MAJOR ARTICLE

Rapid Effectiveness of Minocycline or Doxycycline Against Macrolide-Resistant *Mycoplasma pneumoniae* Infection in a 2011 Outbreak Among Japanese Children

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(See the Editorial Commentary by Bébéar, on pages 1650–1.)

Background. *Mycoplasma pneumoniae* is a major pathogen causing community-acquired pneumonia in children and young adults. Outbreaks typically occur at intervals of several years. In 2011, a widespread outbreak was associated with macrolide-resistant *M. pneumoniae* (MRMP) in Japanese children, often those of school age.

Methods. Two hundred fifty-eight children were diagnosed with *M. pneumoniae*–associated pneumonia based on chest radiography, real-time polymerase chain reaction (PCR), and antibody titers between January and December 2011. *Mycoplasma pneumoniae* cultures obtained from nasopharyngeal samples using appropriate broth were subjected to real-time PCR, by which decreases in *M. pneumoniae* in patients treated with minocycline (MIN), doxycycline (DOX), or tosufloxacin (TFX) were calculated. Mutations of the 23S ribosomal RNA gene that confer high resistance to macrolides in *M. pneumoniae* were identified by DNA sequencing.

Results. Among 202 *M. pneumoniae* isolates from *M. pneumoniae*–associated pneumonia patients, 176 (87.1%) were MRMP. Macrolide-resistant *M. pneumoniae* infection was significantly related to school age ($P < .01$) and initial administration of macrolides ($P < .01$). Minocycline or DOX (n = 125) or TFX or levofloxacin (n = 15) was used for definitive treatment of MRMP patients. Minocycline or DOX was significantly more effective than TFX ($P \leq .05$) in achieving defervescence within 24 hours and in decreasing numbers of *M. pneumoniae* DNA copies 3 days after initiation.

Conclusions. Macrolides are inappropriate as first-choice agents against MRMP in terms of shortening the clinical course and decreasing *M. pneumoniae*. Control and prevention of MRMP outbreaks in children require early decreases in *M. pneumoniae* as well as improvement of clinical findings.

Mycoplasma pneumoniae is the most common cause of community-acquired pneumonia (CAP) in school-aged children [1–3] and young adults [4–6]. *Mycoplasma pneumoniae*–associated pneumonia in children is reported to cause 15%–30% of hospitalizations for CAP [7–9].

Mycoplasma pneumoniae isolates frequently exhibit high resistance to 14-, 15-, and 16-membered ring macrolides and ketolides; this type of *M. pneumoniae* is known as macrolide-resistant *M. pneumoniae* (MRMP), which was first isolated from pediatric patients in 2000 [10]. Prevalence of MRMP in pediatric practice has increased rapidly from 5.0% in 2003 [11] to 39% in 2008 [12]. Macrolide-resistant

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M. pneumoniae also has been isolated from young adult patients with CAP [13].

Like neighboring Japan, China now shows high MRMP prevalence, constituting over 80% of *M. pneumoniae* in children and adults [14, 15]. In contrast, in an epidemic of *M. pneumoniae* infections occurring in Europe and Israel from 2010 to 2011, the *M. pneumoniae* strains were susceptible to macrolides [16, 17]. Prevalence of MRMP has been reported to be relatively low in the West: 8.2% in the United States [18], 0.9% to 2.9% in Denmark [19], 3% in Germany [20], approximately 10% in France [21], 26% in Italy [22], and 32% in Israel [23].

Beginning in spring 2011, signs of an outbreak of *M. pneumoniae*–associated infection in Japanese children were seen in outpatient settings; from the beginning of autumn 2011, this outbreak grew explosively. The National Institute of Infectious Diseases issued an alert (http://idsc.nih.go.jp/idwr/kanja/weeklygraph/18myco-e.html). Japanese continuous surveillance in 2011 showed >80% of *M. pneumoniae* isolates to be MRMP with a mutation at A2063G, A2063T, or A2064G of the 23S ribosomal RNA gene, which confers high resistance to macrolides [12, 24, 25].

