ORIGINAL ARTICLE

Clinical features and seasonal variations in the prevalence of macrolide-resistant *Mycoplasma pneumoniae*

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Abstract

Background: *Mycoplasma pneumoniae* is a common pathogen causing pneumonia; macrolide-resistant strains are rapidly spreading across Japan. However, the clinical features of macrolide-resistant *M. pneumoniae* pneumonia have not been well established. Here, we evaluated the clinical characteristics and seasonal variations in the prevalence of *M. pneumoniae* with macrolide-resistant mutations (MRM).

Methods: The monthly prevalence of MRM in *M. pneumoniae* strains isolated from May 2016 to April 2017 was retrospectively analyzed, and the clinical characteristics of pneumonia cases with MRM were compared to those of cases without MRM. The *M. pneumoniae* isolates and point mutations at site 2063 or 2064 in domain V of 23S rRNA were evaluated by the GENECUBE system and GENECUBE Mycoplasma detection kit.

Results: *Mycoplasma pneumoniae* infection was identified in 383 cases, including 221 cases of MRM (57.7%). The MRM prevalence was 86.3% (44/51) between May and July 2016, demonstrating an apparent decrease in September 2016, subsequently reaching 43.0% (34/79) in November 2016. *Mycoplasma pneumoniae* pneumonia was diagnosed in 275 cases, including 222 pediatric and 53 adult cases. Macrolide use preceding evaluation was found to be the only feature of MRM pneumonia cases both in children (odds ratio [OR] 3.86, 95% confidence interval [CI]:1.72–8.66) and in adults (OR 7.43, 95% CI: 1.67–33.1).

Conclusions: The determination rate of MRM varied widely throughout the year, and our study demonstrated the challenges in predicting *M. pneumoniae* with MRM based on clinical features at diagnosis. Therefore, continuous monitoring of the prevalence of MRM is warranted, which may help in selecting an effective treatment.

KEYWORDS
GENECUBE, macrolide resistance, *Mycoplasma pneumoniae*, point-of-care molecular testing, prevalence

1 | INTRODUCTION

Mycoplasma pneumoniae is one of the most common pathogens in community-acquired pneumonia (CAP), determined in 41.1% of pediatric and 13.0% of adult CAP cases in Japan.[1,2] Most available antimicrobial agents, especially beta-lactam agents,[3] are not effective against *M. pneumoniae*;[4] thus, risk factors and clinical manifestations have been intensively investigated. Till date, school-aged children and adolescents,[3] persistent cough,[5] vomiting or diarrhea,[6] and autumn or winter outbreak[7] have been identified as factors associated with *M. pneumoniae* infections.

Macrolides are used as the first-line therapy for the treatment of *M. pneumoniae* infections, especially in children, owing to their high efficacy[8] and safety profile.[9,10] However, macrolide resistance of *M. pneumoniae*, which is acquired by mutations of the ribosomal target of macrolides,[11] has been spreading rapidly and widely since 2000,[12] resulting in enhanced disease severity with increased complications.[13,14]

The prevalence of macrolide-resistant *M. pneumoniae* is 23.3%-100% in East Asian regions, 3.5%-13.2% in North America, and below 10% in European countries, except Italy, which has a higher rate of 26%.[11] Tanaka et al[15] reported a year-by-year variation in the prevalence of macrolide-resistant *M. pneumoniae* in Japan, demonstrating an increase from 55.6% to 81.6% between 2008 and 2012, with a gradual decrease thereafter, reaching 43.6% by 2015. Despite this decrease, the macrolide resistance rate of *M. pneumoniae* remains high in Japan, and its clinical importance is unaltered. However, the clinical features of macrolide-resistant *M. pneumoniae* at diagnosis are not well established, making it difficult to predict before starting treatment.

To improve the diagnosis and management of macrolide-resistant *M. pneumoniae* pneumonia, this study aimed to determine the seasonal trend in the determination rate of macrolide-resistant mutations (MRM) among 383 *M. pneumoniae* cases diagnosed within a single year (2016/2017). We further compared the clinical characteristics of *M. pneumoniae* pneumonia cases according to the presence of MRM evaluated by the GENECUBE system and GENECUBE Mycoplasma detection kit.

