Macrolide Resistance, Clinical Features, and Cytokine Profiles in Taiwanese Children With *Mycoplasma pneumoniae* Infection

Tsung-Hua Wu,[1,a] Nancy M. Wang,[2,a] Fang-Ching Liu,[3] Hui-Hsien Pan,[4] Fang-Liang Huang,[5] Yu-Ping Fang,[6] Ting-Wei Chiang,[2] Yu-Ying Yang,[7] Chiah-Sing Song,[8] Hsiang-Chin Wu,[9] and Chun-Yi Lee[6]

[1]Department of Pediatrics, Show Chwan Memorial Hospital, Changhua, Taiwan, [2]Department of Biology, National Changhua University of Education, Changhua, Taiwan, [3]Department of Pediatrics, Jen-Ai Hospital, Taichung, Taiwan, [4]Department of Pediatrics, Chung Shan Medical University Hospital, Taichung, Taiwan, [5]Department of Pediatrics, Taichung Veterans General Hospital, Taichung, Taiwan, [6]Department of Pediatrics, Chang Bing Show Chwan Memorial Hospital, Changhua, Taiwan, [7]Department of Clinical Laboratory, Show Chwan Memorial Hospital, Changhua, Taiwan, [8]Department of Clinical Laboratory, Chang Bing Show Chwan Memorial Hospital, Changhua, Taiwan, [9]Department of Laboratory, Chung Shan Medical University Hospital, Taichung, Taiwan

Background. The factors that predict the progression of *Mycoplasma pneumoniae* infection remain inconclusive. Therefore, we investigated macrolide resistance prevalence, *M pneumoniae* genotype, and clinical characteristics of childhood *M pneumoniae* respiratory tract infections in Taiwan.

Methods. A total of 295 children hospitalized with respiratory tract infections with positive serological *M pneumoniae* immunoglobulin M test results were enrolled in this 3-year prospective study. Oropharyngeal swabs were obtained for *M pneumoniae* cultures and polymerase chain reaction tests. All *M pneumoniae* specimens were further characterized by P1 typing, multilocus variable-number tandem-repeat analysis (MLVA), and macrolide resistance genotyping. The clinical characteristics and blood cytokine profiles were analyzed accordingly.

Results. Of 138 *M pneumoniae* specimens, type I P1 was the predominant (136 of 138, 98.6%). The MLVA type P (4-4-5-7-2) was the leading strain (42 of 138, 30.4%), followed by type J, U, A, and X. The overall macrolide-resistant rate was 38.4% (53 of 138); the resistance rate increased dramatically yearly: 10.6% in 2017, 47.5% in 2018, and 62.5% in 2019 ($P < .001$). All macrolide-resistant *M pneumoniae* (MRMP) harbored the A2063G mutation and were MLVA type 4-5-7-2 (49 of 53, 92.5%), especially type U and X. No significant differences in clinical symptoms, duration of hospital stay, and radiographic findings were identified among patients between MRMP and macrolide-sensitive *M pneumoniae* (MSMP) groups. Patients with MRMP infection had more febrile days before and during hospitalization and higher interleukin (IL)-13 and IL-33 levels than patients with MSMP infection ($P < .05$).

Conclusions. Macrolide-resistant *M pneumoniae* surged in Taiwan throughout the study period, but macrolide resistance was not a determinant factor of clinical severity.

Keywords. children; cytokine; macrolide resistance; MLVA; *Mycoplasma pneumonia*.

Mycoplasma pneumoniae is a main pathogen in respiratory tract infections (RTIs), accounting for approximately 10%–30% of community-acquired pneumonia (CAP) in children and young adults [1, 2]. The clinical characteristics of *M pneumoniae* infection vary widely, ranging from self-limiting to severe pneumonia, with extrapulmonary manifestations such as myocarditis, urticaria, hepatitis, hemolytic anemia, arthritis, and encephalitis [3].