Defervescence and disappearance of cough could not be achieved in a number of Japanese patients with *M. pneumoniae*–associated pneumonia who initially received macrolides [26, 27]. Antibiotic treatment was then changed to minocycline (MIN), doxycycline (DOX), or tosufloxacin (TFX), limiting administration to 3–7 days. Although MIN is not approved in the United States or European Union, this agent, administered in the form of granules, was approved in 2004 in Japan for treating *M. pneumoniae* infection in children aged ≥8 years in whom macrolides were ineffective or unusable.

In Japan, oral TFX, a fluoroquinolone agent, was approved for pediatric use by the Ministry of Health, Welfare, and Labor in 2010. Studies of TFX in immature experimental animals reported less potential toxicity, such as abnormalities in articular cartilage and QT intervals, than with ciprofloxacin or norfloxacin [28]. Use was confined strictly to patients with CAP or acute otitis media caused by penicillin-resistant *Streptococcus pneumoniae* or β-lactamase nonproducing, ampicillin-resistant *Haemophilus influenzae* and to patients whose infection did not respond to any agent apart from TFX. Tosufloxacin has not yet been approved for infections with *M. pneumoniae.*

We assessed clinical aspects of pneumonia with MRMP and macrolide-susceptible *M. pneumoniae* (MSMP) as well as differences in bacterial disappearance after administration of MIN, DOX, or TFX, in patients with MRMP-associated pneumonia.

PATIENTS AND METHODS

Patients

A prospective study of pediatric patients aged <15 years old with *M. pneumoniae*–associated pneumonia was conducted by participating pediatricians at 5 medical institutions (National Hospital Organization Tokyo Medical Center, Hakujikai Memorial Hospital, Nihon University Nerima-Hikarigaoka Hospital, Asahikawa-Kosei General Hospital, and Nakafukawa Pediatric Clinic) between January and December 2011. After informed consent was obtained from patients or their parents, nasopharyngeal samples (NPSs) were collected from the patients using a sterile swab (Nippon Becton Dickinson) by each pediatrician. All NPSs were sent to the Laboratory of Molecular Epidemiology for Infectious Agents at Kitasato University. No patients with an underlying disease were enrolled in this study.

A definite diagnosis of *M. pneumoniae*–associated pneumonia was made based on clinical symptoms, chest radiography, and real-time polymerase chain reaction (PCR) results for *M. pneumoniae* when patients first presented at the hospital. Two hundred fifty-eight cases were analyzed in this study.

Real-Time PCR

Mycoplasma pneumoniae was identified in NPSs by comprehensive real-time PCR using a Cycleave probe acting as a modified molecular beacon probe [29]. By this method, 6 bacterial pathogens, including *M. pneumoniae*, could be detected within 2 hours. Sensitivity and specificity of this PCR method based on the antimycoplasmal antibody titer measured by particle agglutination test were 90.2%–99.0% and 95.4%–97.9%, respectively [29, 30].

To eliminate cases of viral pneumonia, 13 respiratory viruses including respiratory syncytial virus subgroups A and B, influenza viruses A, B, and (H1N1)2009, parainfluenza viruses 1–3, human metapneumovirus, human bocavirus, adenovirus, enterovirus, and rhinovirus also were identified by reverse-transcription real-time PCR [31].

Patients coinfected with *M. pneumoniae* and other microorganism were few and were excluded from this study.

Culture and Antibiotic Susceptibility

Nasopharyngeal samples showing a positive reaction to *M. pneumoniae* by real-time PCR were cultured using 2 mL of pleuropneumonia-like organism (PPLO) broth for 4 weeks at 37°C. In PPLO broth where color had changed from red to yellow with glucose consumption, *M. pneumoniae* colonies were purified using a PPLO agar plate. *Mycoplasma pneumoniae* finally was identified by hemadsorption of colonies with a 5% suspension of guinea pig erythrocytes.

Antibiotic susceptibility of MP to erythromycin, clarithromycin, azithromycin, telithromycin, josamycin, MIN, DOX, levofloxacin (LVX), and TFX was measured using microdilution methods and the previously described PPLO broth [11].