2 | MATERIALS AND METHODS

Tsukuba Medical Center Hospital (TMCH; 453 beds), an acute care teaching hospital located in Tsukuba city in Japan, is the primary pediatric emergency center and tertiary emergency medical center in the southern part of the Ibaraki Prefecture. We retrospectively analyzed the clinical data of cases with a positive *M. pneumoniae* result determined by a molecular identification system (GENECUBE, TOYOBO, Co., Ltd., Osaka, Japan) at TMCH between May 2016 and April 2017. This study was approved by the ethics committee of TMCH (approval number: 2016-043).

2.1 | Definition of pneumonia and evaluation of data

The diagnosis of pneumonia was made based on radiological findings and clinical symptoms compatible with pneumonia, without other causes attributed to abnormal radiological findings.[16,17] The radiological finding was reviewed by two independent physicians, and only cases diagnosed as pneumonia by both physicians were included as pneumonia cases. Discordant cases were further reviewed by a board-certified radiologist to make a final diagnosis.

For background data, we collected information on age, gender, comorbidities, evaluation month, history of preceding antimicrobial use, history of symptoms (rhinorrhea, sputum or productive cough, severe cough, hypoxia, diarrhea, skin rashes, high fever [≥38°C]), crackles upon chest auscultation at presentation, duration of symptoms at examination, laboratory findings (white blood cell count [WBC] and C-reactive protein values [CRP]) obtained on the previous or same day of the molecular examination, sources of infections, and requirement of hospitalization before or at presentation. Cases were regarded as having comorbidities when they were diagnosed with chronic diseases defined by the Charlson comorbidity index[18] or equivalent. A severe cough was defined as a cough with vomiting, sleep disturbance, or a constant cough. Hypoxia was defined as a peripheral capillary oxygen saturation level <90% or partial arterial pressure of oxygen <60 mm Hg.

2.2 | Molecular diagnosis of *M. pneumoniae* and identification of MRM

Samples were obtained from the oropharynx using FLOQSwabs 5U005S dual (Sin Corporation, Tokyo, Japan) and were used for molecular examination of *M. pneumoniae* with the GENECUBE system and GENECUBE Mycoplasma detection kit at TMCH, which has been approved in Japan since 2015. In addition to the presence of *M. pneumoniae*, point mutations at domain V of the 23S ribosomal RNA gene (2063 and 2064) of *M. pneumoniae* were analyzed simultaneously with melting curve analysis using the previously validated quenching probe method.[19]

2.3 | Statistical analyses and data evaluation

We compared the clinical characteristics between *M. pneumoniae* pneumonia cases with and without MRM. This comparison was performed separately for pediatric cases (<18 years of age) and adult cases (≥18 years). Categorical variables were compared using Fisher's exact test, and continuous variables were analyzed using the Mann-Whitney *U* test. Variables with a significant association ($P < 0.05$) in the univariate analysis were included in the multivariate logistic regression analysis after considering potentially confounding factors. All statistical analyses were performed using R 3.3.1.
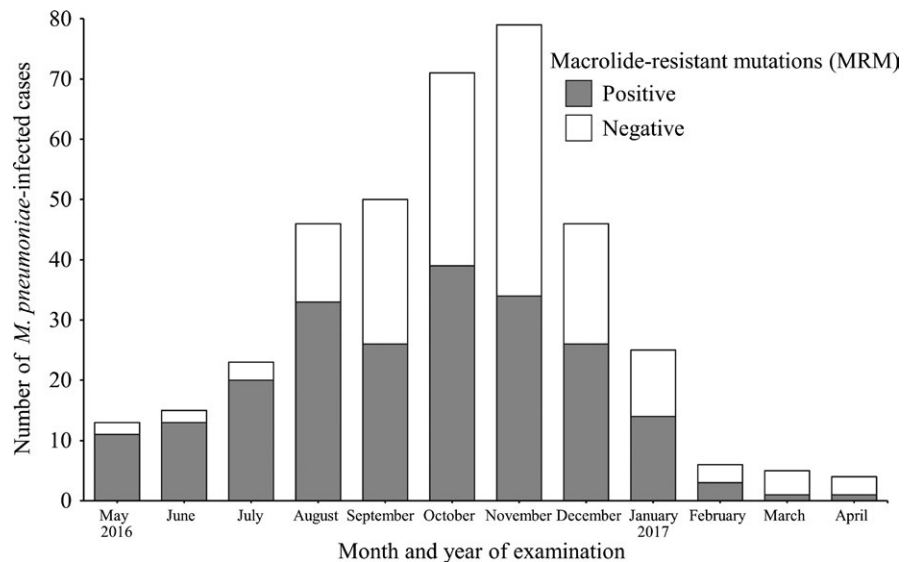
FIGURE 1 Seasonal change in the number of *Mycoplasma pneumoniae*-infected cases and the proportion of cases with macrolide-resistant mutations (MRM)

