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More Complications Occur in Macrolide-Resistant than in Macrolide-Sensitive *Mycoplasma pneumoniae* Pneumonia

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We sought to understand the situation of macrolide-resistant genotypes of *Mycoplasma pneumoniae*, and analyze the relationship between macrolide-resistant genotypes and clinical manifestations of *Mycoplasma pneumoniae* pneumonia (MPP). Full-length sequencing of the 23S rRNA gene of *M. pneumoniae* was performed in 235 nasopharyngeal aspirates (NPAs) from children with MPP. We also retrospectively compared the clinical characteristics of macrolide-resistant (MR) *M. pneumoniae* infections and macrolide-sensitive (MS) *M. pneumoniae* infections. A total of 206 patients had point mutations in the *M. pneumoniae* 23S rRNA gene, and these patients are referred to as MR patients. The remaining 29 patients without point mutations are referred to as MS patients. Among 206 MR patients, 199 (96.6%) had A2063G mutations, 6 had A2063T mutations, and the remaining patients had an A2064G mutation. Among the clinical manifestations, we found that the median fever durations were 8 days (range, 0 to 42 days) and 6 days (0 to 14 days) ($P < 0.01$), the median hospitalization durations were 8 days (2 to 45 days) and 6 days (3 to 16 days) ($P < 0.01$), and the median fever durations after macrolide therapy were 5 days (0 to 42 days) and 3 days (0 to 10 days) ($P < 0.01$), respectively, in the MR and MS groups. We also found that the incidence of extrapulmonary complications in the MR group was significantly higher than that in the MS group ($P < 0.05$). Moreover, the radiological findings were more serious in the MR group than in the MS group ($P < 0.05$). The increasing prevalence of MR *M. pneumoniae* has become a significant clinical issue in the pediatric patients, which may lead to more extrapulmonary complications and severe clinical features and radiological manifestations.

Mycoplasma pneumoniae is one of the most prevalent pathogens causing community-acquired respiratory tract infections in children and young adults (1, 2). *M. pneumoniae* pneumonia (MPP) is usually a benign self-limited disease. However, sometimes it may cause various extrapulmonary complications and progress to a severe life-threatening pneumonia (3–8). These cases might show clinical and radiological deterioration despite of macrolide antibiotic therapy for 7 days or longer (9).

Severe *M. pneumoniae* infections may be related to the occurrence of macrolide-resistant (MR) *M. pneumoniae*. For children, macrolides are the first-choice agents for *M. pneumoniae* infections. However, in recent years, many isolates of *M. pneumoniae* from clinical samples showed resistance to macrolides with a high prevalence of >90% in China (10). The main mechanism of resistance has been shown to be due to mutations in the domain V of 23S rRNA of *M. pneumoniae* (10–13). The mutations that induce a high-level of macrolide resistance are an A-to-G transition at position 2063 and an A-to-G transition at position 2064, whereas low-level resistance is induced by an A-to-G transition at position 2617 and A-to-T transition at position 2063 (13). Several studies have indicated that macrolide resistance in *M. pneumoniae* may have clinical significance in terms of diminishing response to treatment with drugs (14–16). The presence of MR *M. pneumoniae* has been reported to be mainly associated with the persistent clinical symptoms such as fever, causing prolonged hospital stay and elevated antibiotic change rate; to our knowledge, however, no increase in the incidence of complications has been reported.

In the present study, we characterized macrolide resistance directly using *M. pneumoniae*-positive nasopharyngeal aspirates (NPAs) taken from hospitalized children with MPP in our hospital by analyzing the DNA sequence of domain V of 23S rRNA. Furthermore, we retrospectively compared the different clinical characteristics of MR *M. pneumoniae* infections and MS *M. pneumoniae* infections, including symptoms, extrapulmonary complications, and radiological findings.

MATERIALS AND METHODS

Study population and sample collection. A total of 235 NPAs were collected from children with MPP hospitalized in Children's Hospital, Zhejiang University School of Medicine, Hangzhou, China, from 1 April 2009 to 31 March 2010. All patients had signs and symptoms indicative of pneumonia on admission, including fever, cough, abnormal lung auscultation, and a new infiltrate on chest radiograph (17). The *M. pneumoniae* infection was confirmed by serologic testing (detecting *M. pneumoniae* IgM by enzyme-linked immunosorbent assay) and/or *M. pneumoniae* PCR tests of the NPA. All 235 children had positive results for the above-mentioned *M. pneumoniae* tests. Furthermore, other microbiologic tests were performed to exclude other respiratory tract infections and tuberculosis, including the protein purified derivative test, blood cultures, nasopharyngeal aspirate/swab for common respiratory tract virus antigens (respiratory syncytial virus, influenza virus, adenovirus, and parainfluenza virus), and serology for *Chlamydia pneumoniae* and *Legionella pneumophila*. No other pathogens were detected by these tests.

