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ORIGINAL ARTICLE

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A comparative clinical study of macrolide-sensitive and macrolide-resistant *Mycoplasma pneumoniae* infections in pediatric patients

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Abstract In recent years, the increased prevalence of macrolide-resistant *Mycoplasma pneumoniae* (MR-*M. pneumoniae*) has become a significant issue in Japan. We isolated 94 strains of *M. pneumoniae*, and determined the minimum inhibitory concentrations (MICs) of macrolides and other antimicrobial agents for these strains. We also performed a comparative clinical evaluation of macrolide efficacy for cases of MR-*M. pneumoniae* infections and cases of macrolide-sensitive *Mycoplasma pneumoniae* infections (MS-*M. pneumoniae*). Of the 94 isolates of *M. pneumoniae,* 64 (68.1%) were classified as MS-*M. pneumoniae* and 30 (31.9%) as MR-*M. pneumoniae* strains. The clinical study included an assessment of 47 pediatric cases of MS-*M. pneumoniae* and 22 pediatric cases of MR-*M. pneumoniae*. The patient demographics, such as sex, age, the period from the onset of the infection to the first examination, laboratory findings, diagnosis, and the severity of symptoms, showed no significant difference between the two study groups. However, the efficacy of macrolide treatment was 91.5% for MS-*M. pneumoniae* and 22.7% for MR-*M. pneumoniae*, a statistically significant difference ($P < 0.01$). Although *M. pneumoniae* infection is generally considered a treatable condition, the increasing prevalence of macrolide-resistant strains of *M. pneumoniae* has become a significant clinical issue in pediatric patients, and it is therefore necessary to give careful consideration to the appropriate antimicrobial therapy for MR-*M. pneumoniae* infection.

Key words *Mycoplasma pneumoniae* · Macrolide · Drug resistance · Child

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Introduction

Mycoplasma pneumoniae is one of the most prevalent pathogens causing community-acquired respiratory tract infections in children and young adults. Usually, macrolides are used as the first-line agents for treating *M. pneumoniae* infections in children.

Lucier et al.,[1] Okazaki et al.,[2] and Morozumi et al.[3,4] reported previously that transition mutations at A2063G and A2064G in domain V on the 23S rRNA gene were present in macrolide-resistant *Mycoplasma pneumoniae* (MR-*M. pneumoniae*) strains and not in the *M. pneumoniae* M129 strain, currently recognized as the standard strain. Because microbiological investigation and genome sequencing of *M. pneumoniae* is generally not performed at clinical institutions of Japan, we cannot diagnose MR-*M. pneumoniae* infection. However, there are some reports that the prevalence of MR-*M. pneumoniae* has increased recently.[3,4]

It is not appropriate to use tetracycline and fluoroquinolones in pediatric patients because of the adverse effects of these agents. Thus, it is important to evaluate the clinical features and efficacy of antimicrobial agents for treating MR-*M. pneumoniae* infections. We isolated multiple strains of *M. pneumoniae* by cultivation of clinical specimens from

pediatric patients, determined the minimum inhibitory concentrations (MICs) of several oral antimicrobial agents for these strains and identified transition mutations on the 23S rRNA gene in the MR-*M. pneumoniae* strains. We also performed a clinical evaluation of the symptoms of MR-*M. pneumoniae* infections in children and evaluated the efficacy of several antimicrobial agents, including macrolides, for treating these infections.

Patients and methods

Between October 2002 and December 2006, after informed consent was obtained from patients and their parents, clinical specimens were collected from the nasopharynx of pediatric patients with community-acquired respiratory tract infections who visited the Department of Pediatrics, National Hospital Organization Tokyo Medical Center. The specimens were sent to the Laboratory of Molecular Epidemiology for Infectious Agents, Kitasato Institute for Life Sciences, Kitasato University. *M. pneumoniae* strains were isolated by the cultivation of specimens using pleuropneumonia-like organism (PPLO) broth and agar plates,[3,4] and the MICs of three macrolides, erythromycin (EM), clarithromycin (CAM), and azithromycin (AZM) and two other antimicrobial agents, minocycline (MINO) and levofloxacin (LVFX) for the isolated strains were determined by microdilution methods using PPLO broth.[3–5] When the MICs indicated macrolide resistance, the MICs for rokitamycin (RKM) and josamycin (JM) were also determined.