3 | RESULTS

3.1 | Monthly trends in the number of cases infected with *M. pneumoniae* harboring MRM

We examined 1307 individual cases for the determination of *M. pneumoniae* using the GENECUBE system and GENECUBE Mycoplasma detection kit between May 2016 and April 2017; *M. pneumoniae* was identified in a total of 384 cases. Of these, one case was excluded from our study because colonization was highly suspected. Therefore, we included a total of 383 *M. pneumoniae* infection cases in the final analysis.

Figure 1 depicts the monthly trends of the number of *M. pneumoniae* infection cases with and without MRM. The number of *M. pneumoniae* infection cases markedly increased as of August, with 71 cases recorded in November 2016, which is in line with the number of reported cases of *M. pneumoniae* pneumonia in Ibaraki Prefecture.[20]

In total, MRM was determined in 221 cases (57.7%) with high monthly variation. MRM was identified in 86.3% of cases (44/51 cases) between May and July, which apparently decreased as of September, reaching 43.0% (34/79 cases) in November 2016.

3.2 | Demographic data of all cases infected with *M. pneumoniae*

Table 1 summarizes the demographic data of all 383 *M. pneumoniae* infection cases. The median age was 8.0 years (47.8% female), and a large majority of cases (>80%) was pediatric. Asthma was the most common comorbidity identified (11.2%). Antimicrobials were prescribed for over half of the cases before evaluation, with the majority prescribed macrolides (29.2%), and the median duration from symptom onset to evaluation was 7.0 days. Among the observed clinical signs and symptoms, fever ≥38°C was the most frequent, followed by sputum or productive cough, severe cough, rhinorrhea, crackles auscultated upon chest examination, diarrhea, skin rashes,

and hypoxia. The median WBC count and CRP values were 7100/μL and 2.20 mg/dL, respectively. Pneumonia was the most common diagnosis and accounted for 275 cases (71.8%). There was no mortality in any of the cases included in this study, whereas 23.0% of the cases required hospitalization on the day of or before evaluation.

3.3 | Clinical characteristics of pneumonia caused by *M. pneumoniae* with MRM

Among the 275 pneumonia cases determined to be caused by *M. pneumoniae*, 222 were pediatric and 53 were adult cases. Comparison of clinical characteristics of pediatric and adult pneumonia cases, with and without MRM, is shown in Table 2 and Table 3, respectively.

In the 222 pediatric cases, MRM was found in 145 (65.3%) cases. As shown in Table 2, neither the median age nor gender distribution significantly differed between the two groups. Fewer comorbidities were identified among the cases with MRM (7.6% vs 22.1%, *P* = 0.003), whereas these cases showed a higher frequency of history of antimicrobial use, especially macrolide use (43.4% vs 13.0%, *P* < 0.001). No significant difference was obtained with respect to specific clinical signs and symptoms between the groups. In addition, the rate of the requirement of hospitalization was essentially identical. Multivariate logistic regression model showed that macrolide use before evaluation (odds ratio [OR] 3.86, 95% confidence interval [CI]: 1.72-8.66) and the presence of comorbidities (OR 0.23, 95% CI: 0.09-0.60) were significant independent variables for the determination of MRM in pediatric *M. pneumoniae* pneumonia cases.