NPAs were obtained from the patients on admission. An aliquot of DNA was extracted from NPAs and stored at −80°C for determination.

Received 22 August 2013 Returned for modification 19 October 2013
Accepted 21 November 2013
Published ahead of print 25 November 2013
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doi:10.1128/AAC.01806-13

DNA was extracted using the TIANcombi DNA Lyse&Amp PCR kit (Tiangen, Hangzhou, China) in accordance with the manufacturer's instructions.

Amplification of MR genes. A PCR assay, followed by direct amplicon sequencing, was developed to detect point mutations conferring resistance to macrolides in the *M. pneumoniae* 23S rRNA gene. Domain V of the 23S rRNA gene was amplified using the primers 5′-CCTAGTCGGGT AAATTCCGT-3′ and 5′-CCTAGTCGGGTAAATTCCGT-3′ (TaKaRa, Shanghai, China). The primers were used to specifically amplify a 244-bp region of *M. pneumoniae*, including positions 2063 and 2064.

The PCR was carried out in a volume of 50 μl containing 1 μl of total DNA, 5 μl of 10× buffer, 3 μl of 10 mM deoxynucleoside triphosphate, 1 μl of 10 μM concentration of each primer, 38.7 μl of double-distilled H_2O, and 0.3 μl of *Taq* DNA polymerase (Invitrogen, Milan, Italy). The reaction mixture then underwent denaturation for 5 min at 94°C and 35 PCR cycles, each consisting of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C, followed by a final extension step of 5 min at 72°C. The amplified products were analyzed by electrophoresis on a 2% agarose gel (Invitrogen) and visualized by using a Bio-Rad gel-imaging analysis system.

DNA sequencing. All PCR products of 244 bp were sequenced (Yingweijie Co., Ltd., Shanghai, China), and the DNA sequences were compared to the sequence of an *M. pneumoniae* reference strain M129 (GenBank accession no. X68422) by using BLAST. *M. pneumoniae* strain M129 (American Type Culture Collection) served as a positive control.

Evaluation of clinical characteristics. Clinical information was retrospectively collected from the medical records of the patients. Complete information about the antimicrobial agents prescribed, the clinical symptoms, and the radiologic findings was reviewed. The patient demographics, clinical symptoms, the incidence of extrapulmonary complications, and the radiological findings were compared between MR MPP patients and MS MPP patients when discharged.

In our study, all patients were treated with 10 mg of azithromycin/kg/day for the first 3 days, and then treatment ceased for 4 days; this was followed by another 3-day treatment of azithromycin or by treatment with 15 to 30 mg of erythromycin/kg/day for 7 to 14 days. Temperature and respiratory signs and symptoms were examined at study entry and every 8 h thereafter. A febrile day was defined as a day during which the body temperature exceeded 38.0°C at least once (16). Total febrile days, febrile days after macrolide treatment, and hospitalized days were assessed. All patients underwent chest X-ray examination upon admission, and if the patient had large pulmonary lesions evident in the chest radiograph, a second chest X-ray examination was performed about 7 days after admission. A large lesion was defined when the extent of infiltration on chest radiography was more than one-third of the lung (18). The severity of pneumonia was evaluated according to the diagnostic standard for pneumonia advocated by British Thoracic Society (19). During the hospitalization, we also evaluated the extrapulmonary complications of patients. Liver function abnormalities were defined as at least 2-fold increase in glutamic pyruvic transaminase value. Myocarditis was defined as patients with cardiovascular signs and symptoms (such as chest distress, weakness, palpitation, and pale complexion) with an elevated myocardial enzymogram (troponin or creatine kinase cardiac isoenzymes) or abnormal electrocardiographic findings (e.g., diffuse T wave inversions or ST segment elevation). Encephalitis was diagnosed when patients had various neurological signs and symptoms (such as convulsions or paresis) with cerebrospinal fluid positive for *M. pneumoniae* nucleic acid as determined by PCR and any other common pathogens had been excluded (3). Proteinuria was defined as a urine protein/creatinine ratio of >0.2 (20). Hemolytic anemia was defined as patients with hemolytic signs and symptoms with a positive direct Coombs test in the presence of cold agglutinins (20). Arthritis was diagnosed based on clinical symptoms (joint pain and/or swelling). In some cases, we found erythematous rash scattering in the body skin.

