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Macrolide Resistance and Its Impacts on *M. Pneumoniae* Pneumonia in Children: Comparison of Two Recent Epidemics in Korea

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Purpose: The aim of this study was to investigate the change in macrolide resistance rate in pediatric *Mycoplasma pneumoniae* pneumonia and to evaluate the influence of macrolide-resistant *M. pneumoniae* (MRMP) on the clinical course of disease, by comparing 2 recent, consecutive epidemics in Korea. **Methods:** A total of 250 patients with *M. pneumoniae* pneumonia admitted to a single tertiary hospital were enrolled in this study. Detection of MRMP was based on specific point mutations in domain V of the 23S rRNA gene. The medical records of enrolled patients were reviewed retrospectively, and the clinical courses and laboratory data were compared. **Results:** The macrolide resistance rate of *M. pneumoniae* was 51.1% (48/94) in the 2011 epidemic, and 87.2% (136/156) in the 2015 epidemic. All MRMP isolates had the A2063G point mutation. In comparison of 2 epidemics, the mean age of patients with *M. pneumoniae* pneumonia was increased, and the total febrile days and febrile days after initiation of macrolides were prolonged in the 2015 epidemic. Overall severity of MRMP or macrolide-susceptible *M. pneumoniae* (MSMP) pneumonia over 2 epidemics was not significantly changed. However, the proportion of patients who had a fever lasting more than 72 hours after initiation of macrolides and who received corticosteroid treatment were higher in MRMP pneumonia during 2 epidemics. **Conclusions:** The macrolide resistance rate of *M. pneumoniae* has risen rapidly over 2 recent, consecutive epidemics, and this has been associated with a prolonged clinical course and increased use of corticosteroids to treat pediatric *M. pneumoniae* pneumonia.

Key Words: Drug resistance; macrolides; *Mycoplasma pneumoniae*

INTRODUCTION

Mycoplasma pneumoniae is an important cause of community-acquired pneumonia (CAP) in children and young adults. Although *M. pneumoniae* often causes mild to moderate pneumonia, it can also be associated with more serious, life-threatening disease and a wide array of extrapulmonary manifestations.[1,2] *M. pneumoniae* causes up to 40% or more of CAP cases, and as many as 18% of cases requiring hospitalizations in children.[3]

M. pneumoniae is innately resistant to all beta-lactams and glycopeptides due to the lack of a cell wall. In contrast, *M. pneumoniae* is susceptible to antibiotics that interfere with protein or DNA synthesis, such as macrolides, tetracyclines, and quinolones. During the last decade, macrolide-resistant *M. pneumoniae* (MRMP) has been reported worldwide, and its prevalence has generally increased.[4] The prevalence of MRMP is especially high in East Asian countries, such as China, Japan, and Korea.[4-6]

Although the clinical course of MRMP pneumonia appears to be prolonged compared with macrolide-susceptible *M. pneumoniae* (MSMP) pneumonia,[7,8] the clinical relevance of the increased prevalence of MRMP has not been definitely established. Therefore, continuous surveillance of the change in prevalence of MRMP pneumonia and the influence of MRMP on disease outcome are inevitably needed to define its clinical relevance and to develop appropriate treatment strategies at a

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time when MRMP is increasingly being detected.

M. pneumoniae infections occur both endemically and epidemically, in 3-7 years intervals for the latter. In Korea, *M. pneumoniae* epidemics have been observed in 3- to 4-year cycles since the mid-1980s.[9] During the last several *M. pneumoniae* epidemics in Korea, the macrolide resistance rate has increased from 2.9% in 2003 to 62.9% in 2011.[6] However, studies demonstrating changes in the clinical course of disease and in treatment strategies for *M. pneumoniae* pneumonia since 2011 are limited.

The aim of this study was to investigate the change in macrolide resistance rate in pediatric *M. pneumoniae* pneumonia and to evaluate the influence of MRMP on the clinical course of disease by comparing clinical characteristics of *M. pneumoniae* pneumonia that occurred during the epidemics of 2011 and 2015 in Korea.