DNA Sequencing

The full length of the 23S ribosomal RNA gene of all *M. pneumoniae* isolates was sequenced by previously described methods [11]. Major attachment protein (P1) type also was determined by sequencing according to previously described methods [32].

Antibodies

Mycoplasma pneumoniae antibody titers in acute and convalescent phase sera obtained at least 1 week apart were measured with a particle agglutination test using a commercially available kit (Serodia-MycoII kit; Fujirebio). Results were considered positive for *M. pneumoniae* infection based on a 4-fold rise in titer between paired sera or a single titer of at least 1:320.

Data Analysis

We used Microsoft Excel 2010 for Statistics (SSRI) and Prism Version 5.0 (Graph Pad Software) for data analysis. Statistical significance of differences in categorical variables, such as days from onset, age of onset, and initial and secondary antibiotics, was determined by the χ^2 test or Fisher's exact test. Bacterial count of *M. pneumoniae* at 3 points after each antibiotic administration was analyzed with paired *t* test.

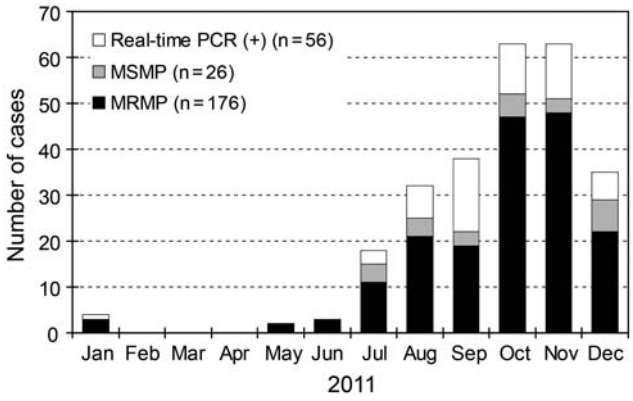

Figure 1. Month-by-month occurrence of *Mycoplasma pneumoniae*–associated pneumonia in children aged <15 years during 2011. Macrolide-resistant *M. pneumoniae* were identified by detecting an A2063G, A2063T, A2063C, or A2064G mutation in domain V of the 23S ribosomal RNA gene. Macrolide-susceptible *M. pneumoniae* included those with none of these mutations. Cases designated "real-time polymerase chain reaction (PCR)" were positive only by PCR without isolation of *M. pneumoniae*. Later, however, all were confirmed to have *M. pneumoniae* by a rise in antibody titer for *M. pneumoniae* using particle agglutination methods. Abbreviations: MRMP, macrolide-resistant *M. pneumoniae*; MSMP, macrolide-susceptible *M. pneumoniae*; PCR, polymerase chain reaction.

RESULTS

In our group of 5 actively participating pediatric departments, 258 patients were diagnosed with *M. pneumoniae*–associated pneumonia in 2011 by real-time PCR and by an increase of antibody titers in paired sera. Among these, *M. pneumoniae* was isolated from 202 (78.3%). The distribution by month is shown in Figure 1.

Some 176 patients with *M. pneumoniae* (87.1%) were diagnosed with MRMP, whereas the remaining 26 (12.9%) had MSMP. Most resistant isolates possessed a mutation in domain V of the 23S ribosomal RNA gene at nucleotide position 2063, with a substitution from adenine (A) to guanine (G) (n = 160), thymine (T) (n = 11), or cytosine (C) (n = 1), or at nucleotide position 2064 (n = 4), with a substitution from A to G. The 90% minimum inhibitory concentrations of erythromycin, clarithromycin, azithromycin, and josamycin for MRMP possessing a mutation A2063 G (C or T) respectively were >64 μg/mL, >64 μg/mL, 64 μg/mL, and 16 μg/mL (Table 1). The 90% minimum inhibitory concentrations of MIN, DOX, LVX, and TFX were 1 μg/mL, 0.5 μg/mL, 1 μg/mL, and 0.5 μg/mL, respectively.

The major attachment protein (P1) type was identified as P1 type 1 in all MRMP and MSMP strains.