Mycoplasma pneumoniae is generally considered to be susceptible to macrolides, tetracyclines, and fluoroquinolones. Among these, macrolides are the first line of antibiotics used to treat children with CAP caused by *M pneumoniae*. Since 2000, however, prevalence of macrolide-resistant *M pneumoniae* (MRMP) isolates in pediatric patients has increased rapidly in Japan [4]. Macrolide resistance has been spreading worldwide, and it is associated with point mutations in the peptidyl-transferase loop of the 23S ribosomal ribonucleic acid (rRNA), which lead to high-level resistance to macrolides [4]. In Europe and North America, the macrolide resistance rate was reported to be between 3.5% and 13.2%, except in Italy where it was 26% [5]. The highest resistant rates occurred in Asia, with a prevalence between 90% and 100% in China and Japan [5]. Other Asian countries have reported resistance rates close to 50% [5, 6].

Molecular characterization is a powerful tool for surveillance and outbreak investigation. Molecular typing of *M pneumoniae* P1 gene has been the most common genotyping method used in

the past 20 years. Because the P1 genotyping method targeting only 1 gene has low discriminatory power [7], a multilocus variable-number tandem-repeat (VNTR) analysis method based on 5 VNTRs, Mpn1-13-14-15-16, which clustered 265 *M pneumoniae* clinical isolates into 26 multilocus variable-number tandem-repeat analysis (MLVA) types, was developed more recently [8]. Molecular epidemiological studies of *M pneumoniae* and macrolide susceptibility have been investigated widely and varied geographically and temporally [6, 9–11].

In *M pneumoniae* infection, the host's cell-mediated immunity plays a key role in pulmonary lesion development [12]. However, the possible pathogenic mechanism of *M pneumoniae* infection has not been fully studied. Previous studies have suggested that the cytokine profile may be associated with the severity of infection [13–15]; nevertheless, factors to predict the progression of *M pneumoniae* infection remain inconclusive.

In this multicenter and prospective study, we analyzed *M pneumoniae* clinical isolates from hospitalized children and investigated the molecular epidemiology and clinical characteristics of childhood *M pneumoniae* RTIs in Taiwan.

METHODS

Study Design

From March 1, 2017 to June 30, 2019, children aged 14 years old or younger were enrolled prospectively in the study at 5 hospitals (Show Chwan Memorial Hospital, Chang Bing Show Chwan Memorial Hospital, Jen-Ai Hospital, Chung Shan Medical University Hospital, and Taichung Veterans General Hospital) situated in central Taiwan. Written informed consent was obtained from parents or caregivers before enrollment, with children providing assent when age appropriate. The study protocol was approved by the institutional review boards (IRBs) at each institution (IRB Numbers 1051007, CF17158A, and CS18084). Children were included in the study if they (1) were admitted to one of the 5 study hospitals, (2) received a diagnosis of an acute RTI, defined as new cough or sputum production, chest pain, dyspnea, tachypnea, abnormal lung examination, or respiratory failure, (3) and had evidence of a positive serological *M pneumoniae* immunoglobulin M (IgM) test result (Biocardä; AniBiotech, Vantaa, Finland). Children were excluded if they had already been enrolled in this study. Trained staff obtained oropharyngeal swabs, blood samples, and acute-phase serum specimens from all the enrolled children as soon as possible after presentation. The samples were stored at −80°C before culture and molecular detection of *M pneumoniae* and cytokine assay. The clinical features, laboratory data, and imaging findings were retrieved from medical records.

Culture and Deoxyribonucleic Acid Extraction

Specimens were cultured in SP-4 medium and incubated in 5% CO_2 at 37°C for 14 days for the development of *Mycoplasma* colonies according to the guidelines (ATCC Medium: 2611 *Spiroplasma* medium) [16]. Nucleic acid was extracted from liquid culture using the deoxyribonucleic acid (DNA) mini kit (QIAGEN, Toronto, Canada) according to the buccal swab protocol, and *M pneumoniae* was confirmed using a repMp1-based real-time polymerase chain reaction (PCR) assay [17]. All culture-positive specimens were further characterized using P1 typing, MLVA typing, and macrolide resistance genotyping.

Detection of Macrolide Resistance

Single-base mutations in the 23S rRNA gene of antibiotic-resistant *M pneumoniae* were detected and analyzed using a nested PCR with the primer as previously described [18]. The purified PCR products were sequenced with ABI 3730 automated sequencer (Applied Biosystems, Thermo Fisher Scientific, Foster City, CA) to detect the mutation sites at positions 2063 and 2064.

P1 Gene Typing

A duplex real-time PCR assay was used for P1 genotyping of *M pneumoniae* as previously described [19]. Isolates were classified as type 1 (*mpn 459*, M129 strain) or type 2 (*mpna 5864*, 309 strain) based on targeting specific DNA fragment insertion or deletion for *M pneumoniae*.