MATERIALS AND METHODS

Study population

A retrospective cross-sectional study was carried out on pediatric patients with *M. pneumoniae* pneumonia admitted to Korea University Guro Hospital between August and December of 2011 and the same time frame in 2015. Among 620 patients diagnosed with *M. pneumoniae* pneumonia based on clinical symptoms, signs of lower respiratory tract infection, chest radiography, and serologic tests for *M. pneumoniae* (either microparticle agglutination assay titer of ≥1:160 or positive specific immunoglobulin M (IgM) against *M. pneumoniae*),[10] 250 patients were randomly selected during each epidemic. Among those patients, 94 patients in 2011 epidemic and 156 patients in 2015 epidemic were *M. pneumoniae* polymerase chain reaction (PCR) positive. These patients were divided into the MRMP and MSMP pneumonia groups based on the presence of specific point mutations in domain V of the *M. pneumoniae* 23S rRNA genes from nasopharyngeal aspirates (Figure).

The medical records of enrolled patients were reviewed retrospectively. Defervescence was defined as a body temperature below 38°C for at least 24 hours without any use of antipyretics. Patients with pre-existing underlying diseases (such as congenital heart disease, bronchopulmonary dysplasia, immunodeficiency, or malignancy) were excluded. This study was approved by the Institutional Review Board of Korea University Guro Hospital.

Specimens and identification of MRMP

All blood samples and nasopharyngeal aspirates were obtained at the time of admission. Nasopharyngeal aspirates were stored at -80°C until they were used for PCR assay at the end of each epidemic. *M. pneumoniae* DNA was detected by conventional PCR targeting a conserved part of the P1 adhesin gene.[11] The point mutations at sites 2063 and 2064 in domain V of 23S rRNA genes were examined using a direct sequencing method as previously reported.[12]

Statistical analysis

All data are expressed as means and standard deviations, unless otherwise indicated. Continuous variables were compared with Student t or the Mann-Whitney tests. Differences in categorical variables were assessed with the χ^2 test or Fisher's exact

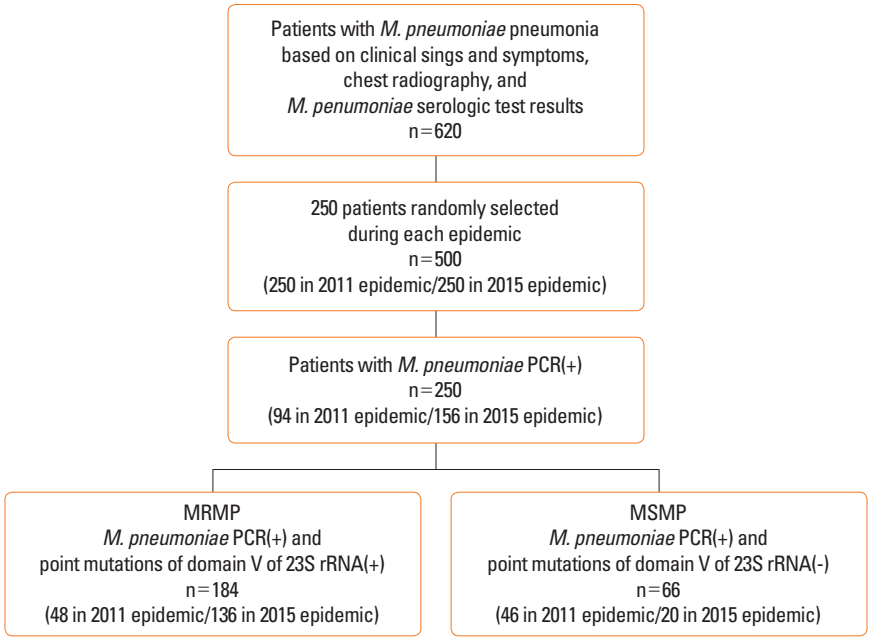

Figure. Study population. MRMP, macrolide-resistant *M. pneumoniae*; MSMP, macrolide-susceptible *M. pneumoniae*; PCR, polymerase chain reaction.

