Journal of Microbiology, Immunology and Infection 56 (2023) 634—640

Available online at www.sciencedirect.com

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Original Article

The clinical significance of and the factors associated with macrolide resistance and poor macrolide response in pediatric *Mycoplasma pneumoniae* infection: A retrospective study

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Received 8 December 2021; received in revised form 28 October 2022; accepted 9 January 2023
Available online 24 January 2023

KEYWORDS
Macrolide resistance;
Mycoplasma pneumoniae;
Pleural effusion;
Poor macrolide response

Abstract *Background:* Macrolide-resistant *Mycoplasma pneumoniae* (MRMP) infection is increasing worldwide. However, its clinical significance is still uncertain.
Methods: The data of the Laboratory Medicine Department of Chang Gung Memorial Hospital in northern Taiwan was searched for children with molecular confirmed macrolide-susceptible *Mycoplasma pneumoniae* (MSMP) and MRMP infections between January 2011 and December 2018. The clinical features, laboratory data, and chest image presentations were compared between patients with MRMP and MSMP infections and between patients with good and poor macrolide response, respectively.

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[1] Contribution of the first 2 authors was equal.

https://doi.org/10.1016/j.jmii.2023.01.010

Results: Records from 158 patients were recovered. Of the enrolled patients 34 (22%) suffered MRMP infection, 27 (17%) had pleural effusions, and 47 (32%) had poor macrolide response. The macrolide resistance rate was 12% in 2011, 20% between 2015 and 2016, and 50% between 2017 and 2018, respectively. Other than a poor macrolide response, the MRMP and MSMP infections are clinically indistinguishable. The presence of pleural effusion and MRMP infections were found to be independently associated with a poor macrolide response, with odds ratios (95% confidence interval) of 14.3 (4.9—42.0) and 14.6 (5.4—40), respectively. The macrolide resistance rate of the patients with a poor macrolide response was 49% and 18% among all the patients enrolled and the patients with a pleural effusion, respectively.
Conclusion: The macrolide resistance rate had possibly increased in recent years in Taiwan and should be continuously monitored. In addition, the macrolide response could be misleading in predicting a macrolide resistance especially for the patients with a pleural effusion.

Introduction

Mycoplasma pneumoniae (MP) is an important pathogen in community acquired pneumonia in children nowadays. In 2010～2011, MP infection accounted for 25～40% of children hospitalized for community acquired pneumonia in Taiwan.[1] Macrolide antibiotics are the first line treatment at present. However, a growing resistance to macrolide antibiotics was observed worldwide since the year 2000.[2] The prevalence of macrolide-resistant *Mycoplasma pneumoniae* (MRMP) infection was higher than 80% or 90% in some Asian countries such as Japan and China in recent decade.[3,4] In Taiwan, the prevalence of MRMP infection ranged around 20%—30% in recent years until 2017.[5-9] Although the clinical course of most Mycoplasma infections are self-limited, approximately 2—5% of pediatric patients suffered severe infections and required intensive care in one tertiary medical center in Taiwan during 2010 and 2019.[7,9]

The Japan guideline suggested that the possibility of a MRMP infection and the switch to alternative antibiotics should be considered if fever lasted longer than 48 h after macrolide use.[10] The antibiotics of choice for MRMP infection include doxycycline, minocycline, and quinolones.[10] In Taiwan, doxycycline and ciprofloxacin were also the alternative antibiotics suggested for MRMP infections in children elder or lower than 8 years old, respectively.[11] However, the characteristics and clinical significance of MRMP infection are still unclear. The clinical impact of MRMP infection on disease severity or outcome is inconsistent.[5-8,12—15]

Point mutations in the 23sRNA gene, including particularly A2063G, A2064G, and A2063T mutations, are reported in cases of macrolide resistance and are usually used to identify MRMP infection clinically.[3,6,8,15] Molecular diagnosis provides a timely, highly sensitive, and highly specific means of diagnosing community acquired MP infection in clinical contexts.[10] In Chang Gung Memorial Hospital (CGMH) the investigation of the molecular diagnosis of MP infection and associated genome sequences to detect macrolide resistant genes was commenced in 2011 and available clinically since 2015.[5] Furthermore, for molecular diagnosed MP infection, full genome sequencing to identify macrolide resistant genes is additionally available on request if MRMP infection is suspected clinically.