Ethics. This study was approved by the ethics committee of the Children's Hospital, Zhejiang University School of Medicine. Written informed consent was obtained from at least one guardian of each patient before enrollment.

Statistical analysis. Statistical analyses were performed using SPSS software (v15.0). Skewed distribution data were expressed as median values (with a range from minimum to maximum). The comparisons were made by using the Mann-Whitney U test. Chi-squared tests were used to compare categorical data. Statistical significance was defined as $P < 0.05$.

RESULTS

Clinical characteristics. Upon admission, all patients had symptoms and signs indicative of pneumonia, including fever (>38°C per axilla), cough, and abnormal breath sounds on auscultation. No patients were transferred to the intensive care unit or received mechanical ventilation during hospitalization. All patients were PCR positive, and for 199 patients there were positive serological results. The median age of the 235 children was 4 years (range, 0 to 14 years). A total of 149 patients were males.

Analysis of MR genotypes. The 244-bp bands were detected in all samples. Of the 235 patients with MPP, 206 (87.7%) showing point mutations in domain V of 23S rRNA were defined as MR patients. The remaining 29 patients (12.3%), who showed no point mutations, were defined as MS patients. Of the MR patients, 199 (96.6%) were positive for an A-to-G transition mutation at position 2063 in domain V of 23S rRNA gene (A2063G), 6 showed an A-to-T transition mutation at position 2063 (A2063T), and the remaining one had an A-to-G transition mutation at position 2064 (A2064G). In general, the mutation rate at position 2063 reached 99.5% (205/206).

Comparison of clinical characteristics between MR and MS patients. Patients were categorized in one of two groups, MR or MS, on the basis of presence or absence of 23S rRNA gene mutations. A total of 206 patients were in the MR group (132 males, 74 females), with a median age of 4 years (range, 0 to 14 years). Another 29 patients were in the MS group (17 males, 12 females), with a median age of 5 years (range, 0 to 11 years). There were no significant differences between the two groups with respect to age and sex distribution (Table 1).

Clinical characteristics in the MR and MS patients, including incidence of complications, radiologic findings, and clinical course, are summarized in Table 1. Of the 206 MR patients, extrapulmonary complications were found in 61 cases (29.6%), including liver function abnormalities in 27, myocarditis in 15, rash in 11, encephalitis in 4, proteinuria in 2, hemolytic anemia in 1, and arthritis in 1. In the MS group, only 3 patients (10.3%) who had liver function abnormalities had extrapulmonary complications. There was a significant difference in the incidence of extrapulmonary complications between the two groups ($P < 0.05$). We also evaluated the severity of MPP in all patients. The incidences of severe MPP were 18.4% in the MR group and 3.4% in the MS group, with a significant difference ($P < 0.05$). In addition to clinical symptoms, the radiological findings were more severe in the MR group than in the MS group, with 61.7% of the patients in the MR group showing large lesions versus 41.4% in the MS group ($P < 0.05$).

Concerning clinical course, we found that the median durations of fever were 8 days (0 to 42 days) in the MR group and 6 days (0 to 14 days) in the MS group ($P < 0.01$). The median hospitalization durations were 8 days (2 to 45 days) and 6 days (3 to 16 days) ($P < 0.01$), respectively. The median fever duration after