Table 1. Changes in clinical characteristics of *M. pneumoniae* pneumonia over 2 consecutive epidemics

Characteristics	2011 epidemic (n=94)	2015 epidemic (n=156)	*P* value
Age (year)	5.3±3.1	6.0±2.7	0.049
No. of cases			
≤2	24 (25.5)	19 (12.2)	0.004*
3-5	40 (42.6)	65 (41.7)	
≥6	30 (31.9)	72 (46.1)	
Sex (male/female)	49/45	74/82	NS
Total febrile days	7.2±5.4	8.9±3.3	<0.001
Febrile days after initiation of macrolides	3.6±4.8	4.3±3.1	0.001
No. of patients with fever lasting >72 hours after initiation of macrolides	37 (39.3)	83 (53.2)	0.037
Proportion of MRMP cases	48 (51.1)	136 (87.2)	<0.001
Initial antibiotics			
Macrolide alone	1 (1.1)	108 (69.2)	<0.001
Macrolide+β-lactams	93 (98.9)	48 (30.8)	
Change from macrolide to tetracycline or fluoroquinolone	0 (0.0)	1 (0.6)	NS
Steroid use	6 (6.4)	30 (19.2)	0.005
Laboratory findings			
WBC (%)	8,524±3,328	8,381±3,640	NS
Neutrophil	59.1±14.2	62.5±12.0	0.042
LymphocyteCRP (mg/L)	30.3±12.5	26.9±10.2	NS
	28.9±27.7	33.1±28.6	NS
Chest X-ray			
Consolidation, effusion	18 (19.1)	36 (23.1)	NS
Extrapulmonary complication	12 (12.8)	20 (12.8)	NS
Rash	3	10	
Liver function abnormality	8	4	
Proteinuria	1	5	
Arthralgia	0	1	

Values are presented as number (%).
MRMP, macrolide-resistant *M. pneumoniae*; WBC, white blood cell; CRP, C-reactive protein; NS, not significant.
*Cochran-Armitage trend test.

test. SigmaPlot software (Systat Software Inc., San Jose, CA, USA) was used for statistical analysis, and *P* values <0.05 were considered statistically significant.

RESULTS

Change in the macrolide resistance rate in *M. pneumoniae* pneumonia over 2 consecutive epidemics

Among 250 patients with positive *M. pneumoniae* PCR results, the resistant strain was detected in 51.1% (48/94) in the 2011 epidemic and 87.2% (136/156) in the 2015 epidemic. All MRMP isolates had the A2063G point mutation.

Changes in the clinical characteristics of *M. pneumoniae* pneumonia over 2 consecutive epidemics

The clinical course and laboratory findings of patients with *M. pneumoniae* pneumonia in each epidemic are summarized in Table 1. Compared with the 2011 epidemic, the mean age of patients was increased, and both the total febrile days and the febrile days after initiation of macrolides were prolonged in the 2015 epidemic. The proportion of patients who had a fever lasting more than 72 hours after initiation of macrolides and who received corticosteroid treatment were also higher in the 2015 epidemic. However, chest radiologic findings and the incidence of extrapulmonary complications were not significantly different between the 2 epidemics. Among laboratory findings, the total white blood cell (WBC) count was not different between the 2 epidemics, though the relative proportion of neutrophils was higher in the 2015 epidemic.

Comparison of the clinical characteristics of MSMP or MRMP pneumonia between the 2 epidemics

The clinical course and laboratory findings of patients with MSMP or MRMP pneumonia in each epidemic are summa-

Table 2. Comparison of clinical characteristics of MSMP pneumonia during 2 epidemics

Characteristics	2011 MSMP (n=46)	2015 MSMP (n=20)	*P* value
Age (year)	5.4±2.8	5.7±1.9	NS
No. of cases			
≤2	10 (21.7)	2 (10.0)	NS
3-5	21 (45.7)	9 (45.0)	
≥6	15 (32.6)	9 (45.0)	
Sex (male/female)	23/23	5/15	NS
Total febrile days	6.5±3.4	8.0±1.9	0.030
Febrile days after initiation of macrolides	1.9±2.0	2.9±2.1	NS
No. of patients with fever lasting >72 hours after initiation of macrolides	7 (15.2)	5 (25.0)	NS
Steroid use	0 (0.0)	2 (10.0)	NS
Laboratory findings			
WBC	8,204±2,708	9,411±4,254	NS
CRP (mg/L)	30.9±27.2	27.2±30.3	NS
Chest X-ray			
Consolidation, effusion	9 (19.6)	7 (35.0)	NS
Extrapulmonary complication	7 (15.2)	1 (5.0)	NS
Rash	2	1	
Liver function abnormality	4	0	
Proteinuria	1	0	
Arthralgia	0	0	

Values are presented as number (%).
MSMP, macrolide-susceptible *M. pneumoniae*; WBC, white blood cell; CRP, C-reactive protein; NS, not significant.