Multilocus Variable-Number Tandem-Repeat Analysis Typing

The MLVA typing was performed using Fast-Run Taq Master Mix (Protech Technology Enterprise Co., Ltd., Taiwan) on all isolates as previously described [8]. The 5 selected loci (Mpn1, Mpn13-16) containing tandem repeats were amplified using nested PCR. Flank primers targeting the surrounding genome regions of each loci were used for the first amplification with annealing temperature at 55°C. The amplified products were then purified with the PCR cleanup kit (Nucleospin Kit; Machrey-Nagel, Düren, Germany), and 30 ng of the purified DNA was used as the template for the nested PCR using additional inner primers. The amplified products were purified and subjected to cycle sequencing (ABI Prism 3730; Thermo Fisher Scientific), and the data were used for Basic Local Alignment Search Tool (BLAST) analysis.

Phylogenetic Tree

The number of repeat units for each locus from MLVA was used as character data in BioNumerics software (version 5.0; Applied Maths) for cluster analysis using a minimum spanning tree (MST) algorithm. The dendrogram was generated based on the categorical coefficient with a priority rule of the first link types set as the highest number of single-locus variants [20].

Cytokine Detection

Serum concentrations of chemokines and cytokines were measured using Human Cytokine Protein Array (RayBiotech, Inc., Norcross, GA) according to the manufacturer's instructions.

The Protein Array kit was used to simultaneously detect 9 cytokines and 1 gelatinase, namely, interleukin (IL)-10, IL-12p40, IL-13, IL-17, IL-33, IL-6, IL-8, macrophage-inflammatory protein (MIP)-1a, tumor necrosis factor (TNF)α, and matrix metalloproteinase (MMP)-9.

Statistical Analysis

IBM SPSS Statistics for Windows, version 24.0 (IBM Corp., Armonk, NY) was used for the statistical analyses. Changes in incidence over time were assessed using Poisson regression. The mean age of patients and laboratory data were compared using t test. Continuous data obtained are expressed as the mean ± standard deviation, and categorical data were analyzed using the χ^2 test. Fisher's exact test was performed when any expected count was less than 5. Skewed distribution data were expressed as median values (25th–75th interquartile ranges). The comparisons were made using the Mann-Whitney U test. Variables associated with MRMP in univariate analyses were included in the model of multiple logistic regression. A $P < .05$ indicates statistically significant differences.

RESULTS

Of the 295 enrolled patients (age ≤14 years) with RTIs, 144 (48.8%) had evidence of *M pneumoniae* isolate (Supplementary Figure). Among these *M pneumoniae*-positive patients, the mean age was 5.2 ± 3.2 years, 65 (45.1%) were male, and 142 (98.6%) received a diagnosis of lower RTI.

Genotyping and Macrolide Susceptibility

The macrolide resistance could not be determined in 6 of the 144 *M pneumoniae*-positive specimens owing to poor amplification of the target sequence. Of the 138 specimens, 85 (61.6%) were sensitive to macrolides and 53 (38.4%) were resistant. Sequencing analysis revealed that all isolates with macrolide resistance harbored an A2063G mutation in 23s rRNA. The macrolide resistance rate increased dramatically with time during the study period, with 10.6% in 2017, 47.5% in 2018, and 62.5% in 2019 ($P < .001$) (Figure 1a).