Characteristics of children with MSMP- and MRMP-associated pneumonia are shown in Table 2. Days from clinical onset and ratios of inpatients to outpatients did not differ significantly between the 2 groups. In contrast, the median age of onset of symptoms of MRMP was 8 years and was significantly more frequent in the school-age group (≥6 years; $P < .01$). As for antibiotics initially used at other clinics, the macrolides clarithromycin and azithromycin predominated in the MRMP group (n = 114; 64.8%) compared with the MSMP group (n = 5; 19.2%) ($P < .01$). Of 159 patients in the MRMP group (90.3%), treatment for 140 cases was changed to MIN or DOX (n = 125; 78.6%), TFX, or LVX (n = 15; 9.4%) when fever or clinical findings failed to improve for 3 days or chest radiographic findings worsened.

Dosage of MIN, DOX, and TFX was twice-daily administration of 4 mg/kg/day, 4 mg/kg/day, and 12 mg/kg/day, respectively, according to package inserts accompanying these drugs.

Duration of hospitalization did not differ significantly between the 2 groups. Among the 3 patients hospitalized for >10 days, TFX was administered to 2 and AZM to 1. No recurrences were observed among the 258 patients.

Patients' blood test results according to presence or absence of steroid use are summarized in Supplementary Table 1. White blood cell counts, neutrophil counts, lymphocyte counts, C-reactive protein values, creatine kinase values, and ferritin values did not differ significantly between the 2 groups. Only lactate dehydrogenase was significantly higher in the steroid group than the nonsteroid group.

Table 1. Minimum Inhibitory Concentrations of 8 Antimicrobial Agents Against *Mycoplasma pneumoniae*

Macrolide Resistance Class	MIC_{90} (range), μg/mL							
	ERY	CLR	AZM	JOS	MIN	DOX	LVX	TFX
MSMP (n = 26)	0.016 (0.002–0.031)	0.008 (0.001–0.031)	0.001 (0.0001–0.002)	0.063 (0.016–0.063)	1 (0.031–2)	0.5 (0.063–0.5)	1 (0.125–1)	0.5 (0.25–0.5)
MRMP (n = 176)								
Position of mutation: A2063G, C, or T	>64 (32–>64)	>64 (32–>64)	64 (4–>64)	16 (0.063–64)	1 (0.063–1)	0.5 (0.125–0.5)	1 (0.5–1)	0.5 (0.25–0.5)
A2064G	>64 (64–>64)	64 (64–>64)	64 (16–64)	>64 (64–>64)	0.5 (0.031–1)	0.5 (0.25–0.5)	1 (0.5–1)	0.5 (0.25–0.5)

Abbreviations: AZM, azithromycin; CLR, clarithromycin; DOX, doxycycline; ERY, erythromycin; JOS, josamycin; LVX, levofloxacin; MIC, minimum inhibitory concentration; MIN, minocycline; MRMP, macrolide-resistant *Mycoplasma pneumoniae*; MSMP, macrolide-susceptible *Mycoplasma pneumoniae*; TFX, tosufloxacin.

As shown in Table 3, defervescence times in patients with MRMP-associated pneumonia were compared for each antimicrobial agent group (macrolides, MIN, DOX, and TFX), excluding patients given steroids. Defervescence and other improvements in clinical findings occurred within 24 hours after initiation of MIN (57.7%) or DOX (81.3%), with poorer results using TFX (30.8%) or macrolides (30.8%) ($P < .05$). Average duration of treatment was 5 days for MIN, 3 days for DOX, 5 days for TFX, and 6 days for macrolides in patients with MRMP pneumonia.

Figure 2 shows *M. pneumoniae* numbers estimated from real-time PCR results at 3 time points—before MIN, DOX, or TFX was administered, after 3 days, and after 5 days—using box-and-whisker plots. These results were calculated based on serial 10-fold dilutions of pMP01 plasmid carrying a 225-base pair target DNA fragment from the 16S ribosomal RNA gene in the M129 standard strain [30]. The lower limit of detection was calculated to be 10 colony-forming units per reaction tube.