TABLE 1 Clinical information of MR and MS patients

Clinical information	MR group ($n = 206$)	MS group ($n = 29$)	P
Median age in yrs (range)	4 (0–14)	5 (0–11)	NS[b]
No. of patients (male/female)	132/74	17/12	NS
% Patients with severe MPP	18.4 (38/206)	3.4 (1/29)	0.042
% Patients with large lesions on chest radiography[a]	61.7 (127/206)	41.3 (12/29)	0.038
Median durations in days (range)			
Fever duration	8 (0–42)	6 (0–14)	0.001
Hospitalization duration	8 (2–45)	6 (3–16)	0.007
Fever duration after macrolide therapy	5 (0–42)	3 (0–10)	0.007
% Patients presenting with extrapulmonary complications	29.6 (61/206)	10.3 (3/29)	0.029
Digestive system (liver function abnormalities)	44.3 (27/61)	100 (3/3)	
Cardiovascular system (myocarditis)	24.6 (15/61)	0 (0/3)	
Rash	18.0 (11/61)	0 (0/3)	
Nervous system (encephalitis)	6.6 (4/61)	0 (0/3)	
Urinary system (proteinuria)	3.3 (2/61)	0 (0/3)	
Hematologic system (hemolytic anemia)	1.6 (1/61)	0 (0/3)	
Joint system (arthritis)	1.6 (1/61)	0 (0/3)	

[a] A large lesion was determined when the extent ofinfiltration on chest radiography was more than one-third of the lung.
[b] NS, not significant.

macrolide therapy was significantly longer in the MR patients than in the MS patients (median of 5 days versus 3 days, $P < 0.01$).

DISCUSSION

Although *M. pneumoniae* infection was traditionally thought to be a self-limited process, more and more severe cases even fatal cases of *M. pneumoniae* infections were reported in recent years (3–8). The reasons why more severe *M. pneumoniae* infections occurred remain unclear, but resistance to macrolides does have a close association with the emergence and increase of MR strains. Since 2000, the emergence of macrolide resistance has been reported mainly in Asia (21). In China, prevalence of MR *M. pneumoniae* isolated in pediatric cases has increased rapidly. In 2009, Xin et al. (22) reported that 46/50 (92%) *M. pneumoniae* isolates from pediatric patients were resistant to macrolides. In 2010, Liu et al. (10) reported that 90/100 (90%) *M. pneumoniae* isolates were resistant to macrolides. In our study, we found that 206 (87.7%) NPAs from patients with MPP showed point mutations in domain V of 23S rRNA, which were considered MR. The high prevalence of MR was similar to the data reported previously.

MR genotypes are defined by specific point mutations in the V domain of the *M. pneumoniae* 23S rRNA gene. Several potential transition mutations in the complete sequence of the 23S rRNA were found: A2063G, A2064G, and A2063T. A2063G and A2064G mutations are responsible for the high-level macrolide resistance in *M. pneumoniae* (23). Morozumi et al. (24) found that among 380 *M. pneumoniae* isolates from 3,678 pediatric patients with community-acquired pneumonia, 50 MR strains had an A2063G transition in domain V of the 23S rRNA, whereas 5 had an A2064G transition. In our study, we amplified the full length of the 23S rRNA fragment from 235 NPAs of patients with MPP and compared it to the sequence of M129. We found that among the 206 MR patients 199 (96.6%) possessed an A2063G transition in domain V of the 23SrRNA, 6 cases showed A2063T mutation, and 1 showed an A2064G mutation. Furthermore, the point mutations at position 2063 in domain V of the 23S rRNA gene accounted for 99.5% (205 cases) of all mutations, which was consistent with that reported in the literature (25, 26). The other less frequent point mutation, at position 2617, was also detected, but no positive results were found (data not shown).

As for the current situation of macrolide resistance of *M. pneumoniae*, it is important to evaluate the clinical significance of MR *M. pneumoniae*. Suzuki et al. (16) found the total febrile days and the numbers of febrile days during macrolide administration to be longer in MR patients than in MS patients. Ensuing clinical studies were reported with similar results. In our study, MR *M. pneumoniae*-infected patients showed much a longer median duration of fever, a longer median hospitalization duration, and a longer median fever duration after macrolide therapy than were observed for patients with MS *M. pneumoniae*. These results implied that MR *M. pneumoniae* was refractory to treatment and could result in a prolonged clinical course.

More interestingly, we found that the incidence of extrapulmonary complications in the MR patients was higher than in the MS patients. A total of 29.6% (61/206) of the MR patients had extrapulmonary complications versus 10.3% in the MS group. Moreover, only one patient (3.4%) demonstrated severe pneumonia in the MS group, and 38 of 206 MR patients (18.4%) were diagnosed as severe having MPP. In addition to the clinical symptoms, 61.7% of patients in the MR group had large lesions observed radiologically versus 41.4% in the MS group. To our knowledge, this is the first report showing severe clinical course and more extrapulmonary complications in the MR patients.