In this study, we examined the medical records of 158 children with molecular diagnosed macrolide-susceptible *Mycoplasma pneumoniae* (MSMP) and MRMP infections hospitalized in 2011 and between 2015 and 2018 at CGMH. The aim of the study was to evaluate the prevalence of MRMP infection of the children in Taiwan, compare the clinical features between MRMP infections and MSMP infections, and to identify the potential risk factors and clinical significance of poor macrolide response in the care of the children with MP pneumonia.

Methods

Ethical statement

The study was approved by the Institutional Review Board of CGMH (Reference Number 104-A223B and 202001378B0). Written informed consent was waived since the study was limited to a retrospective chart review.

Patient details and information collection

The database records of the laboratory medicine department of CGMH were retrieved for children (less than 18 years old) with molecular diagnosed MRMP and MSMP infection. The nucleic acid amplification (NAA) of specimens and genome sequencing of positive specimens were performed using conventional techniques, as described previously.[5] A2063G, A2063T, and A2064G mutations of the 23sRNA gene were considered to indicate macrolide resistance.

On the basis of the retrieved records, 59 patients were enrolled in 2011, where these patients were previously described.[5] A further 99 patients with community acquired MP infection were retrieved from January 2015 to December 2018, including 75 enrolled between 2015 and 2016 and 24 patients between 2017 and 2018. For these 99 patients the decision of detecting macrolide resistant gene was made at the discretion of physicians. Thus, a total of

158 patients were enrolled in the study. Clinical information, including demographic data, clinical characteristics, laboratory and imaging information, treatment, and so on, were collected for each patient. The accuracy of the image diagnoses, including those of chest X-rays (CXRs) and computed tomography (CT) images, was confirmed by an experienced pediatric radiologist. Patients who required ICU care, or exhibited severe pulmonary complications such as necrotizing pneumonitis, were classified as severe infection cases. A good macrolide response was defined as the fever subsided after and within 48 h of initiating macrolide treatment while a poor macrolide response was defined as the fever persisted longer than 48 h after initiating macrolide therapy.

Statistical analysis

The collected data were analyzed using SPSS Version 12.0. The data pertaining to the clinical features and laboratory items were presented as median (inter-quartile range) or frequency (%) values. A Mann-Whitney U test was applied to process the continuous variables, while a chi-square or Fisher's exact test was used to process the categorical data. The clinical profiles (including the demographic data, clinical manifestation, laboratory data, and therapy timing) were compared for three conditions: (1) patients with MRMP and MSMP infection, respectively; (2) patients with good macrolide response and poor macrolide response, respectively; and (3) patients with pleural effusion and without pleural effusion, respectively. Possible prognostic factors were screened using univariate analysis. Furthermore, factors with a p-value of 0.2 or less were incorporated into stepwise multiple logistic regression to adjust for confounding factors simultaneously and to calculate the multivariate-adjusted odds ratio for each risk factor. The αlevel of model selection was set at 0.2 in every case. All statistical tests were 2-sided, and $P < 0.05$ was considered significant.

Results

Demographic and clinical information

Of the 158 patients enrolled in the study, 34 (22%) had MRMP infections and 47 (32%) had a poor macrolide response. Of the 34 patients with MRMP infections, 33 were A2063G and one was A2063T mutations. The macrolide resistance rate was 12% (7 in 59 patients) in 2011, 16% (7 in 43 patients) in 2015, 22% (15 in 32 patients) in 2016, and 50% (12 in 24 patients) between 2017 and 2018, respectively (Fig. 1). A total of 157 patients received macrolide antibiotics (azithromycin or clarithromycin) and 29 patients (15 MRMP infections and 14 MSMP infections) also received alternative antibiotics including doxycycline (27 patients) and ciprofloxacin (two patients). Among the patients who received alternative antibiotics, one patient received doxycycline as initial therapy and the other 28 patients received the alternative antibiotics due to persistent fever in spite of macrolide therapy. No apparent adverse effects associated with antibiotics treatment were noted during

