



Clinical Evaluation of Macrolide-resistant *Mycoplasma pneumoniae* Infections in Pediatric Japanese Patients

**Yuichirou Tsuji^{1*}, Chitose Karasawa¹, Masami Kurokawa²
and Hiroshi Takahashi²**

¹*Department of Pediatrics, Tokyo Takanawa Hospital, Japan.*

²*Department of Clinical Laboratory, Tokyo Takanawa Hospital, Japan.*

Authors' contributions

This work was carried out in collaboration between all authors. Author YT designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author CK managed the analyses of the study. Authors MK and HT managed the laboratory findings. All authors read and approved the final manuscript.

Original Research Article

Received 18th April 2014
Accepted 19th May 2014
Published 4th June 2014

ABSTRACT

Background: In recent years, an increase in *Mycoplasma pneumoniae* infections among children has become a social issue in Japan.

Methods: During a 2-year collection period (2011-2012), we evaluated trends during the first and second halves of both years. Only patients with positive rapid antigen detection test results (ImmunoCard *Mycoplasma*: Meridian Bioscience, Inc Cincinnati, OH, USA) were included. The evaluation items included the patient number, sex, radiography findings, white blood cell (WBC) count, C-reactive protein (CRP) level, and clinical macrolide-resistance rate.

Results: The patient number increased significantly during the latter halves of the years. The macrolide-resistance rate also increased during the same periods. A similar trend was observed with respect to radiography findings but not with respect to the WBC count and CRP level.

Conclusions: It is important to monitor the macrolide-resistance trends in case of *Mycoplasma pneumoniae* infections in children.

*Corresponding author: Email: yuitsuji@ybb.ne.jp;

Keywords: Macrolide-resistant; pediatric; *Mycoplasma pneumoniae*.

1. INTRODUCTION

Mycoplasma pneumoniae is the main causative pathogen of respiratory tract infections in children. Generally, macrolides are the first treatment agents used against *M. pneumoniae* infections in children. In recent years, however, macrolide-resistant *M. pneumoniae* has been reported to comprise more than 40% of all *M. pneumoniae* isolates from *M. pneumoniae* infections in Japanese children. This trend has been reported not only in Japan but also in other countries. Therefore, this increased incidence of macrolide-resistant *M. pneumoniae* infections required attention with respect to the treatment of mycoplasmal infections in children.

2. MATERIALS AND METHODS

We enrolled all pediatric patients with acute respiratory lower tract infections who visited our pediatric section between January 2011 and December 2012. The patient characteristics are listed in Table 1.

Table 1. Patient characteristics

	I	II	III	IV	P-value
n	26	70	26	67	
Age	6.2±3.8	8.1±3.7	8.4±3.2	7.8±3.6	0.103
Male(%)	13(50%)	35(50%)	10(38.5%)	29(43.3%)	
Female	13	35	16	38	

Statistical analysis: one-way factorial ANOVA. Age values are shown as means±standard deviations

We evaluated the blood test results and chest radiography findings for suspected *M. pneumoniae* infections.

Mycoplasma infections diagnoses were based on chest radiography findings, blood laboratory findings, ImmunoCard *Mycoplasma* test results (Meridian Bioscience, Inc., Cincinnati, OH, USA), and clinical symptoms. The ImmunoCard *Mycoplasma* test was performed according to the manufacturer's instructions. Patients with negative ImmunoCard *Mycoplasma* test results were excluded from the study. Findings suggestive of pneumonia that were visible on radiographs were classified into 3 grades as follows: 1, mild; 2, moderate; and 3, heavy and/or atelectasis. The white blood cell (WBC) counts and C-reactive protein (CRP) levels were estimated using the blood laboratory findings.

Determinations of macrolide-resistant *M. pneumoniae* infections were made for cases in which no improvements with respect to clinical efficacy were observed during a 5-day period and/or the chest radiography findings indicated aggravation in response to macrolides therapy. Clarithromycin was the first-line macrolide therapy. Erythromycin, rokitamycin, or azithromycin was prescribed only when internal clarithromycin administration proved difficult. If the patient was determined to harbor a macrolide-resistant *M. pneumoniae* infection, we switched from macrolide therapy to minocycline (MINO) or tosufloxacin (TFLX). We prescribed MINO to patients who were at least 8 years of age and TFLX to patients who were younger than 8 years of age to avoid tooth discoloration.

Concomitant infections with other bacteria were possible in patients with *Mycoplasma pneumoniae* infections. We excluded patients with abnormally WBC counts and CRP levels. We also excluded patients who had been treated with MINO or TFLX prior to macrolide therapy.

The study period was divided into 4 parts: I, the first half of the year of 2011; II, the latter half of 2011; III, the first half of 2012; and IV, the latter half of 2012.

This single-institution study was conducted at the Department of Pediatrics, Tokyo Takanawa Hospital.

Consent was obtained from the parents' parents, who were provided with information about our treatment policy.

Statistical analyses were performed using StatView 4.0 software (SAS Institute, Inc., Cary, NC, USA).