Several reasons may explain the difference between previous reports and the findings of our study. First, the children with MPP enrolled in our study reflected a natural clinical course. Because of potential side effects, fluoroquinolones are contraindicated in all children, and tetracyclines, such as doxycycline and minocycline, can only be used in children ≥8 years old; there are almost no alternatives for treating MR *M. pneumoniae* infection in children, especially pre-school-aged children. Therefore, all of the patients in our study received only macrolides. Local *M. pneumoniae* loads at 48 h after macrolide therapy in the MR patients were reported to be much higher than those in the MS patients (14). The higher and more persistent *M. pneumoniae* stimulation may induce a much stronger host response, especially interleukin-8 (IL-8)- and IL-18-

associated inflammation, which is related to the severity of MPP in children (27). In previous studies, the initial macrolide treatment was usually switched to other antibiotics such as levofloxacin, ciprofloxacin, minocycline, or doxycycline within 2 to 3 days after macrolide medication, with an antibiotic change rate of 63.6 to 85.7% (14, 16, 28). Secondarily, our hospital is a tertiary hospital, and the enrolled patients had a much longer period prior to hospitalization at our hospital than in previous studies. Again, the persistent *M. pneumoniae* antigen stimulation and/or invasion greatly increased the possibilities of severe lung lesions and pulmonary and extrapulmonary complications. Third, our study had the largest numbers of children with MPP, and we focused on the incidence of extrapulmonary complications. Until now, no other study focusing on the real state of extrapulmonary complications in the MR children has been reported. Our study was the first to demonstrate a higher incidence of extrapulmonary complications in MR patients compared to MS patients.

One limitation of our study is that the MIC values were not measured, because *M. pneumoniae* was not isolated from all these patients. However, it is widely acknowledged that the presence of mutations at positions 2063 and 2064 is usually associated with macrolide resistance.

In conclusion, we amplified and analyzed the full-length sequence of the 23S rRNA gene from 235 NPAs and compared it to that of the *M. pneumoniae* reference strain M129. We have shown that the increasing prevalence of MR *M. pneumoniae* has become a significant clinical issue in pediatric patients, and it may lead to more extrapulmonary complications, severe clinical features, and radiological manifestations. The impact of macrolide resistance on clinical outcome for children with MPP needs to be further investigated.

ACKNOWLEDGMENTS

We are grateful to our colleagues in the Department of Pulmonology for collecting nasopharyngeal aspirates from patients.

This study was partially supported by the National Natural Science Foundation (grants 81070004 and 81000765), the Zhejiang Province Natural Science Fundation of China (LQ13H100002), and the Zhejiang Medicine and Health Research Fund (grant 201340691) and by the scientific research fund of the Zhejiang provincial education department (Y201330161).