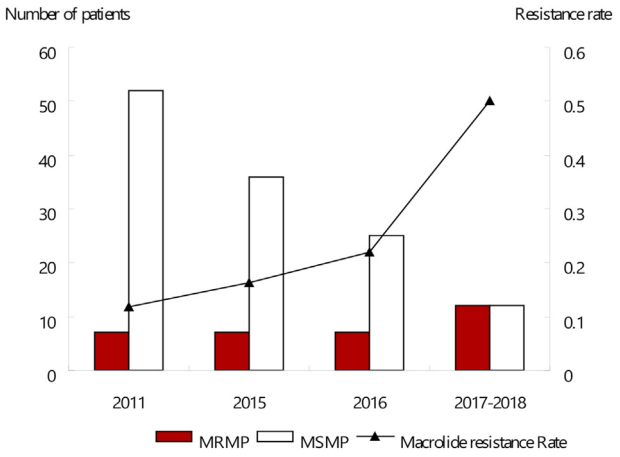

Figure 1. Distribution of patients with Macrolide resistance infection by calendar year.

hospitalization. Of the 29 patients receiving alternative antibiotics, 10 (67%) of the 15 MRMP infected patients and 7 (50%) of the 14 MSMP infected patients became afebrile within 24 h of therapy. None of these patients received parenteral or oral steroid for the purpose of anti-mycoplasma treatment. No significant differences were observed between the patients enrolled in 2011 and 2015—2018 in terms of age and gender. Throat swab specimens for MP NAA were sampled at a median of 5 (3—7) days after fever onset. Co-infection was noted in one boy, who got MSMP and Influenza B co-infection and his fever subsided within 48 h after azithrocin use. The level of serum IgG and IgM for MP was measured in 134 patients on admission and of them 82 (61%) showed IgM positivity. Among the seronegative patients, the antibody level was rechecked in 23 patients 4—10 days later and all 23 patients showed IgM seroconversion. The presence of mycoplasma IgM supported that *Mycoplasma pneumoniae* was responsible for the illness of these patients. Most of the enrolled patients experienced atypical pneumonia and recovered smoothly. However, four patients suffered severe infection and three of them required intensive care (Table 1). All the four patients with severe illness suffered MSMP infections and exhibited persistent pulmonary lesions following discharge. Significant extra-pulmonary complication was present in one patient who suffered respiratory distress and complicated by infection associated hemophagocytic syndrome (patient 4, Table 1).

The chest X-ray findings of the enrolled patients ranged from multi-lobar distribution of interstitial infiltration to total opacity/consolidation in one or several lobes. Lower lobe involvement was the most common finding and was seen in 99 (63%) patients. CT studies and chest sonography were performed for patients with more complicated or severe illnesses. Pleural effusion was noted in 27 (17%) patients. The amount of pleural effusion was minimal and or less than 10 mm in thickness in all patients, except an 8 year old girl who had a severe illness (patient 3 in Table 1). Pleural effusion analyses were available in two patients with severe illness and were exudative and monocyte-predominant (Table 1).

Table 1 Clinical information of the patients with severe *Mycoplasma pneumoniae* infection.