The 2 main P1 genotypes of *M pneumoniae*, types 1 and 2, accounted for 98.6% and 1.4% of total *M pneumoniae*-positive specimens (n = 138), respectively. Typing with MLVA targeting 5 loci revealed 17 distinct MLVA types in the 138 *M pneumoniae* isolates. The most prevalent MLVA type was type P (4-4-5-7-2; 30.4%, 42 of 138), followed by type J (3-4-5-7-2; 18.8%, 26 of 138), type U (5-4-5-7-2; 11.6%, 16 of 138), type 3-4-5-7-3 (8.7%, 12 of 138), and type A (1-4-5-7-2, 7.2%, 10 of 138). Because the Mpn1 locus is variable [21], if Mpn1 was eliminated from the typing, only 7 MLVA types were identified. The most prevalent 4-loci scheme MLVA type was 4-5-7-2 (73.2%, 101 of 138). The distribution of each MLVA type varied during the study period (Figure 1b). MLVA type P (4-4-5-7-2) was predominant in 2017
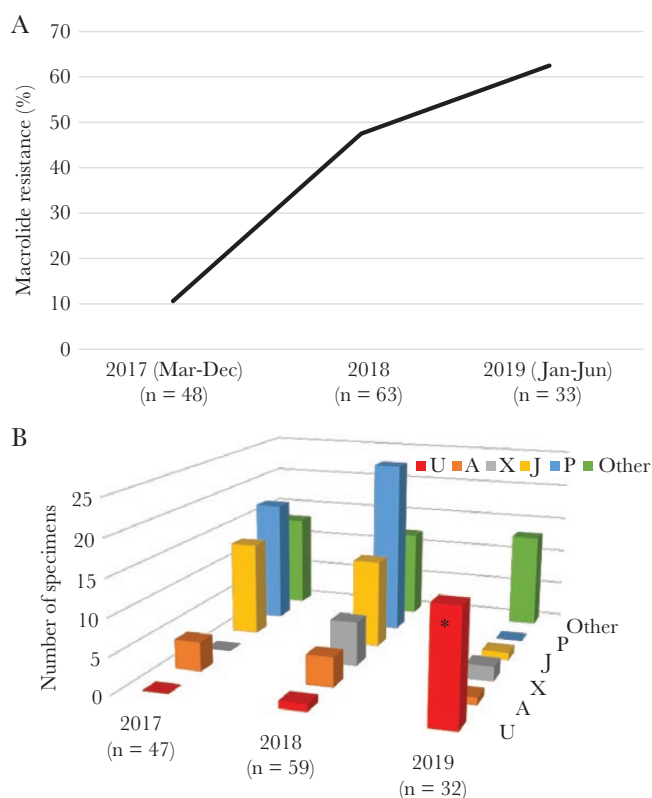

Figure 1. (a) Rate of macrolide resistant *Mycoplasma pneumoniae* infections identified during 2017–2019. (b) Distribution of the 5 predominant multilocus variable-number tandem-repeat analysis (MLVA) types (U, A, X, J, and P) over the study period. Other types include the following: type K, R, etc. An asterisk indicates significant difference ($P < .001$) for the trend over time.

and 2018, but there was none in 2019. The numbers of MLVA type P (4-4-5-7-2) and J (3-4-5-7-2) decreased significantly between 2017 and 2019 ($P < .001$). However, only the numbers of MLVA type U (5-4-5-7-2) isolates increased between 2017 and 2019 ($P < .001$).

Difference in distributions of MLVA profiles between macrolide-sensitive *M pneumoniae* (MSMP) and MRMP is presented in Table 1. The prevalence of MLVA type U (5-4-5-7-2) and X (6-4-5-7-2) was substantially higher in the MRMP group than in the MSMP group ($P < .001$ and $P = .005$, respectively). In contrast, type 1-4-5-7-3 and 3-4-5-7-3 were more prevalent in the MSMP group than in the MRMP group ($P = .023$ and $P = .003$, respectively). For the 4-loci scheme, the most prevalent type was type 4-5-7-2 in the MRMP group ($P < .001$). In contrast, type 4-5-7-3 was more dominant in the MSMP group than in the MRMP group ($P < .001$). The relationships among MLVA types were demonstrated with an MST (Figure 2). The MST modeling for the 5-loci scheme showed diversity of the isolated strains. MLVA type U (5-4-5-7-2) and X (6-4-5-7-2) had high macrolide resistance rates (16 of 16, 100% and 7 of 8, 87.5%, respectively), and type P (4-4-5-7-2) was the most prevalent in MSMP (23 of 85, 27.1%).

