Clinic), Kota Saito (Saito Pediatric Clinic), Masahiko Nitta (Yaotokushukai Hospital and Osaka Medical College), Shigeru Ohnari (Nakafukawa Pediatric Clinic), Takao Morinobu (Seikeikai Hospital), Masato Nonoyama (Ebina General Hospital), and Tomohiro Oishi (Niigata Kenritsu Shibata Hospital).

This study was supported by a grant from Kawano Masanori Memorial Foundation for the Promotion of Pediatrics.

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