Patients plotted in the figure are limited to those in whom *M. pneumoniae* was isolated before any agent was administered. Estimated *M. pneumoniae* amounts after 3 days clearly decreased from 2×10^5 to 2×10^2 in patients receiving MIN (n = 37) and from 10^6 to 5×10^2 in those receiving DOX (n = 6). In contrast, the *M. pneumoniae* amounts were only

Table 2. Characteristics of Japanese Children With *Mycoplasma pneumonia*–Associated Pneumonia From January to December 2011

Variable	Total	Macrolide-Susceptible *M. pneumoniae*, n = 26	Macrolide-Resistant *M. pneumoniae*, n = 176	*P* Value
Sex, male/female	106/96	15/11	91/85	.58
Inpatients/outpatients (%)	136/66	19/7 (73.1)	117/59 (66.5)	.50
Days from onset (range)	6 (2–15)	6 (3–12)	6 (2–15)	.67
Median age, years (range)	8 (1–14)	5 (1–13)	8 (1–14)	
≤1 (%)	6 (3.0)	3 (11.5)	3 (1.7)	.03
2–5	52 (25.7)	12 (46.2)	40 (22.7)	.01
≥6	144 (71.3)	11 (42.3)	133 (75.6)	.001
Initial antibiotics (%)				
Not used	32 (15.8)	12 (46.2)	20 (11.4)	<.001
β-lactams	33 (16.3)	8 (30.8)	25 (14.2)	.07
Macrolides	119 (58.9)	5 (19.2)	114 (64.8)	<.001
Quinolones	8 (4.0)	0	8 (4.5)	.57
Other	10 (5.0)	1 (3.8)	9 (5.1)	.84
Secondary antibiotics (%)	182	23	159	.96
Macrolides	21 (11.5)	4 (17.4)	17 (10.7)	.56
Minocycline or doxycycline	141 (77.5)	16 (69.6)	125 (78.6)	.33
Tosufloxacin or levofloxacin	17 (9.3)	2 (8.7)	15 (9.4)	.79
Other[a]	3 (1.6)	1 (4.3)	2 (1.3)	.83
Hospitalization, days (range)	6 (3–19)	6 (5–13)	6 (3–14)	

[a] Inappropriate antibiotics.

Table 3. Time to Defervescence After Initiation of a Secondary Agent and Duration of Antibiotic Use in Macrolide-Resistant *Mycoplasma pneumoniae*–Associated Pneumonia[a]

Time to Defervescence, Hours	Macrolides[b] (n = 13)	Minocycline (n = 52)	Doxycycline (n = 16)	Tosufloxacin[c] (n = 13)	*P* Value[d]
0–24	4 (30.8)	30 (57.7)	13 (81.3)	4 (30.8)	.03
25–48	2 (15.3)	17 (32.7)	1 (6.3)	5 (38.5)	.13
49–72	3 (23.1)	3 (5.8)	2 (12.5)	3 (23.0)	.98
>72	4 (30.8)	2 (3.8)	0	1 (7.7)	
Duration of administration, days (range)	6 (3–10)	5 (2–7)	3 (3–7)	5 (2–7)	

[a] Patients receiving a steroid together with an antimicrobial agent are not included.

[b] Clarithromycin (n = 8) or azithromycin (n = 5) was used.

[c] Includes 1 patient given levofloxacin.

[d] Minocycline or doxycycline vs tosufloxacin.

slightly reduced by TFX (n = 6), from 10^6 to 2×10^4 after 3 days to 1.5×10^3 after 5 days. The decrease in *M. pneumoniae* was significantly more rapid in patients receiving MIN ($P = .05$) or DOX ($P = .04$) than in those receiving TFX ($P = .16$).

DISCUSSION

Most often *M. pneumoniae* infection in children and young adults is characterized by comparatively mild disease, with high prevalence occurring at approximately 4-year intervals. Since their development, 14-membered and azalide macrolides have been used as first-line antibiotic treatment for this infection. Only a small portion of patients—those with severe or prolonged symptoms, underlying diseases, or mixed infections including other causative organisms—needed hospitalization for treatment.