Table 1. The Relationship Between MLVA Types and Macrolide Resistance in Children With Respiratory Tract Infections

MLVA			MSMP (n = 85)	MRMP (n = 53)	
Mpn13-16	Mpn1, 13-16	All	n (%)	n (%)	P Value
4-5-7-2		101	52 (61.2%)	49 (92.5%)	<.001
	1-4-5-7-2 (A)	10	9 (10.6%)	1 (1.9%)	.088
	3-4-5-7-2 (J)	26	19 (22.4%)	7 (13.2%)	.182
	4-4-5-7-2 (P)	41	23 (27.1%)	18 (34%)	.388
	5-4-5-7-2 (U)	16	0	16 (30.2%)	<.001
	6-4-5-7-2 (X)	8	1 (1.2%)	7 (13.2%)	.005
4-5-7-3		27	25 (29.4%)	2 (3.8%)	<.001
	1-4-5-7-3	8	8 (9.4%)	0	.023
	3-4-5-7-3	12	12 (14.1%)	0	.003
	4-4-5-7-3	3	3 (3.5%)	0	.285
	5-4-5-7-3	4	2 (2.4%)	2 (3.8%)	1
1-5-6-2		3	2 (2.4%)	1 (1.9%)	1
	4-1-5-6-2	1	0	1 (1.9%)	.384
	6-1-5-6-2	2	2 (2.4%)	0	.521
4-5-6-2		3	3 (3.5%)	0	.282
	3-4-5-6-2 (K)	2	2 (2.4%)	0	.523
	4-4-5-6-2	1	1 (1.2%)	0	1
4-5-5-2		2	1 (1.2%)	1 (1.9%)	1
	4-4-5-5-2	1	1 (1.2%)	0	1
	6-4-5-5-2	1	0	1 (1.9%)	.384
3-5-7-2	5-3-5-7-2 (R)	1	1 (1.2%)	0	1
1-5-7-2	5-1-5-7-2	1	1 (1.2%)	0	1

Abbreviations: MLVA, multilocus variable-number tandem-repeat analysis; MRMP, macrolide-resistant *Mycoplasma pneumoniae*; MSMP, macrolide-sensitive *M pneumoniae*.

Clinical Characteristics

The demographic and clinical characteristics of patients with *M pneumoniae* RTI according to macrolide susceptibility are shown in Table 2. There were no statistical differences regarding sex, clinical symptoms, laboratory characteristics, initially prescribed antibiotics, duration of hospital stay, and presence of radiographic findings in chest x-ray between the MSMP and MRMP groups. The rate of MSMP infection was higher in children aged 0–6 years (*P* = .025). Patients with MRMP infection had more days from fever onset to admission (3.94 ± 3.76 days, *P* = .006) and longer duration of fever after the start of antibiotic treatment (65.52 ± 35.67 hours, *P* = .04) than those with MSMP infection. In addition, 33% of patients with MRMP infection switched from azithromycin to doxycycline treatment during hospitalization.

Cytokine Response

A comparison of serum cytokines between the MSMP (n = 80) and MRMP (n = 48) groups is presented in Table 3. Serum IL-13 (median 0.63 pg/mL, 25th–75th interquartile ranges 0.00~3.05 pg/mL) and IL-33 (median 17.45 pg/mL, 25th–75th interquartile ranges 1.30~64.94 pg/mL) levels were significantly higher in the MRMP group than in the MSMP group (*P* = .015 and *P* < .001, respectively). Serum IL-17 and TNFα levels in the

vast of *M pneumoniae*-infected patients were below the limits of detection (the limits of detection of IL-17 and TNFα are 10 and 2 pg/mL, respectively). However, serum IL-10, IL-12p40, IL-6, IL-8, MIP-1a, TNFα, and MMP-9 concentrations did not differ significantly between the MSMP and MRMP groups.

Multiple Logistic Regression Analysis for the Related Factors Associated With Macrolide-Resistant *Mycoplasma pneumoniae*

To further evaluate the predictors associated with MRMP, multiple logistic regression was performed. The older children and duration of fever from onset to admission played a significant role in predicting the MRMP, with the odds ratio values of 2.671 and 1.408 (Supplementary Table).

DISCUSSION

To our knowledge, this is the first study on the molecular epidemiology, clinical characteristics, and cytokine profiles of childhood *M pneumoniae* infection in Taiwan. We analyzed 138 isolates from 5 health centers and identified the predominant strain MLVA type P (4-4-5-7-2), accounting for 38.8% of isolates, between March 2017 and June 2019. The MRMP isolates harboring the A2063G mutation were significantly associated with MLVA type U (5-4-5-7-2) and X (6-4-5-7-2).