	Patient 1	Patient 2	Patient 3	Patient 4
Age	2y4m	9y2m	8y4m	3y7m
Gender	Female	male	female	female
Hospitalization days	19	38	8	25
Total fever days	23	13	8	11
WBC ($\times$1000/mm^3)	15.2	11.6	7.2	14.8
Neutrophils (%)	86	72	80	89
Lymphocytes (%)	6	11	12	8
Hb (gm/dL)	10.1	12.8	12.3	13.4
Platelet ($\times$1000/mm^3)	176	405	224	222
Peak CRP (mg/L)	298	126	279	77
Imaging findings				
CXR patterns/CT findings	Necrotizing pneumonitis, RLL, LLL,	Necrotizing pneumonitis, LLL	Total opacity of RUL and RML	Multiple consolidation of RUL, RML, LLL
Pleural effusion (thickness)	Bilateral (2–3 mm)	No	Right side (30 mm)	Bilateral (10 mm)
Significant Complications				1. ARDS 2. IAHS
Management		ICU cared	ICU cared	ICU cared. ECMO, IVIG
Antibiotics used	AZI, DOXY	AZI, TAZ	AZI, TEC, CRO	AZI, DOXY, CRO, MERO, MOXI, TEC
Macrolide resistance	No	No	No	No
Pleural effusion analysis				
PH	–	–	8.3	8.4
Appearance	–	–	Cloudy, yellow	Turbid, red
WBC/RBC (/mm^3)	–	–	590/1350	115/38160
N/L/M (%)	–	–	19/22/59	37/18/45
Gram stain/culture	–	–	negative	negative
Glucose (mg/dL)	–	–	108	122
LDH (IU)	–	–	2601	2604

Abbreviations: ARDS: acute respiratory distress syndrome; AZI, azithrocin; CRO, ceftriaxone; DOXY, doxycycline; IAHS, infection associated hemophagocytic syndrome; MERO, meropenem; MOXI, moxicycline; N/L/M: neutrophils/lymphocyte/monocyte; RUL, RML and LLL: right upper lobe, right middle lobe, and left lower lobe respectively; TAZ, piperacillin + tazobactam; TEC, teicoplanin.

Comparison between MRMP and MSMP infection cases

Table 2 compares the demographic and clinical information between the patients with MRMP and MSMP infection, respectively. The rate of MRMP infections were significantly higher in patients enrolled after year 2015. Of the two groups of patients, those with MRMP infection were more likely to experience a poor macrolide response. Except a poor macrolide response, however, patients of the two groups were indistinguishable clinically in terms of their demographic characteristics, CXR findings and hemograms.

Risk factors and clinical significance of poor macrolide response

Table 3 compares the demographic and clinical information between the patients with good and poor macrolide response, respectively. No significant difference was noted between the two groups in terms of their age, gender, date of infection, hemograms, CRP level and timing of macrolide therapy intervention. However, the patients with a poor macrolide response exhibited more complete consolidation or necrosis of lung, more pleural effusion, more MRMP infections, and a longer duration of fever and hospital stay. According to a multivariate analysis, pleural effusion and macrolide resistance were both independently associated with a poor macrolide response (Table 4). Of the 47 patients with a poor macrolide response 24 (51%) were MSMP infections and 23 (49%) were MRMP infections.

Characteristics of patients with pleural effusion compared to those without pleural effusion

Of all the patients enrolled a total of 27 patients had a pleural effusion. No significant differences were found between the patients with and without pleural effusions in terms of their age, timing of macrolide therapy intervention, hemograms and presence of macrolide resistant genes. However, the patients with a pleural effusion were characterized by a higher CRP level (71 (31–125) vs. 30 (12–55) mg/L, $p < 0.001$), a greater association with complete consolidation or necrotizing pneumonitis, and a higher incidence of poor macrolide response (68% vs. 24%, $p < 0.001$). Of the patients with a pleural effusion a total of 17 patients had a poor macrolide response and among them 3 (18%) were MRMP infections.

Table 2 Comparison of demographic data and clinical information of the patients with MRMP and MSMP infections.