3. RESULTS

This clinical study included 189 patients. The patients' ages and genders are shown in Table 1. A statistically significant difference was observed with respect to the average age but not the sex.

The antimicrobial agents used in the study are listed in Table 2. The number of cases that required MINO or TFLX increased during the latter halves of the study years.

The WBC counts, CRP levels, and radiography grades are listed in Table 3. During the 4 study periods, a statistically significant difference was observed with respect to the radiography grades but not the WBC counts and CRP levels.

The number of macrolide-resistant cases and the resistance rate are shown in Table 4. The clinical macrolide-resistance rate increased by more than 70% during the latter halves of the 2 study years.

Table 2. Antimicrobial agent usage

	I	II	III	IV
CAM	21	14	10	23
AZM	2	4	1	2
RKM	1	4	0	0
EM	0	3	2	3
MINO	3	49	10	26
TFLX	3	22	7	30

CAM, clarithromycin; AZM, azithromycin; RKM, rokitamycin; EM, erythromycin; MINO, minocyclin; TFLX, tosufloxacin

Table 3. White blood cell (WBC) counts, C-reactive protein (CRP) levels, and radiography grades

	I	II	III	IV	P-value
n	26	70	26	67	
WBC	7591±3337	7379±2995	7980±1975	7283±2677	0.694
CRP	0.87±1.42	1.06±1.23	1.06±1.47	0.92±1.17	0.837
Radiography grade	1.50±0.65	2.00±0.82	1.73±0.78	1.84±0.75	0.027

Values are shown as means±standard deviations. Statistical analysis: one-way factorial ANOVA. WBC, white blood cell count; CRP, C-reactive protein

Table 4. Number of macrolide-resistant infections and the resistance rate

	I	II	III	IV
n	24	25	13	29
Macrolide-resistant cases	4	20	3	21
Resistance rate	16.7%	80.0%	23.1%	72.4%

4. DISCUSSION

Recently in Japan, the incidence of macrolide-resistant *M. pneumoniae* infections has increased in pediatric patients [1,2]. Morozumi et al. reported that the number of macrolide-resistant strains increased rapidly from April 2002 to December 2006 [3]. Furthermore, the incidence rates of macrolide-resistant *M. pneumoniae* infections also increased in other countries [4-7].

Until 2010, we generally prescribed macrolides to the pediatric patients with *M. pneumoniae* infections, and most patients were successfully cured without switching from macrolide therapy. However, during the latter period of 2011, many cases required switching from macrolides to MINO or TFLX because of persistent fevers and coughing or abnormalities on the chest radiograph.

Matsubara et al. reported that the clinical efficacy of macrolides for the treatment of macrolide-resistant *M. pneumoniae* was significantly lower than the clinical efficacy for cases of macrolide-sensitive *M. pneumoniae* [8].

In the present study, however, more than 70% of the patients during the latter halves of the 2 study years required switching to MINO or TFLX.

The administration of MINO to children less than 8 years of age is inappropriate because of the risk of tooth discoloration; therefore, MINO was only prescribed to patients who were at least 8 years of age. Accordingly, we prescribed TFLX to patients with clinical macrolide-resistant *M. pneumoniae* infections who were aged less than 8 years. MINO and TFLX exhibited good antimycoplasmal activities against the clinical macrolide-resistant *M. pneumoniae* infections in our study.

Regarding problems associated with this study, it is initially difficult to diagnose macrolide-resistant *M. pneumoniae* infections. Additionally, there were no apparent differences in the clinical symptoms, laboratory data, and radiography findings between the cases with macrolide-sensitive and resistant *M. pneumoniae* infections. Therefore, we performed a

second evaluation of the clinical macrolide efficacy after a 3-day interval and consequently determined whether the patient required a switch from macrolide therapy. However, changes with respect to macrolide therapy for children with clinical macrolide-resistant *M. pneumoniae* infections are controversial.

M. pneumoniae infections are difficult to diagnose because there are no specific early-stage clinical, epidemiological, or laboratory observations. Similarly, the radiographic findings are not specific. Therefore, our diagnoses of *M. pneumoniae* infections were based on results obtained using the Meridian ImmunoCard *Mycoplasma* test, a card-based enzyme-linked immunosorbent assay designed to detect IgM antibodies against *M. pneumoniae*. Matas et al. reported that the Meridian ImmunoCard *Mycoplasma* test appeared to be a good screening assay for *M. pneumoniae* IgM titers in children [9]. However, this test cannot detect IgM antibodies at early stages and yields positive results for a long period after an infection has been resolved. Jacobs reported that IgM titers could be detected beginning at 7 days after the onset of symptoms in patients with primary *M. pneumoniae* infections [10].

Given the above information, we generally diagnosed *M. pneumoniae* infections based on the clinical symptoms, blood test results, radiography findings, and Meridian ImmunoCard *Mycoplasma* test results. Our diagnostic criteria for *M. pneumoniae* infection included prolonged coughing, abnormalities on the radiograph, and positive Meridian ImmunoCard *Mycoplasma* test results. In our study, the radiography findings were statistically significant with respect to the incidence of macrolide-resistant *M. pneumoniae* infections.