A significant increase in macrolide resistance rate (38.8%) was observed in our study compared with the 12%–24% rate observed during 2011–2016 in Taiwan [5, 6]. Our data also revealed that the MRMP infection rate had increased by more than 5-fold from 10.9% in 2017 to 61.5% in 2019. The increase in MRMP rate emphasizes the need for establishing a continuous surveillance system with a more formative protocol. The prevalence of macrolide resistance in Taiwan was higher than that in North America and Europe (10%) but lower than that in Japan and China (90%–100%) [5]. An increase in MRMP has been reported in other Asian countries, such as South Korea and Hong Kong [5, 22, 23]. The trend and pattern of MRMP infection rates varied among these countries, which may reflect the genetic characteristics of *M pneumoniae* and antimicrobial pressure in different geographic regions.

In the present study, 138 *M pneumoniae* clinical isolates were successfully sequenced and divided into 17 MLVA types. The types P (4-4-5-7-2), J (3-4-5-7-2), U (5-4-5-7-2), and A (1-4-5-7-2) were the most common. However, the most common types reported in China and France were U (5-4-5-7-2), X (6-4-5-7-2), P (4-4-5-7-2), J (3-4-5-7-2), and E (2-4-5-7-2) [24, 25], which might be attributed to geographical differences. Several studies have proposed the 4-loci classification scheme by exclusion of the unstable Mpn1 locus to improve classification accuracy [21]. By using this 4-loci typing scheme, the most prevalent MLVA genotype in this study was 4-5-7-2, which was consistent with a previous study in Taiwan [6] and other reports worldwide, including in the United States, Asia, and Europe [9–11, 22, 26].
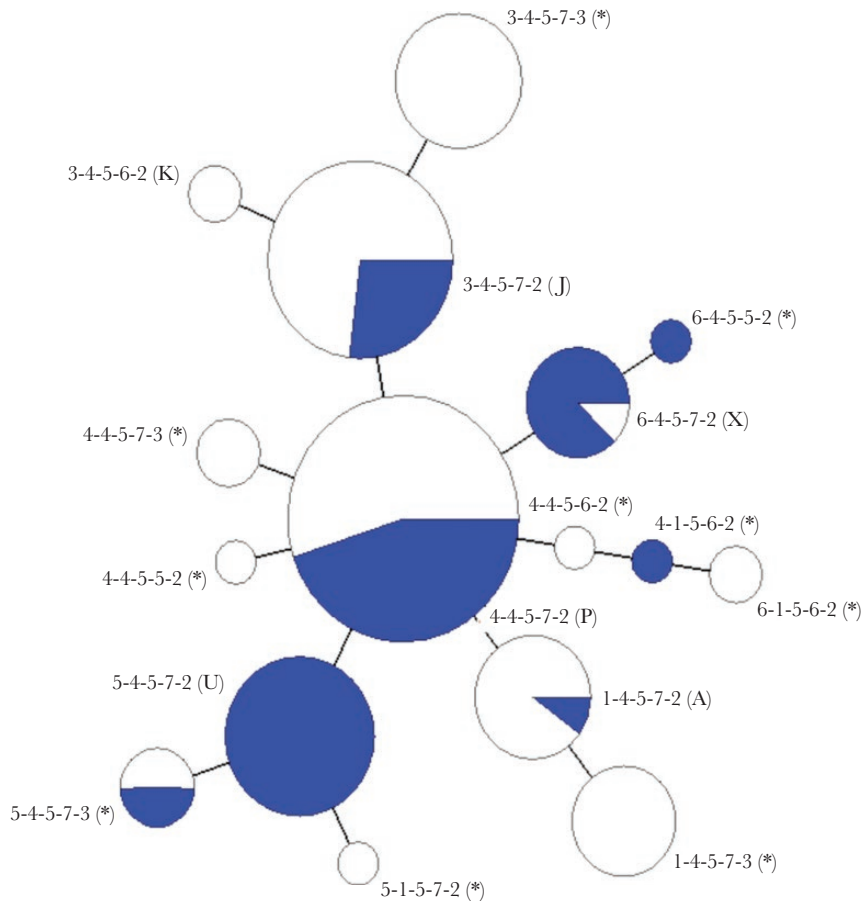
Figure 2. Minimum spanning tree of the multilocus variable-number tandem-repeat analysis (MLVA) profiles of 138 *Mycoplasma pneumoniae* isolates. Each circle represents a particular MLVA type, and similar types are connected by lines. The color of the circles indicates susceptible (white) and resistant (blue) isolates.