	ALL patients (n = 158)		MRMP (n = 34)		MSMP (n = 124)		P
Year 2011	59	(37%)	7	(21%)	52	(42%)	0.027
Age	6.3	(4.6–8.4)[a]	6.1	(3.9–7.5)	6.3	(4.7–8.6)	0.252
Males	74	(47%)	18	(53%)	56	(45%)	0.421
Fever days before macrolide use	5	(3–7)	4.5	(3–7)	5	(3–7)	0.558
Hospitalization days	5	(4–6)	6	(3–7)	4	(4–6)	0.035
Total fever days	7	(5–9)	8	(6–10)	7	(5–9)	0.033
WBC ($\times$1000/mm^3)	7.2	(5.8–9.1)	7.2	(5.4–8.3)	7.2	(5.9–9.4)	0.370
Neutrophil (%)	65	(57–72)	65	(57–69)	65	(56–72)	0.742
Lymphocytes (%)	25	(19–32)	27	(21–33)	25	(18–32)	0.498
Hb (gm/dL)	12.5	(11.7–12.9)	12.5	(11.7–13.2)	12.5	(11.6–12.9)	0.486
Platelet ($\times$1000/mm^3)	222	(196–270)	202	(190–254)	230	(198–275)	0.128
Peak CRP (mg/L)	33	(15–59)	33	(17–49)	35	(12–66)	0.666
CXR patterns							0.347
Interstitial pattern	6	(4%)	1	(3%)	5	(4%)	
Mixed interstitial and alveolar pattern	62	(39%)	18	(53%)	44	(35%)	
Incomplete consolidation	70	(44%)	12	(35%)	58	(47%)	
Complete consolidation/Necrotizing pneumonitis	19	(12%)	3	(9%)	16	(13%)	
Pleural effusion	27	(17%)	3	(9%)	24	(19%)	0.148
Poor macrolide response[b]	47	(30%)	23	(68%)	24	(19%)	<0.001

[a] Median (inter-quartile range).
[b] Fever persisted longer than 48 h after initiating macrolide therapy.
Abbreviation: MRMP, macrolide-resistant *Mycoplasma pneumoniae*; MSMP, macrolide-susceptible *Mycoplasma pneumoniae*.

Discussion

This study showed that the macrolide resistance rate had possibly increased progressively in recent years in Taiwan. MRMP and MSMP infections seemed were indistinguishable from each other clinically except the response to initial macrolide therapy. Pleural effusion was noted in 17% of the patients, and was associated with more severe radiological findings such as complete lung consolidation or necrosis. The presences of pleural effusion and macrolide resistance were both independent predictors of poor macrolide response, while a poor macrolide response was not necessarily a reliable indicator of a MRMP infection.

The clinical significance and appropriate treatment of MRMP infection is still unclear, and the association between a macrolide resistance and severe infection is inconsistent in literature.[5-7,12–15] The pathogenesis and pulmonary damage caused by MP infection is related to the immune response/inflammation elicited by MP cell membrane.[16–21] In a recent report Yang showed that a MRMP infection itself is neither associated with more extra-pulmonary manifestations nor a good indicator of disease severity and prognosis for the children with MP infections.[7] Our results show similar findings and suggest that macrolide resistance is possibly not the only determinant of pulmonary damage, the most important determinant of disease severity in MP infections.

Identification and appropriate therapy of MRMP infections is of value in shortening disease course and infection control issues but the optimal timing to initiate the alternative antibiotics in the absence of genotypic result to guide treatment is not clear.[7,22] The response to macrolide therapy is commonly relied on to predict a possible MRMP infection. Under the circumstance of a high MRMP infection rate, Japanese guidelines suggest the switching to tetracycline antibiotics if fever persists longer than 48 h after macrolide treatment.[10] However, the present study showed that the fever duration after macrolide therapy might be misleading in predicting MRMP infections, especially when the patient had a pleural effusion or the local prevalence of MRMP infections is not high. In Japan, the rate of MRMP infection reduced from 81% to 44% over the period of 2012–2015.[23] In Taiwan, the macrolide resistance rate reported was lower than 30% until 2017.[5,6,7] In addition, potential side effects of these alternative antibiotics including the cutaneous effects associated with doxycycline and the musculoskeletal and cardiovascular effects associated with fluoroquinolones, raised concerns about their use especially in children.[24] Considering that most MP infections were mild and recovered smoothly, the second line antibiotics should be used judiciously. In addition, an earlier study has shown that extra-pulmonary manifestations were presented more commonly in the patients with delayed appropriate antibiotics treatment after 6 days of illness.[7] Therefore for the patients who encountered a poor macrolide response, immediate switching to alternative antibiotics may not be necessary if the clinical condition, laboratory data, and image presentation remains stable or had been improved, especially when it was still within 6 days of disease onset.