To our best knowledge, MRMP first was isolated in Japan from patients with pneumonia in 2000 [10]. Continuous Japanese surveillance indicated that cases requiring hospitalization for treatment have gradually increased in parallel with an increase of MRMP in children (Supplementary Figure 1) [33].

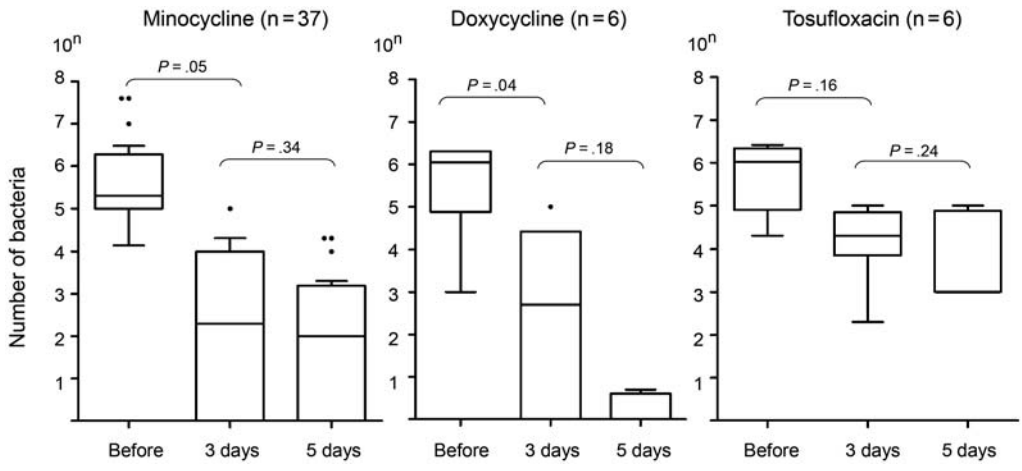

Figure 2. Decreasing numbers of macrolide-resistant *Mycoplasma pneumoniae* (MRMP) estimated from real-time polymerase chain reaction results at 3 points during administration of minocycline (n = 37), doxycycline (n = 6), or tosufloxacin (n = 6). "Before" indicates before receiving antibiotics; "3 days" indicates 3 days after initial administration; "5 days" indicates 5 days after initial administration. Data are displayed as box-and-whisker plots. All bacterial cultures were positive before administration of each antibiotic. Subsequent culture positivity was 57.1% 3 days after minocycline and 31.3% 5 days after minocycline; 16.7% 3 days after doxycycline and 0% 5 days after doxycycline; and 100% 3 days after tosufloxacin and 75% 5 days after tosufloxacin.

Reportedly, MRMP is prevalent in China, not only in children but also in adults [14, 15, 34]; similar problems with treatment have been encountered there. In the United States and the European Union, prevalence of MRMP has remained low, although MRMP may become a treatment issue in the future.

In Japan, prevalence of penicillin-resistant *S. pneumoniae* possessing an *ermB* or *mefA* gene, which confers macrolide resistance, is 80% [35]. Rapidly increasing macrolide resistance in pathogens causing respiratory infections is closely related to the fact that macrolides account for 30% of all prescribed oral antibiotics in Japan.

Since early summer 2011, the National Institute of Infectious Diseases has warned in its Infectious Diseases Weekly Reports that MRMP infection is increasing. Our study group also experienced an increase in patients with *M. pneumoniae* infection whose clinical findings did not improve after 3 days of administration of initial agent; almost all were hospitalized, and medication was changed to MIN, DOX, or TFX.

In the United States and the European Union, use of MIN for *M. pneumoniae* infections is not approved; only DOX is recommended. In Japan, however, only MIN, marketed as granules, is approved by the Ministry of Health, Welfare, and Labor for pediatric *M. pneumoniae* infections, whereas DOX, LVX, and garenoxacin are approved for *M. pneumoniae* infections in adults. When used in children, MIN and DOX rarely may cause side effects, such as yellow staining of developing teeth and dizziness, whereas side effects of new quinolones include arthritic disorders [36, 37]. Because spontaneous healing may occur in *M. pneumoniae* infection, the agents above may be used for patients with *M. pneumoniae* infection only under the condition that maximum clinical benefit is achieved over the shortest possible period to minimize side effects.