The MLVA type 4-5-7-2 (Mpn13-16) had the highest macrolide resistance rate in our study. The prevalence of MLVA type 4-5-7-2 infection increased with time, which was also reported in Hong Kong, China, and Japan [9, 11, 22]. Although MLVA type 4-5-7-2 was also the predominant type in the United States and Europe, the macrolide resistance rate was low [10, 24]. By using 5-loci scheme to further differentiate MLVA type 4-5-7-2 isolates, MLVA type U (5-4-5-7-2) and X (6-4-5-7-2) were found to be significantly associated with high macrolide resistance rate. Moreover, the prevalence rate of MLVA type U drastically increased from 0% in 2017 to 45% in 2019; this increasing rate trend was positively correlated with MRMP rate. In China, MLVA type U (5-4-5-7-2) was also reported as the prevalent type in patients with CAP in combination with a high macrolide resistance rate and clinical severity [25]. Therefore, we speculated that the emergence of MLVA type U (5-4-5-7-2) was associated with macrolide resistance epidemics in Taiwan.

Among the clinical features of MRMP infection, fever duration has been reported as significantly longer in MRMP infection due to the decreased efficacy of macrolides [27]. In our data, fever duration from onset to admission and after antibiotic therapy initiation in MRMP RTIs showed a significant difference; the clinical manifestations, chest radiographic findings, and laboratory data were not significantly affected by macrolide resistance. Although a few studies showed more severe radiological findings or complications after MRMP infection [28], there appeared to be no significant difference in the pooled data of a previous meta-analysis [27]. Moreover, the chest radiographic findings of *M pneumoniae* infection in children were nonspecific and variable [29]. Doxycycline or a fluoroquinolone should be considered in patients with fever lasting >72 hours after macrolide treatment because of its good activity against MRMP isolates. In this study, only 33% of patients with MRMP who received macrolide treatment needed to use doxycycline alternatively. The reason for this still needs to be further studied.

Cell-mediated immunological response plays an important role in the progression of *M pneumoniae* infection. Several studies demonstrated that certain serum cytokines and chemokines, such as IL-6, IL-10, IFN-γ, and serum lactate dehydrogenase, are considered good biomarkers for predicting severity. However, because these studies generally addressed refractory *M pneumoniae* infection [14, 15], the results cannot be

Table 2. Comparison of Patient Characteristics Between MSMP and MRMP Respiratory Tract Infections

Characteristic	MSMP (n = 85)	MRMP (n = 53)	P Value
Age Group (Years)			
0–6	58 (68.2%)	26 (49.1%)	.025
7–14	27 (31.8%)	27 (50.9%)	
Gender (male/female)	42/43	21/32	.262
Duration of Symptom From Onset to Admission (Days)			
Fever	2.39 ± 1.53	3.94 ± 3.76	.006
Cough	4.98 ± 5.86	7.13 ± 7.40	.061
Shortness of breath	0.49 ± 1.20	0.79 ± 1.97	.325
Diagnosis			.384
Upper respiratory tract infection	0	1 (1.9%)	
Lower respiratory tract infection	85 (100%)	52 (98.1%)	
Signs and Symptoms			
Rhinorrhea	60 (70.6%)	40 (75.5%)	.532
Cough	83 (97.6%)	50 (94.3%)	.372
Rales breath sounds	31 (36.5%)	25 (47.2%)	.213
Wheeze breath sounds	28 (32.9%)	11 (20.8%)	.122
Dyspnea	22 (25.9%)	9 (17%)	.223
Cyanosis	2 (2.4%)	0	.523
GI tract discomfort	18 (21.2%)	5 (9.4%)	.072
Rash	5 (5.9%)	3 (5.7%)	1
Neurologic symptom	2 (2.4%)	1 (1.9%)	1
Laboratory Findings			
White blood cell count (k/μL)	12.55 ± 13.45	11.46 ± 14.58	.653
C-reactive protein (mg/dL)	2.74 ± 4.12	2.53 ± 2.82	.724
Initially Prescribed Antibiotics			.704
Azithromycin	60 (70.6%)	39 (73.6%)	
Doxycycline	25 (29.4%)	14 (26.4%)	
Antibiotic switch from azithromycin to doxycycline	10/60 (16.7%)	13/39 (33.3%)	.055
Fever duration after the start of antibiotic therapy (hour)	50.19 ± 40.07	62.72 ± 35.67	.04
Hospital stay (days)	5.99 ± 3.11	5.60 ± 2.40	.411
Abnormal findings in chest x-ray	55 (64.7%)	39 (73.6%)	.374
Focal reticular pattern	5	6	.339
Pneumonia patch	13	12	.32
Perihilar peribronchial opacification	14	6	.35
Consolidation or pseudoconsolidation	2	2	1
Diffuse interstitial pattern	19	16	.361
Hilar lymphadenopathy	0	1	.393
Pleural effusion	2	2	1