The chest radiological findings were known to be correlated with the disease severity and the clinical course in children with MP pneumonia.[25–27] However the

Table 3 Comparison of demographic data and clinical information of the patients with good and poor macrolide response.

	Good macrolide response[a] (n = 101)		Poor macrolide response[b] (n = 47)		P
Age	6.2	(4.6—8.7)[c]	6.3	(4.6—7.1)	0.230
Males	48	(48%)	21	(45%)	0.747
Fever days before macrolide use	6	(3—7)	5	(3—7)	0.300
Hospitalization days	4	(3—5)	7	(6—9)	<0.001
Total fever days	6	(5—8)	10	(8—12)	<0.001
WBC (x 1000/mm^3)	7.3	(5.9—9.3)	7.0	(4.8—8.1)	0.052
Neutrophils (%)	65	(56—72)	65	(57—72)	0.816
Lymphocytes (%)	25	(19—34)	26	(19—32)	0.981
Hb (gm/dL)	12.4	(11.6—12.9)	12.5	(11.7—13)	0.927
Platelet (×1000/mm^3)	240	(204—276)	205	(180—256)	0.023
CRP (mg/L)	35	(13—56)	34	(16—71)	0.451
CXR patterns					0.001
Interstitial pattern	5	(5%)	1	(2%)	
Mixed interstitial and alveolar pattern	42	(42%)	14	(30%)	
Incomplete consolidation	48	(48%)	19	(40%)	
Complete consolidation/Necrotizing pneumonitis	5	(5%)	13	(28%)	
Pleural effusion	8	(8%)	17	(36%)	<0.001
Macrolide Resistance	11	(11%)	23	(49%)	<0.001

[a] Indicates fever subsided after and within 48 h of initiating macrolide therapy.
[b] Indicates fever persisted longer than 48 h after initiating macrolide therapy.
[c] Median (inter-quartile range).

radiological findings is seemed not a good indicator for predicting a macrolide resistance. The lack of association between chest X ray findings and macrolide resistance had been shown before.[25] On the contrary, chest radiological findings itself have a significant impact on clinical course. Yoon's report suggested that a homogenous lobar consolidation and pleural effusion were important determinants of prolonged clinical course including fever lasted longer than 7 days after macrolide therapy regardless of macrolide resistance.[25] Our results show similar findings, which suggest that the macrolide response itself is not reliable in predicting a macrolide resistance or guiding the treatment for patients with lobar consolidation or pleural effusion.

Several limitations of the present study should be acknowledged. Firstly, gene sequencing was not performed for all of the molecular diagnosed patients in 2015～2018. Thus, the presence of selection bias is possible. Secondly, macrolide resistant gene mutations other than A2063G or A2063T were not enrolled in this study. Thirdly, the presence of IgM for *Mycoplasma pneumoniae* is helpful in defining the pathogenic role of mycoplasma in current infection. However, not all patients in this study received antibody test at admission or were followed up when the first IgM test was negative. Finally, the association between the cytokine profile and MRMP infection was unable to be addressed in this study.

Nonetheless, the present study shows the increase of macrolide resistance rate in recent years especially between 2017 and 2018 in Taiwan. In addition, this study also suggests that severe MP infection is not limited to MRMP infection, and a poor macrolide response might be misleading in predicting a MRMP infection especially for the patients complicated with a pleural effusion. For the children with Mycoplasma infections, the alternative antibiotics should be used cautiously and maybe better reserved for the patients with severe infection rather than rely solely on the duration of fever after macrolide therapy. The appropriate management for the patient without a rapid macrolide response in the absence of a genotypic result to guide the treatment needs further evaluation and discussion.

Table 4 Factors[a] independently significant for poor macrolide response.

Multiple logistic	P value	Odds ratio	95.0% confidence interval	
Pleural effusion	<0.001	14.3	4.9	- 42.0
MRMP infection	<0.001	14.6	5.4	- 40.0

[a] A total of 5 factors (WBC count, platelet count, pneumonia patch on chest radiography, resistant strain, and pleural effusion) were selected into the multiple logistic regression analysis.

Funding

The authors have no funding source to declare.

Declaration of competing interest

No conflict of interest exists in the present study.

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