We therefore sought to gain an accurate understanding of bacterial decrease in *M. pneumoniae* pneumonia cases after changing antimicrobial agents. As indicated by our results in Table 1, antibacterial activities of MIN and DOX are not much greater than that of TFX at 0.25–1.0 μg/mL. However, within 3 days after these agents were administered to patients with MRMP, bacterial counts decreased 100–1000-fold. This clearly was superior to results after administration of TFX. The advantage of MIN was attributable to a relatively high blood concentration (2.3 μg/mL after giving 4 mg/kg) and to a very long half-life (10 hours) [36]. In contrast, when TFX was administered at 6 mg/kg, the maximum blood concentration was 1.0 μg/mL, and the half-life was 3.8 hours [37]. When MIN or DOX was administered, almost 90% of patients showed defervescence and other improvements in clinical findings within 48 hours. No recurrences were observed after drug administration for an average of 5 days. From this experience, we concluded that 5–7 days of administration were adequate for MRMP infection.

The 2011 MRMP outbreak in Japan was likely fostered by frequent initial prescription of macrolides to school-aged patients with *M. pneumoniae* infection. Macrolide administration did not decrease bacterial numbers in the nasopharynx, and prolonged coughing caused further spread of MRMP. T-helper 1–type cytokines induced when *M. pneumoniae* infection is prolonged strengthen the inflammatory response [38], but rarely the cytokines may exacerbate pneumonia.

Diagnosis of *M. pneumoniae* infection has, for a long time, been serologic; analysis of paired sera may require more than 2 weeks. Prevention of more extensive outbreaks of MRMP infection requires more rapid diagnosis. Immunoglobulin M kits available in the clinic are useful but limited by low specificity [39]. Semi-nested PCR [40], real-time PCR [29], and loop-mediated isothermal amplification [41] are highly sensitive and specific alternatives to serology that permit early diagnosis of *M. pneumoniae* infections. Identification of specimens as *M. pneumoniae*–positive by PCR is an early indication to perform *M. pneumoniae* cultures.

Recently, several real-time PCR methods [21, 23, 42–44] and pyrosequencing assays [45] were developed to directly detect mutations in the 23S ribosomal RNA gene, which is associated with macrolide resistance in *M. pneumoniae*, without waiting for *M. pneumoniae* cultures. Those direct methods are reliable guides for choosing a therapeutic agent.

In some countries, *M. pneumoniae* isolates have been typed molecularly by PCR restriction fragment-length polymorphism of the adhesion P1 gene [46–48] and by multilocus, variable-number, tandem-repeat analysis [49, 50]. Although no clear association was observed between macrolide-resistant isolates and the PI subtypes, more discriminating multilocus, variable-number, tandem-repeat analysis will be necessary for studying MRMP strains in the future.

Steroids were used for 1 or 2 days in 44% of prolonged cases in our patient population. In these patients, lactate dehydrogenase tended to increase, suggesting a need to more clearly define indications for administration of steroids.

We presently await development of an antimicrobial agent that is more effective against MRMP infections in children.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Potential conflicts of interest. H. S. is on the speakers' bureau of Meiji Seika Pharma, Shionogi, and Taisho Toyama Pharmaceutical. S. I. consults

for Pfizer Japan and Quintiles East Asia; has grants with Pfizer Japan, Dainippon Sumitomo Pharma, GlaxoSmithKline, Taisho Toyama Pharmaceutical, Meiji Seika Pharma, Benesis, Shionogi, Daiichi Sankyo, Chugai Pharmaceutical, Toyama Chemical, and Astellas Pharma; is on the speakers' bureau of Meiji Seika Pharama, Pfizer Japan, Dainippon Sumitomo Pharma, GlaxoSmithKline, Taisho Toyama Pharmaceutical, Shionogi, and Daiichi Sankyo; and has received payment for manuscript preparation from GlaxoSmithKline and Meiji Seika Pharma. K. U. has received grants from Becton Dickinson and is on the speakers' bureau of Meiji Seika Pharma. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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