The characteristics of 53 adult *M. pneumoniae* pneumonia cases are presented in Table 3, including 26 cases with MRM (49.1%). There was no significant difference between the cases with and without MRM in terms of age, gender distribution, presence of comorbidities, and any clinical signs and symptoms. The significant variables in univariate analyses were the use of macrolides before

		Macrolide-resistant mutations	
	Total	Positive	Negative
N	383	221/383	162/383
Age (y)	8.0 [5.0-13.0]	7.0 [5.0-12.0]	9.0 [5.0-16.0]
Children (<18 y old)	315 (82.2)	190 (86.0)	125 (77.2)
Female	183 (47.8)	101 (45.7)	82 (50.6)
Comorbidities	54 (14.1)	22 (10.0)	32 (19.8)
Asthma	43 (11.2)	18 (8.1)	25 (15.4)
Immunosuppressive state	2 (0.5)	2 (0.9)	0 (0.0)
Preceding antimicrobial use	214 (55.9)	148 (67.0)	66 (40.7)
Macrolides	112 (29.2)	93 (42.1)	19 (11.7)
Quinolones/Tetracyclines	65 (17.0)	46 (20.8)	19 (11.7)
Onset to evaluation (d)	7.0 [5.0-8.0]	7.0 [6.0-8.0]	6.0 [4.0-8.0]
Rhinorrhea	64 (16.7)	31 (14.0)	33 (20.4)
Sputum or productive cough	98 (25.6)	56 (25.3)	42 (25.9)
Severe cough	90 (23.5)	48 (21.7)	42 (25.9)
Hypoxia	9 (2.3)	4 (1.8)	5 (3.1)
Diarrhea	19 (5.0)	13 (5.9)	6 (3.7)
Skin rashes	12 (3.1)	9 (4.1)	3 (1.9)
Fever (≥38°C)	289 (75.5)	174 (78.7)	115 (71.0)
Crackles on chest auscultation	45 (11.7)	29 (13.1)	16 (9.9)
White blood cell count (/μL)	7100 [5700-8800]	6800 [5500-8450]	7400 [5900-9425]
C-reactive protein (mg/dL)	2.20 [1.10-4.58]	2.00 [1.04-4.13]	2.52 [1.27-5.40]
Diagnosis			
Pneumonia	275 (71.8)	171 (77.4)	104 (64.2)
Bronchitis/URI/sinusitis	99 (25.8)	46 (20.8)	53 (32.7)
Others	9 (2.3)[a]	4 (1.8)	5 (3.1)
Requirement of hospitalization	88 (23.0)	55 (24.9)	33 (20.4)

TABLE 1 Demographic data of all cases of *Mycoplasma pneumoniae* infection with and without macrolide-resistant mutations

URI, upper respiratory infection.

Categorical data are presented as the number (proportion, %). Continuous data are presented as the median [interquartile range].

[a]Only fever (five cases), chronic cough (two cases), tonsillitis (one case), and exudative erythema multiforme (one case) are included.

evaluation (53.8% vs 11.1%, *P* = 0.001) and the median time from symptom onset to evaluation (8.0 days vs 5.0 days, *P* = 0.002). Multivariate logistic regression model revealed that in adults, only preceding macrolide use was significantly associated with *M. pneumoniae* pneumonia cases with MRM (OR 7.43, 95% CI: 1.67-33.1).

4 | DISCUSSION

In this study, we demonstrated a high variation in MRM determination rate in *M. pneumoniae* within a single year. In parallel with the increasing number of *M. pneumoniae* infections determined between August and November 2016, the proportion of *M. pneumoniae* with MRM substantially decreased. In addition, macrolide use preceding evaluation was the only characteristic finding among the cases of *M. pneumoniae* pneumonia with MRM, observed both in children and in adults.

Clinical characteristics of macrolide-resistant *M. pneumoniae* have been evaluated in previous studies. Cao et al[21] described that the presence of macrolide resistance in *M. pneumoniae* did not alter the clinical presentation, including age, gender distribution, fever grade, cough, sputum, shortness of breath, and chest pain. Furthermore, WBC count, CRP values, and radiological findings were reported not to be influenced by macrolide resistance.[22] Consistent with previous studies, our results indicated that clinical signs and symptoms were almost identical between pneumonia cases with and without MRM,[21,22] except for the macrolide prescription rate before evaluation.[23] The higher macrolide prescription rate before evaluation, observed in MRM pneumonia cases, could be explained by the poor clinical response to the initial treatment, which may contribute to increased hospital visits.