REFERENCES

1. **Michelow IC, Olsen K, Lozano J, Rollins NK, Duffy LB, Ziegler T, Kauppila J, Leinonen M, McCracken GH, Jr.** 2004. Epidemiology and clinical characteristics of community-acquired pneumonia in hospitalized children. Pediatrics **113:**701–707. http://dx.doi.org/10.1542/peds.113.4.701.
2. **Defilippi A, Silvestri M, Tacchella A, Giacchino R, Melioli G, Di Marco E, Cirillo D, Di Pietro P, Rossi GA.** 2008. Epidemiology and clinical features of *Mycoplasma pneumoniae* infection in children. Resp. Med. **102:** 1762–1768. http://dx.doi.org/10.1016/j.rmed.2008.06.022.
3. **Socan M, Ravnik I, Bencina D, Dovc P, Zakotnik B, Jazbec J.** 2001. Neurological symptoms in patients whose cerebrospinal fluid is culture- and/or polymerase chain reaction-positive for *Mycoplasma pneumoniae*. Clin. Infect. Dis. 15;. **32:**E31–E35. http://dx.doi.org/10.1086/318446.
4. **Azumagawa K, Kambara Y, Murata T, Tamai H.** 2008. Four cases of arthritis associated with *Mycoplasma pneumoniae* infection. Pediatr. Int. **50:**511–513. http://dx.doi.org/10.1111/j.1442-200X.2008.02622.x.
5. **Hwkins S, Rausch CM, McCanta AC.** 2011. Constrictive pericarditis secondary to infection with *Mycoplasma pneumoniae*. Curr. Opin. Pediatr. **23:**126–129. http://dx.doi.org/10.1097/MOP.0b013e328341579c.
6. **Metz G, Kraft M.** 2010. Effects of atypical infections with *Mycoplasma* and *Chlamydia* on asthma. Immunol. Allergy Clin. N. Am. **30:**575–585. http://dx.doi.org/10.1016/j.iac.2010.08.003.
7. **Radisic M, Torn A, Gutierrez P, Defranchi HA, Pardo P.** 2000. Severe acute lung injury caused by *Mycoplasma pneumoniae*: potential role for steroid pulses in treatment. Clin. Infect. Dis. **31:**1507–1511. http://dx.doi.org/10.1086/317498.
8. **Wang RS, Wang SY, Hsieh KS, Chiou YH, Huang IF, Cheng MF, Chiou CC.** 2004. Necrotizing pneumonitis caused by *Mycoplasma pneumoniae* in pediatric patients: report of five cases and review of literature. Pediatr. Infect. Dis. J. **23:**564–567. http://dx.doi.org/10.1097/01.inf.0000130074.56368.4b.
9. **Tamura A, Matsubara K, Tanaka T, Nigami H, Yura K, Fukaya T.** 2008. Methylprednisolone pulse therapy for refractory *Mycoplasma pneumoniae* pneumonia in children. J. Infect. **57:**223–228. http://dx.doi.org/10.1016/j.jinf.2008.06.012.
10. **Liu Y, Ye X, Zhang H, Xu X, Li W, Zhu D, Wang M.** 2010. Characterization of macrolide resistance in *Mycoplasma pneumoniae* isolated from children in Shanghai, China. Diagn. Microbiol. Infect. Dis. **67:**355–358. http://dx.doi.org/10.1016/j.diagmicrobio.2010.03.004.
11. **Dumke R, von Baum H, Lück PC, Jacobs E.** 2010. Occurrence of macrolide-resistant *Mycoplasma pneumoniae* strains in Germany. Clin. Microbiol. Infect. **16:**613–616. http://dx.doi.org/10.1111/j.1469-0691.2009.02968.x.
12. **Wolff BJ, Thacker WL, Schwartz SB, Winchell JM.** 2008. Detection of macrolide resistance in *Mycoplasma pneumoniae* by real-time PCR and high-resolution melt analysis. Antimicrob. Agents Chemother. **52:**3542–3549. http://dx.doi.org/10.1128/AAC.00582-08.
13. **Morozumi M, Hasegawa K, Kobayashi R, Inoue N, Iwata S, Kuroki H, Kawamura N, Nakayama E, Tajima T, Shimizu K, Ubukata K.** 2005. Emergence of macrolide-resistant *Mycoplasma pneumoniae* with a 23S rRNA gene mutation. Antimicrob. Agents Chemother. **49:**2302–2306. http://dx.doi.org/10.1128/AAC.49.6.2302-2306.2005.
14. **Kawai Y, Miyashita N, Yamaguchi T, Saitoh A, Kondoh E, Fujimoto H, Teranishi H, Inoue M, Wakabayashi T, Akaike H, Ogita S, Kawasaki K, Terada K, Kishi F, Ouchi K.** 2012. Clinical efficacy of macrolide antibiotics against genetically determined macrolide-resistant *Mycoplasma pneumoniae* pneumonia in pediatric patients. Respirology **17:**354–362. http://dx.doi.org/10.1111/j.1440-1843.2011.02102.x.
15. **Matsubara K, Morozumi M, Okada T, Matsushima T, Komiyama O, Shoji M, Ebihara T, Ubukata K, Sato Y, Akita H, Sunakawa K, Iwata S.** 2009. A comparative clinical study of macrolide-sensitive and macrolide resistant *Mycoplasma pneumoniae* infections in pediatric patients. J. Infect. Chemother. **15:**380–383. http://dx.doi.org/10.1007/s10156-009-0715-7.
16. **Suzuki S, Yamazaki T, Narita M, Okazaki N, Suzuki I, Andoh T, Matsuoka M, Kenri T, Arakawa Y, Sasaki T.** 2006. Clinical evaluation of macrolide-resistant *Mycoplasma pneumoniae*. Antimicrob. Agents Chemother. **50:**709–712. http://dx.doi.org/10.1128/AAC.50.2.709-712.2006.
17. **Nelson S, Belknap SM, Carlson RW, Dale D, Deboisblanc B, Farkas S, Fotheringham N, Ho H, Marrie T, Movahhed H, Root R, Wilson J, CAP Study Group.** 1998. A randomized controlled trial of filgrastim as an adjunct to antibiotics for treatment of hospitalized patients with community-acquired pneumonia. J. Infect. Dis. **178:**1075–1080. http://dx.doi.org/10.1086/515694.
18. **Suzuko U, Keisuke S, Hiroyuki E, Kazunobu O, Kenji O, Tomomichi K, Hiroshi S, Hiroyuki T, Tsunekazu H, Toshihiro M, Tsutomu Y.** 2011. Japanese guidelines for the management of respiratory infectious diseases in children 2007 with focus on pneumonia. Pediatr. Int. **53:**264–276. http://dx.doi.org/10.1111/j.1442-200X.2010.03316.x.
19. **British Thoracic Society of Standards of Care Committee.** 2002. BTS guidelines for the community acquired pneumonia in childhood. Thorax **57**(Suppl):S1–S34.
20. **Hu YM, Jiang ZF.** 2002. Zhu futang textbook of pediatrics, 8th ed. People's Medical Publishing House, Beijing, China.
21. **Okazaki N, Narita M, Yamada S, Izumikawa K, Umetsu M, Kenri T, Sasaki Y, Arakawa Y, Sasaki T.** 2001. Characteristics of macrolide-resistant *Mycoplasma pneumoniae* strains isolated from patients and induced with erythromycin in vitro. Microbiol. Immunol. **45:**617–620. http://dx.doi.org/10.1111/j.1348-0421.2001.tb01293.x.
22. **Xin D, Mi Z, Han X, Qin L, Li J, Wei T, Chen X, Ma S, Hou A, Li G, Shi D.** 2009. Molecular mechanisms of macrolide resistance in clinical isolates of *Mycoplasma pneumoniae* from China. Antimicrob. Agents Chemother. **53:**2158–2159. http://dx.doi.org/10.1128/AAC.01563-08.
23. **Cao B, Zhao CJ, Yin YD, Zhao F, Song SF, Bai L, Zhang JZ, Liu YM, Zhang YY, Wang H, Wang C.** 2010. High prevalence of macrolide resistance in *Mycoplasma pneumoniae* isolates from adult and adolescent pa-