Full-length sequencing of the 23S rRNA gene in *M. pneumoniae* strains showing macrolide resistance was performed by methods reported previously.[3,4] These genome sequences had transition mutations at A2063G, A2064G, or other locations in domain V which were previously reported.[1–5] The MICs and the molecular genomic analysis of most strains in this study have already been reported by Morozumi et al.,[4] so we focused on the clinical courses of the patients from whom those strains were isolated, adding several cases and strains to the earlier study.[4]

The patient demographics, clinical symptoms, and clinical efficacy of macrolides were compared for MR-*M. pneumoniae* and macrolide-sensitive *M. pneumoniae* (MS-*M. pneumoniae*) cases. These parameters were analyzed according to the criteria for clinical trials of therapeutic agents in pediatrics advocated by the Japanese Society of Chemotherapy[6] (Table 1). The determination of the disease severity of pneumonia and bronchitis followed the guidelines for the treatment of pediatric community-acquired pneumonia published in 2007 by the Japanese Society of Pediatric Infectious Diseases and the Japanese Society of Pediatric Respiratory Infectious Diseases.[7]

There is a possibility of dual infection with other bacteria or viruses in patients with *M. pneumoniae* infection. So, for the evaluation of clinical efficacy, we excluded patients who were treated with beta-lactams together with macrolides, considering the possibility of dual bacterial infections. The presence of dual viral infections was checked by real-time PCR[8] for clinical specimens or by serological diagnosis. For the evaluation of clinical efficacy, we also excluded patients who had been treated with antimicrobials having anti-mycoplasmal activities, such as LVFX or MINO, before using macrolides.

Statistical analysis was performed using StatMate III (ATMS, Tokyo, Japan).

Table 1. Japanese Society of Chemotherapy[6] criteria for the clinical efficacy of antimicrobial agents in childhood infectious diseases

Clinical efficacy	Period before main symptoms resolve	
	Improving	Almost disappearing
Excellent	1 Day or less	3 Days or less
Good	3 Day or less	5 Days or less
Fair	More than 3 days	More than 5 days
Poor	No change or exacerbation	

Table 2. Minimum inhibitory concentrations of *Mycoplasma pneumoniae*

Antimicrobial agents	MIC (μg/ml)		
	Range	50%	90%
MS-*M. pneumoniae* (n = 64)			
EM	0.00195–0.0313	0.0078	0.0156
CAM	0.00049–0.0156	0.0039	0.0078
AZM	0.00024–0.00195	0.000488	0.000975
LVFX	0.25–1.0	1.0	1.0
MINO	0.0313–1.0	0.5	1.0
MR-*M. pneumoniae* (n = 30)[a]			
EM	32–>64	64	>64
CAM	16–>64	64	>64
AZM	16–>64	32	64
RKM	0.0313–16	0.125	16
JM	2.0–>64	8	64
LVFX	0.5–1.0	1.0	1.0
MINO	0.0313–2.0	1.0	1.0

EM, erythromycin; CAM, clarithromycin; AZM, azithromycin; RKM, rokitamycin; JM, josamycin; LVFX, levofloxacin; MINO, minocycline; MR, macrolide resistant; MS, macrolide susceptible

[a] MICs of RKM, JM, and MINO were not measured in seven strains of MR-*M. pneumoniae*

Results

Ninety-four strains of *M. pneumoniae* were isolated by the cultivation of samples from 94 patients. Of the 94 strains, 64 (68.1%) were classified as MS-*M. pneumoniae* and 30 strains (31.9%) as MR-*M. pneumoniae* by MIC measurements (Table 2) and genome sequences. All MS-*M. pneumoniae* were susceptible to the three macrolides (EM, CAM, and AZM), but MR-*M. pneumoniae* had very high MICs for 14- and 15-membered ring macrolides and moderately high MICs for two 16-membered ring macrolides. MR-*M.* and MS-*M pneumoniae* strains showed equal susceptibilities to LVFX and MINO. Of the MR-*M. pneumoniae* strains, 26 had the A2063G mutation and 3 the A2064G mutation. The strains with the A2063G mutation had lower MICs for RKM and JM than those with the

Table 3. Demographics of patients with MS-*M. pneumoniae* and MR-*M. pneumoniae* infections

	MS-*M. pneumoiniae* ($n = 47$)		MR-*M. pneumoniae* ($n = 22$)		*P* value
	Male/Female		Male/Female		
Sex	23/24		13/9		NS
	Mean	Range	Mean	Range	*P* value
Age (years)	7.5	0–14	7.7	3–13	NS
Period from the infection onset to the first examination (days)	5.8	3–10	5.8	3–10	NS
Clinical symptoms and laboratory findings at the first examination					
Temperature (°C)	37.8	36.5–39.8	37.9	36.8–39.4	NS
WBC (/μl)	6574	2,800–14,400	6482	3,600–11,100	NS
CRP (mg/dl)	2.06	0.1–6.8	1.95	0.1–6.1	NS
Maximum temperature during treatment period (°C)	38.9	37.0–40.5	38.9	38.0–40.2	NS
	n	%	*n*	%	*P* value
Severity of symptoms					
Mild	14	29.8	7	31.8	NS
Moderate	33	70.2	15	68.2	NS
Severe	0	0.0	0	0.0	NS

WBC, white blood cell; CRP, C-reactive protein; NS not significant

Table 4. Clinical efficacy of macrolides for treatment of MS-*M. pneumoniae* and MR-*M. pneumoniae* infections

	MS-*M. pneumoniae* ($n = 47$)		MR-*M. pneumoniae* ($n = 22$)		*P* value
	Mean	Range	Mean	Range	
No. of days from start of macrolide treatment to symptom disappearance					
Fever	1.5	0–8	4.0	1–8	<0.01
Cough	7.0	4–13	11.4	8–22	<0.01
					P value
Efficacy rate[a]	91.5%	(43 cases)	22.7%	(5 cases)	<0.01

[a] Efficacy rate = number of excellent and good cases divided by total no. of cases

A2064G mutation (data not shown; 1 strain was not sequenced).