Collecting the updated data of MRM determination rates among cases of *M. pneumoniae* infections may help to predict macrolide

TABLE 2 Factors associated with the determination of macrolide-resistant mutations among pediatric cases with *Mycoplasma pneumoniae* pneumonia

	Macrolide-resistant mutations		P value	Adjusted P value
	Positive	Negative		
N	145	77		
Age (y)	7.0 [5.0-9.0]	6.0 [3.0-10.0]	0.32	
School age (6-17 y old)	100 (69.0)	45 (58.4)	0.14	
Female	73 (50.3)	38 (49.4)	1.00	
Comorbidities	11 (7.6)	17 (22.1)	0.003	0.003
Asthma	9 (6.2)	13 (16.9)	0.02	
Immunosuppressive state	2 (1.4)	0 (0.0)	0.55	
Preceding antimicrobial use	101 (69.7)	34 (44.2)	<0.001	
Macrolides	63 (43.4)	10 (13.0)	<0.001	0.001
Quinolones/Tetracyclines	35 (24.1)	11 (14.3)	0.12	
Onset to evaluation (d)	7.0 [6.0-8.0]	7.0 [5.0-8.0]	0.15	
Rhinorrhea	16 (11.0)	15 (19.5)	0.10	
Sputum or productive cough	31 (21.4)	19 (24.7)	0.61	
Severe cough	34 (23.4)	22 (28.6)	0.42	
Hypoxia	4 (2.8)	3 (3.9)	0.70	
Diarrhea	6 (4.1)	3 (3.9)	1.00	
Skin rashes	6 (4.1)	0 (0.0)	0.10	
Fever (≥38°C)	122 (84.1)	62 (80.5)	0.58	
Crackles on chest auscultation	24 (16.6)	13 (16.9)	1.00	
White blood cell count (/µL)	6800 [5550-8450]	7650 [6225-10 000]	0.008	0.05
C-reactive protein (mg/dL)	1.80 [1.08-3.63]	2.23 [1.44-4.00]	0.22	
Requirement of hospitalization	44 (30.3)	23 (29.9)	1.00	

Categorical data are presented as the number (proportion, %). Continuous data are presented as the median [interquartile range].

resistance and select effective treatment strategy accordingly. In Japan, the Infectious Disease Control Law mandates that the number of *M. pneumoniae* pneumonia cases diagnosed at designated sentinel sites be reported weekly, although these data do not include the prevalence trends of macrolide resistance. The rate of macrolide resistance was shown to vary from 50% to 93% across the seven districts of Japan,[24] as well as among areas in the same prefecture ranging from 0 to 100% (Hokkaido).[23] In this study, we employed the GENECUBE system and GENECUBE Mycoplasma detection kit for point-of-care molecular examinations, which can determine the presence of *M. pneumoniae* and MRM within only 1 hour. The reliability of mutation analysis with the GENECUBE system and GENECUBE Mycoplasma detection kit has previously been demonstrated, showing results identical with those obtained with a conventional sequence method (absence of mutations 82/173, A2063G 90/173, A2064G 1/173). Our results revealed that the rate of *M. pneumoniae* with MRM varied remarkably from season to season within the same year, which was possibly because of the change in endemic strains.[25] Therefore, determining the exact trend of prevalence of *M. pneumoniae* with MRM requires continuous monitoring, conducted locally throughout the year, which could be helpful for the antimicrobial stewardship in primary care settings.

Macrolides and tetracyclines have an excellent therapeutic effect against *M. pneumoniae*,[26] and defervescence is usually achieved within 48-72 hours in successful *M. pneumoniae* pneumonia cases.[27] However, tetracyclines are contraindicated in children less than 8 years of age, who are at a high risk of *M. pneumoniae* infections. Tosufloxacin is effective against both macrolide-susceptible and macrolide-resistant strains, although its weak bactericidal effect limits its therapeutic efficacy.[27] Taken together, especially in pediatric patients, first-line treatment for macrolide-susceptible *M. pneumoniae* pneumonia should be macrolides, whereas tetracycline or tosufloxacin should be preserved for macrolide-resistant *M. pneumoniae* pneumonia, and tetracycline is preferred choice for cases older than 8 years of age.