M. pneumoniae infections generally occur throughout the year. However, the results of our study demonstrated that the infection incidence increased during the latter halves of both years. The reason for this phenomenon is unclear, however, the number of patients increased along with the increased incidence of macrolide-resistant *M. pneumoniae* infections. Therefore, an etiological explanation for the increased incidence of macrolide-resistant *M. pneumoniae* infections during the latter half of the year needs to be identified.

Specific point mutations were reported in all macrolide-resistant *M. pneumoniae* strains [3,11]. Morozumi et al. reported that these macrolide-resistant strains carried either an A2063G or an A2064G transition in domain V of the 23S rRNA gene and that the prevalence of these resistant strains had increased rapidly in Japan [3]. Akaike et al. reported that the macrolide-resistant strains exhibited high resistance to erythromycin, clarithromycin, and azithromycin. Conversely, TFLX exhibited potent antimycoplasmal activity [11]. In our study, the clinical efficacies of the antimicrobial agents displayed similar tendencies to those reported previously.

5. CONCLUSION

In conclusion, macrolides were previously considered an appropriate first-line agent for the treatment of *M. pneumoniae* infections in children. Therefore, it is important to monitor the incidence of macrolide-resistant *M. pneumoniae* infections in the community and to select appropriate therapeutic agents according to the macrolide-resistant or macrolide-sensitive status of the infectious *M. pneumoniae* strain.

CONSENT

The personal information that can identify an individual does not include it in this article.

ETHICAL APPROVAL

This study was performed with the permission of the Tokyo Takanawa Hospital Ethics Committee including the outside member consisting of school teachers and the lawyer. All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Available: Idsc.nih.gov/iasr/rapid/graph/pf38141.gif.
2. Okazaki N, Ohya H, Sasaki T. Mycoplasma pneumoniae Isolated from Patients with Respiratory Infection in Kanagawa Prefecture in 1976-2006: Emergence of Macrolide-Resistant Strains. *Jan. J. Infect. Dis.* 2007;60:325-326.
3. Morozumi M, Iwata S, Hasegawa K, Chiba N, Takayanagi R, Matsubara K, Nakayama E, Sunakawa K, Ubukata K. And the Acute Respiratory Diseases Study Group. Increased Macrolide Resistance of Mycoplasma pneumoniae in Pediatric Patients with Community-Acquired Pneumonia. *Antimicrob. Agents Chemother.* 2008;52:348-350.
4. Averbuch D, Hidalgo-Grass C, Moses AE, et al. Macrolide resistance in *Mycoplasma pneumoniae*, Israel, 2010. *Emerg. Infect. Dis.* 2011;17:1079-1082.
5. Dumke R, von Baum H, Luck PC, et al. Occurrence of macrolide-resistant *Mycoplasma pneumoniae* strain in Germany. *Clin. Microbiol. Infect.* 2010;16:613-616.
6. Lin Y, Ye X, Zhang H, et al. Characterization of macrolide resistance in *Mycoplasma pneumoniae* isolated from children in Shanghai, China. *Diagn. Microbiol. Infect. Dis.* 2010; 67:355-358.
7. Peuchant O, Menard A, Renaudin H, et al. Increased macrolide resistance of *Mycoplasma pneumoniae* in France directly detected in clinical specimens by real-time PCR and melting curve analysis. *J. Antimicrob. Chemother.* 2009;64:52-58.
8. Matsubara K, Morozumi M, Okada T, Matsushita T, Komiyama O, Shoji M, Ebihara T, Ubukata K, Sato Y, Akita H, Sunakawa K, Iwata S. A comparative clinical study of macrolide-sensitive and macrolide-resistant *Mycoplasma pneumoniae* infections in pediatric patients. *J Infect Chemother.* 2009;15:380-383.
9. Matas L., Dominguez J., Ory FD., Garcia N., Gali N., Cardona PJ., Hernandez A., Rodrigo C, Ausina V. Evaluation of Meridian ImmunoCard *Mycoplasma* Test for the Detection of *Mycoplasma Pneumoniae*-specific IgM in Paediatric Patients. *Scand J Infect Dis.* 1998;30:289-293.
10. Jacobs E. Serological diagnosis of *Mycoplasma pneumoniae* infections: A critical review of current procedures. *Clin Infect Dis.* 1993;17 (Suppl 1):S79-82.

11. Akaike H, Miyashita N, Kubo M, Kawai Y, Tanaka T, Ogita S, Kawasaki K, Nakano T, Terada K, Ouchi K. And the Atypical Pathogen Study Group. In vitro activities of 11 antimicrobial agents against macrolide-resistant *Mycoplasma pneumoniae* isolates from pediatric patients: Results from a multicenter surveillance study. *Jpn. J. Infect. Dis* 2012;65:535-538.

© 2014 Tsuji et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://www.sciencedomain.org/review-history.php?iid=549&id=12&aid=4798>