Seventeen cases of MS-*M. pneumoniae* and 8 cases of MR-*M. pneumoniae* infection were excluded because we were not able to judge the efficacy of the macrolide. In 12 cases macrolides and beta lactams were used together, in 9 cases LVFX or MINO was used before using macrolides, 3 cases lacked the clinical information that was necessary for analysis, and in 1 case a beta-lactam was used alone. There were no cases of viral dual infection. So the analysis of the clinical study was performed for 47 cases of MS-*M. pneumoniae* infection and 22 cases of MR-*M. pneumoniae* infection. All cases were diagnosed as pneumonia, and the patient demographics, such as sex, age, period from the infection onset to the first examination, laboratory findings, and the severity of symptoms, showed no significant difference between the two groups (Table 3). The mean febrile period after macrolide administration was 1.5 days for MS-*M. pneumoniae* and 4.0 days for MR-*M. pneumoniae,* a statistically significant difference ($P < 0.01$). The mean period of persistent cough after macrolide administration was 7.0 days for MS-*M. pneumoniae* and 11.4 days for MR-*M. pneumoniae,* respectively, again a statistically significant difference ($P < 0.01$). The clinical efficacy of macrolide administration for MS-*M. pneumoniae* was determined to be excellent for 22 cases (46.8%), good for 21 cases (44.7%), fair for 3 cases (6.4%), and poor for 1 case (2.1%), compared with excellent for 1 case (4.5%), good for 4 cases (18.2%), fair for 7 cases (31.8%), and poor for 10 cases (45.5%) for MR-*M. pneumoniae*. Thus, the efficacy rate for macrolide therapy was 91.5% in MS-*M. pneumoniae* and 22.7% in MR-*M. pneumoniae*, and a significant difference was observed between the two study groups ($P < 0.01$; Table 4).

Discussion

Historically, Ikejima et al.[9] reported that all *M. pneumoniae* strains isolated were sensitive to macrolides; however, more recently, several reports of MR-*M. pneumoniae* in Japan have been published.[2–5,10] Nevertheless, there are still few reports of clinical studies of MR-*M. pneumoniae*,[4,10] because the cultivation of *M. pneumoniae* and identification of its genome sequences are not generally performed at the clinical laboratories of medical facilities. In addition, *M. pneumoniae* infections tend to resolve naturally without the administration of antibacterial agents. Therefore, care should be exercised in comparing the efficacy of the antibacterial agents.

Morozumi et al.[4] and Suzuki et al.[10] have reported that the efficacy of macrolides for treating MR-*M. pneumoniae* infections is decreasing. In the present study, we evaluated

the clinical severity of *M. pneumoniae* infections and clinical symptoms in pediatric patients, and examined the efficacy of macrolides for treating these *M. pneumoniae* infections. We found that the clinical efficacy of macrolides for treating cases of MR-*M. pneumoniae* was significantly lower than the clinical efficacy for cases of MS-*M. pneumoniae*. In the present study, it was necessary to administer MINO or LVFX in 10 of the 22 patients with MR-*M. pneumoniae* infection (45.5%), as their primary symptoms did not improve after primary treatment with macrolides. However, it is not appropriate to use tetracyclines and fluoroquinolones for MR-*M. pneumoniae* infections in children, as tetracyclines are not recommended for use in children less than 8 years old and almost all fluoroquinolones are not indicated for administration to children. In addition, because there were no observed differences in clinical symptoms and disease severities between the cases of MS-*M. pneumoniae* and MR-*M. pneumoniae* infection, it was difficult to diagnose MR-*M. pneumoniae* infection at the start of treatment. Although there was no patient in the study in whom *M. pneumoniae* infections caused severe complications, it is still a significant problem that the clinical courses of respiratory tract infections with MR-*M. pneumoniae* in children are prolonged when they are treated with macrolides, the first-line antibiotics for mycoplasmal infections in childhood.

There is a likelihood that the prevalence of MR-*M. pneumoniae* will increase, as macrolides are used as the first-line agents for the treatment of pediatric community-acquired respiratory infections. The incidence of MR-*M. pneumoniae* should therefore be carefully monitored, and in addition, it is important to develop new agents that will be effective in treating MR-*M. pneumoniae* infections, as well as being safe for administration to children.

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