Table 3. Comparison of clinical characteristics of MRMP pneumonia during 2 epidemics

Characteristics	2011 MRMP (n=48)	2015 MRMP (n=136)	*P* value
Age (year)	5.2±3.4	6.1±2.9	NS
No. of cases			
≤2	14 (29.2)	17 (12.5)	0.009*
3-5	19 (39.6)	56 (41.2)	
≥6	15 (31.2)	63 (46.3)	
Sex (male/female)	26/22	69/67	NS
Total febrile days	7.3±5.0	8.9±3.4	0.035
Febrile days after initiation of macrolides	4.1±3.8	4.4±3.1	NS
No. of patients with fever lasting >72 hours after initiation of macrolides	26 (54.2)	78 (57.4)	NS
Change from macrolide to tetracycline or fluoroquinolone	0 (0.0)	1 (0.7)	NS
Steroid use	6 (12.5)	28 (20.6)	NS
Laboratory findings			
WBC	8,829±3,866	8,187±3,542	NS
CRP (mg/L)	27.2±28.5	34.3±28.4	NS
Chest X-ray			
Consolidation, effusion	9 (18.8)	29 (21.3)	NS
Extrapulmonary complication	5 (10.4)	19 (14.0)	NS
Rash	1	9	
Liver function abnormality	4	4	
Proteinuria	0	5	
Arthralgia	0	1	

Values are presented as number (%).
MRMP, macrolide-resistant *M. pneumoniae*; WBC, white blood cell; CRP, C-reactive protein; NS, not significant.
*Cochran-Armitage trend test.

rized in Tables 2 and 3. Febrile days after initiation of macrolides, proportion of patients with a fever lasting more than 72 hours after initiation of macrolides, chest radiologic findings, and the incidence of extrapulmonary complications were not significantly different between the 2 epidemics in both groups. However, compared with the 2011 epidemic, total febrile days were prolonged in both groups, and number of patients with MRMP pneumonia increased significantly as the age increased in the 2015 epidemic.

Comparison of the clinical characteristics of MSMP and MRMP pneumonia during 2 epidemics

The clinical course and laboratory findings of patients with MSMP or MRMP pneumonia during the 2 epidemics are summarized in Table 4. Demographic data were not significantly different between the 2 groups. However, compared with patients with MSMP pneumonia, patients with MRMP pneumonia showed longer total febrile days and longer febrile days after initiation of macrolides. Both the proportion of patients with fever lasting more than 72 hours after initiation of macrolides, and the proportion of patients with corticosteroid use were higher in patients with MRMP pneumonia. Chest radiologic findings and the incidence of extrapulmonary complications were not significantly different between the 2 groups. The proportion of patients with asthma or allergic rhinitis was also not significantly different between the 2 groups.

DISCUSSION

In this study, we found that the macrolide resistance rate of *M. pneumoniae* rose from 51.1% in 2011 to 87.2% in 2015. In comparison of 2 epidemics, the mean age of patients with *M. pneumoniae* pneumonia was increased, and the total febrile days and febrile days after initiation of macrolides were prolonged in the 2015 epidemic. The overall severity of MRMP or MSMP pneumonia over 2 epidemics was not significantly changed. However, the proportion of patients who had a fever lasting

Table 4. Comparison of clinical characteristics between MSMP and MRMP pneumonia during 2 epidemics

Characteristics	MSMP (n=66)	MRMP (n=184)	*P* value
Age (year)	5.5±2.6	5.9±3.0	NS
No. of cases			
≤2	12 (18.2)	31 (16.8)	NS
3-5	30 (45.4)	75 (40.8)	
≥6	24 (36.4)	78 (42.4)	
Sex (male/female)	28/38	95/89	NS
Total febrile days	2.2±2.1	4.4±3.3	0.003
Febrile days after initiation of macrolides	3.6±4.8	4.3±3.1	<0.001
No. of patients with fever lasting >72 hours after initiation of macrolides	12 (18.2)	104 (56.5)	<0.001
Change from macrolide to tetracycline or fluoroquinolone	0 (0.0)	1 (0.5)	NS
Steroid use	2 (3.0)	34 (18.5)	0.002
Laboratory findings			
WBC	8,570±3,267	8,354±3,629	NS
CRP (mg/L)	29.8±28.0	32.5±28.5	NS
Chest X-ray			
Consolidation, effusion	16 (24.2)	38 (20.7)	NS
Extrapulmonary complication	8 (12.1)	24 (13.0)	NS
Rash	3	10	
Liver function abnormality	4	8	
Proteinuria	1	5	
Arthralgia	0	1	
Patient with allergic disease	2 (3.0)	7 (3.8)	NS
Asthma	2	4	
Allergic rhinitis	0	3	