Abbreviations: MSMP, macrolide-sensitive *Mycoplasma pneumoniae*; MRMP, macrolide-resistant *M pneumoniae*.

extrapolated to all *M pneumoniae* infection. The reason for the significantly high serum IL-13 and IL-33 levels in the MRMP group as well as the underlying mechanisms of these cytokines should be investigated in the future. Despite being limited to severe cases, analysis from our clinical features and cytokine profiles presented a general view of *M pneumoniae* lower RTI. Although a few differences, such as febrile days and expression

of certain cytokines, were found, our study shows that macrolide resistance does not seem to be a factor affecting overall clinical severity, which is in agreement with a previous meta-analysis [27].

There are some limitations to our study. First, the use of single positive serum *Mycoplasma* IgM result as inclusion criteria may not be appropriate owing to the low sensitivity and specificity of this test. Thus, the diagnosis of *M pneumoniae* infection was defined by PCR and culture reports in this study, which has been accepted as a practical way to diagnose *M pneumoniae* infection. Measurement of *M pneumoniae*-specific IgM-secreting cells using enzyme-linked immunospot assay has been proposed as a better way to determine *M pneumoniae* RTI but is not available in most clinical practices [30]. Second, potential viral or bacterial coinfection was not investigated in our study. The coinfection rate with *M pneumoniae* is approximately 20% in some studies, the most common of which is human rhinovirus [31, 32]. Third, pathogen detection and comparison of clinical features of the other 151 patients with positive serum *M pneumoniae* IgM report but negative *M pneumoniae* PCR or culture report were not further analyzed.

CONCLUSIONS

In conclusion, macrolide resistance rate of *M pneumoniae* has increased significantly in Taiwan. The MLVA type 4-5-7-2 is the macrolide-resistant clone prevailing in Taiwan, and type U (5-4-5-7-2) is associated with the surge of resistance. Moreover, our results suggested that certain inflammatory cytokines, such as IL-13 and IL-33, may play some roles in MRMP infection. The older children and febrile periods from onset to admission were significantly associated with MRMP infection. However, no differences in severity between MRMP and MSMP infection were found. Further research on the immunopathogenic mechanism of *M pneumoniae* infection is needed.

Table 3. Comparing Serum Cytokine Profiles Between MSMP and MRMP Respiratory Tract Infections

Cytokine	MSMP (n = 80)	MRMP (n = 48)	P Value
IL-10	5.18 (1.73–10.52)	4.70 (0.56–11.64)	.433
IL-12p40	129.45 (67.61–284.22)	134.30 (74.71–316.79)	.457
IL-13	0.08 (0–0.93)	0.63 (0–3.05)	.015
IL-33	0.85 (0–15.91)	17.45 (1.30–64.94)	<.001
IL-6	10.78 (2.99–20.05)	10.19 (0.69–40.61)	.799
IL-8	15.04 (1.97–98.38)	14.81 (3.23–88.84)	.906
MIP-1a	38.56 (11.17–100.90)	39.87 (12.62–82.85)	.976
MMP-9[a]	5578.29 (1252.71–12 912.86)	7416.87 (2879.65–20 050.22)	.137

Abbreviations: MMP, matrix metalloproteinase; MSMP, macrolide-sensitive *Mycoplasma pneumoniae*; MRMP, macrolide-resistant *M pneumoniae*.

[a]MMP-9 is a gelatinase.

NOTE: Data are presented as median values (25th–75th interquartile ranges) of cytokine concentration (pg/mL).

Supplementary Material

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Supplementary Figure. Enrollment flow diagram. IgM, immunoglobulin M; RTI, respiratory tract infection.

Supplementary Table. Multiple logistic regression analysis of the factors associated with MRMP.

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