Excessive use of macrolides and quinolones for respiratory infections has been a major concern in Japan,[28] because of their possible association to high macrolide resistance rates among *Streptococcus pneumoniae*[29] and *M. pneumoniae*,[7] and increased quinolone resistance among *Enterobacteriaceae*.[30] Besides the reduction in antibiotic use, we consider that rapid diagnoses and early initiation of effective treatment may prevent the transmission of *M. pneumoniae* and consequently reduce the spread of macrolide-resistant *M. pneumoniae*. Combination of clinical judgment and point-of-care molecular

TABLE 3 Factors associated with the determination of macrolide-resistant mutations among adult cases with *Mycoplasma pneumoniae* pneumonia

	Macrolide-resistant mutations		P value	Adjusted P value
	Positive	Negative		
N	26	27		
Age (y)	27.5 [21.0-33.8]	31.0 [25.0-37.5]	0.17	
Female	12 (46.2)	14 (51.9)	0.79	
Comorbidities	3 (11.5)	4 (14.8)	1.00	
Asthma	2 (7.7)	1 (3.7)	0.61	
Immunosuppressive state	0 (0.0)	0 (0.0)	-	
Preceding antimicrobial use	18 (69.2)	13 (48.1)	0.17	
Macrolides	14 (53.8)	3 (11.1)	0.001	0.009
Quinolones/Tetracyclines	5 (19.2)	2 (7.4)	0.25	
Onset to evaluation (d)	8.0 [6.0-9.0]	5.0 [4.0-6.0]	0.002	0.06
Rhinorrhea	1 (3.8)	6 (22.2)	0.10	
Sputum or productive cough	18 (69.2)	14 (51.9)	0.26	
Severe cough	5 (19.2)	3 (11.1)	0.47	
Hypoxia	0 (0.0)	2 (7.4)	0.49	
Diarrhea	3 (11.5)	0 (0.0)	0.11	
Skin rashes	0 (0.0)	0 (0.0)	-	
Fever (≥38°C)	20 (76.9)	21 (77.8)	1.00	
Crackles on chest auscultation	3 (11.5)	1 (3.7)	0.35	
White blood cell count (/μL)	6850 [5750-7975]	6700 [5300-8600]	0.76	
C-reactive protein (mg/dL)	6.51 [4.67-12.17]	9.00 [5.09-11.21]	0.93	
Requirement of hospitalization	10 (38.5)	6 (22.2)	0.24	

Categorical data are presented as the number (proportion, %). Continuous data are presented as the median [interquartile range].

examination may assist in the appropriate prescription of antimicrobials, because of their high diagnostic performance for *M. pneumoniae* infections and determination of MRM. Further studies are required to investigate the optimal time for molecular examinations and favorable treatment strategy for *M. pneumoniae* pneumonia with MRM.

The present study has certain limitations. First, because of the nature of our institution and limitations of the retrospective study, the results could be biased and may not be comparable to those obtained in other hospitals. Second, our patients are likely to be cases with poor clinical response cases or those with unstable conditions. Cases without MRM or comorbidities could have been successfully treated at primary clinics or hospitals; thus, selection bias may have facilitated the prevalence of comorbidities, including asthma and the rate of MRM determination. Third, we did not evaluate the mutations at site 2617 of the 23S rRNA region, as it could not be determined by the GENECUBE system and GENECUBE Mycoplasma detection kit. However, a previous study showed that mutations at site 2617 were rarely determined in Japan (0.3%),[15] and these mutations are also known to confer only weak resistance to macrolides.[31] Therefore, any undetermined mutations at site 2617 were considered to have a negligible impact on the general conclusions of our study.

5 | CONCLUSION

The results of this study point out the difficulty in the diagnosis of *M. pneumoniae* pneumonia cases with MRM based on clinical signs and symptoms alone, because the only distinctive feature of *M. pneumoniae* with MRM was found to be a preceding use of macrolides. Therefore, local and continuous monitoring of the rate of MRM among *M. pneumoniae* clinical isolates is warranted to improve infection management.

research expenses for the quality evaluation of the GENECUBE and GENECUBE Mycoplasma systems.

CONFLICT OF INTEREST

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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