Values are presented as number (%).
MRMP, macrolide-resistant M. pneumoniae; MSMP, macrolide-susceptible M. pneumoniae; WBC, white blood cell; CRP, C-reactive protein; NS, not significant.

more than 72 hours after initiation of macrolides and who received corticosteroid treatment were higher in MRMP pneumonia during 2 epidemics.

Macrolide resistance is mainly due to transition mutations at positions A2063 or A2064 in domain V of the 23S rRNA gene, a binding site of macrolide antibiotics.[13] Based on previous studies to confirm that point mutations at the sites resulted in macrolide resistance, detection of these point mutations have been used as a marker for macrolide resistance.[4,14,15] An A-G transition at A2063 or A2064 confers a high level of resistance to macrolides. The A2063G transition is the most common mutation, followed by the A2064G transition.[4] Although mutations at position A2067 and/or C2617 in domain V of the 23S rRNA gene are also associated with macrolide resistance, these mutations have rarely been reported. All MRMP isolates in this study had the A2063G point mutation in both epidemics, which is consistent with those of previous reports.

In Korea, the macrolide resistance rate of *M. pneumoniae* was 51.6%-62.9% in 2011.[6,16] To our knowledge, there are no available data for the macrolide resistance rate since 2011. In the present study, the macrolide resistance rate in children with *M. pneumoniae* pneumonia was 87.2% in the 2015 epidemic, which is equivalent to that in China or Japan according to recent reports.[17,18] Considering the resistance rate was 51.1% in the 2011 epidemic, the macrolide resistance rate appears to be rising continuously and rapidly in Korea.

In this study, the mean age of patients with *M. pneumoniae* pneumonia was increased in the 2015 epidemic as compared with the 2011 epidemic. The change can be explained by increased proportion of MRMP pneumonia over the 2 epidemics because the peak age of MRMP pneumonia incidence was increased in the 2015 epidemic, in contrast to that of MSMP pneumonia incidence. This trend is different from those of recent *M. pneumoniae* epidemics in Korea. During the last several epidemics, the peak age of incidence appeared to be falling.[19] However, there have been few studies to investigate the age distribution of patients with MRMP pneumonia. Thus, further studies are necessary to ascertain whether older age group is more susceptible to MRMP pneumonia as shown in the present study.

Although the prescription pattern of initial antibiotics was changed over 2 epidemics, this change was associated with change in the principles of empirical antimicrobial therapy in CAP at our hospital, but not associated with change in the severity of pneumonia at admission. Because there was limited information about the macrolide resistance rate of *M. pneumoniae* until the end of the 2011 epidemic in Korea, this affected the choice of initial antibiotics. Macrolide/β-lactam combination was used as initial antibiotics in most patients (93/94) in the 2011 epidemic, whereas macrolide monotherapy was used in most patients (108/156) in the 2015 epidemic, along with awareness that the main reason for prolonged clinical course in most patients has been the macrolide resistance, not co-infection with other bacteria. However, macrolide/β-lactam combination was prescribed to all patients with consolidation or effusion on chest radiography in both epidemics.

Information about the influence of macrolide resistance on clinical outcomes is critical to determining whether there is a need to change current treatment recommendations for pediatric *M. pneumoniae* pneumonia. Although previous data from patients with MRMP pneumonia suggest that the presence of resistance did not increase the overall severity of disease, recent reports have demonstrated that disease progression during therapy, with increased complications, occurred in patients with MRMP pneumonia.[20,21] Surveillance data from Japan have also shown that cases requiring hospitalization have gradually