tients with respiratory tract infection in China. Clin. Infect. Dis. **51:**189–194. http://dx.doi.org/10.1086/653535.

24. **Morozumi M, Iwata S, Hasegawa K, Chiba N, Takayanagi R, Matsubara K, Nakayama E, Sunakawa K, Ubukata K.** 2008. Increased macrolide resistance of *Mycoplasma pneumoniae* in pediatric patients with community-acquired pneumonia. Antimicrob. Agents Chemother. **52:**348–350. http://dx.doi.org/10.1128/AAC.00779-07.
25. **Zhao F, Lv M, Tao X, Huang H, Zhang B, Zhang Z, Zhang J.** 2012. Antibiotic sensitivity of 40 *Mycoplasma pneumoniae* isolates and molecular analysis of macrolide-resistant isolates from Beijing, China. Antimicrob. Agents Chemother. **56:**1108–1109. http://dx.doi.org/10.1128/AAC.05627-11.
26. **Matsuoka M, Narita M, Okazaki N, Ohya H, Yamazaki T, Ouchi K, Suzuki I, Andoh T, Kenri T, Sasaki Y, Horino A, Shintani M, Arakawa Y, Sasaki T.** 2004. Characterization and molecular analysis of macrolide-resistant *Mycoplasma pneumoniae* clinical isolates obtained in Japan. Antimicrob. Agents Chemother. **48:**4624–4630. http://dx.doi.org/10.1128/AAC.48.12.4624-4630.2004.
27. **Narita M, Tanaka H.** 2007. Cytokines involved in the severe manifestations of pulmonary diseases caused by *Mycoplasma pneumoniae*. Pediatr. Pulmonol. **42:**397. http://dx.doi.org/10.1002/ppul.20445.
28. **Okada T, Morozumi M, Tajima T, Hasegawa M, Sakata H, Ohnari S, Chiba N, Iwata S, Ubukata K.** 2012. Rapid effectiveness of minocycline or doxycycline against macrolide-resistant *Mycoplasma pneumoniae* infection in a 2011 outbreak among Japanese children. Clin. Infect. Dis. **55:**1642–1649. http://dx.doi.org/10.1093/cid/cis784.