increased as MRMP pneumonia in children increased.[8] The reason for the difference in the influence of MRMP on clinical outcomes in previous studies could be partially explained by the fact that patients with MRMP pneumonia had more persistent signs and symptoms, which in turn led physicians to add an adjunctive treatment, such as corticosteroids, or to replace antibiotics in order to obtain a more rapid clinical improvement. In the present study, although the overall severity, including extrapulmonary complications between MSMP and MRMP pneumonia, was similar (except for the prolonged clinical course), the proportion of patients who received corticosteroid treatment was also higher in patients with MRMP pneumonia than in those with MSMP pneumonia. Because we used corticosteroids for cases with rapid progression despite the initiation of macrolides or cases with high fever lasting more than 5 days regardless of the macrolide resistance state, the higher proportion of corticosteroid use in MRMP pneumonia cases may reflect a higher disease severity in this group.

A previous study demonstrated that atopic sensitization and history of asthma were associated with macrolide treatment failure in patients with *M. pneumoniae* pneumonia.[22] However, there is no study to investigate whether patients with allergic disease are more susceptible to MRMP infection. In the present study, there was no significant difference in the proportion of patients with allergic disease between the MSMP and MRMP pneumonia groups. Therefore, macrolide treatment failure in the patients with asthma does not seem to be associated with MRMP infection. However, because the numbers of patients with allergic disease in those studies were relatively small, further studies are necessary to confirm the results and to elucidate underlying mechanisms for macrolide treatment failure in *M. pneumoniae* pneumonia patients who have asthma.

The adjunctive use of corticosteroids is based on previous studies suggesting that the host immune response plays a role in severe *M. pneumoniae* pneumonia.[23-25] In the current study, this therapeutic approach did result in rapid clinical improvements and resolution of pulmonary lesions associated with *M. pneumoniae* pneumonia, consistent with previous studies. Only 1 patient showed disease progression despite steroid use, and this patient also showed clinical improvement after switching from macrolide to fluoroquinolone antibiotics. Considering possible adverse effects, there is a need to more clearly define indications for corticosteroid use and to use alternative antibiotics with caution until new antibiotics that are safe and effective against MRMP in children are available.

One interesting result from this study is that patients with MSMP pneumonia showed increased total febrile days and a weak trend of increased febrile days after initiation of macrolides, over the 2 consecutive epidemics. One explanation for this result is the possible acquisition of resistance to macrolides during treatment. In fact, several recent *in vitro* and *in vivo* studies have demonstrated that *M. pneumoniae* acquires macrolide resistance within a few days of administration of macrolide antibiotics.[26-28] However, we could not confirm this due to a lack of samples which were collected at different time points during the treatment course. Therefore, to properly evaluate the influence of MRMP on clinical outcomes, re-testing of the macrolide resistance state during treatment based on the individual clinical situation should be done in future studies.

The inclusion of patients from 2 recent, consecutive epidemics and a relatively large sample size are the strengths of this study. However, there were some limitations in this study, including its retrospective design. First, not all children with *M. pneumoniae* pneumonia were included because we enrolled only moderate to severe PCR-positive patient who needed hospitalization. Although some previous studies included outpatients, those did not present data about the macrolide resistance rate in outpatients separately, which makes it difficult to estimate the resistance rate in mild cases. Therefore, future studies are still necessary to investigate the difference in the resistance rate between mild and moderate-to-severe cases. Secondly, other mutations associated with macrolide resistance were not searched because resistance to macrolides in East Asia has been mainly due to A2064G and A2064G, and thus there is a possibility that the resistance rate may be underestimated. Thirdly, all patients were from a single tertiary hospital and thus do not represent the general pediatric population in Korea. However, the macrolide resistance rates in this study sufficiently suggest that the prevalence of MRMP is rising rapidly in Korea because the resistance rate was measured in the same tertiary hospital over 2 consecutive epidemics.

In conclusion, the macrolide resistance rate of *M. pneumoniae* has risen rapidly over 2 recent, consecutive epidemics, and this was associated with a prolonged clinical course of disease in pediatric *M. pneumoniae* pneumonia. The relative proportion of patients receiving corticosteroids as an adjunctive therapy was significantly higher in cases of MRMP pneumonia during both epidemics, suggesting that the severity of MRMP pneumonia may be higher than that of MSMP pneumonia. Large-scale surveillance studies including outpatients with mild disease are needed to investigate the prevalence of MRMP pneumonia and its true impact on clinical outcomes in Korean children.

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