European **Rev**iew for **Med**ical and **Pharmacol**ogical **Sci**ences 2022; 26: 5844-5856

Regional and annual patterns in respiratory virus co-infection etiologies and antibiotic prescriptions for pediatric mycoplasma pneumoniae pneumonia

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Abstract. – OBJECTIVE: *Mycoplasma pneumoniae (M. pneumoniae)* pneumonia is the second-most common cause of community-acquired pneumonia (CAP). This study aimed at investigating into the prevalence of macrolide-resistant *M. pneumoniae* (MRMP) with respiratory virus co-infection and the antibiotic prescriptions in children with CAP in four provinces in Korea, and to assess the variations in the findings across regions and throughout the year.

PATIENTS AND METHODS: This prospective study was conducted in 29 hospitals in Korea between July 2018 and June 2020. Among the enrolled 1,063 children with CAP, all 451 patients with *M. pneumoniae* underwent PCR assays of M. pneumoniae and respiratory viruses, and the presence of point mutations of residues 2063 and 2064 was evaluated.

RESULTS: Gwangju-Honam (88.6%) showed the highest prevalence of MRMP pneumonia, while Daejeon-Chungcheong (71.3%) showed the lowest, although the differences in prevalence were not significant (p=0.074). Co-infection of *M. pneumoniae pneumonia* and respiratory virus was observed in 206 patients (45.4%), and rhinovirus co-infection (101 children; 22.2%) was the most frequent. The prevalence of MRMP pneumonia with respiratory virus co-infection and the antibiotic prescriptions differed significantly among the four provinces ($p < 0.05$). The monthly rate of MRMP pneumonia cases among all cases of *M. pneumoniae* pneumonia and tetracycline or quinolone prescriptions did not differ significantly among the four regions (trend $p > 0.05$) during the study period.

CONCLUSIONS: The prevalence of *M. pneumoniae pneumonia* with virus co-infection and antibiotic prescriptions could differ according to region, although the MRMP pneumonia rate showed no difference within Korea.

Key Words:
Mycoplasma pneumoniae, Macrolide-resistant *Mycoplasma pneumoniae*, Macrolide-sensitive *Mycoplasma pneumoniae*, Children.

Abbreviation
CAP: community-acquired pneumonia; *M. pneumoniae*: *Mycoplasma pneumoniae;* MRMP: macrolide-resistant *M. pneumoniae*; PCR: polymerase chain reaction;RSV: Respiratory syncytial virus.

Introduction

The most common causes of pediatric community-acquired pneumonia (CAP) in high-income countries are *Mycoplasma pneumoniae* (*M. pneumoniae*) and respiratory viruses[1]. Epidemics of *M. pneumoniae* infections follow a 3-7-year cycle, and the most recent outbreaks in Korea occurred in 2015 and 2019[2,3]. *M. pneumoniae* infection is mild or self-limiting in almost all cases, but approximately 10% of *M. pneumoniae* infections develop clinical signs of pneumonia with an "atypical" radiographic finding[4]. Macrolides are primarily used for treating *M. pneumoniae* infection because they show low minimum inhibitory concentrations, toxicity, and contraindications in the pediatric population[5].

However, since 2003, macrolide-resistant *M. pneumoniae* (MRMP) pneumonia has become prevalent globally[6,7]. The overuse of macrolide antibiotics in East Asia has increased the proportion of MRMP pneumonia cases to > 97% of the CAP cases in China[8], while in Japan the proportion of MRMP pneumonia cases in children was

reported to be 43.6% in 2015[9]. Additionally, the MRMP pneumonia rate in 2011 was over 50%[10,11], but over 60% of children were reported to have the A2063G mutation during 2018-2020 in Korea[12]. While 30% of MRMP pneumonia cases may improve without secondary treatment[13], prolonged fever and lobar MRMP pneumonia can endanger the patient's life[14]. Therefore, the treatment regimen for MRMP pneumonia for children in Korea has been revised to include secondary antibiotics or steroid administration[14-16].

Because of the increasing prevalence of MRMP pneumonia in Korea, the Korean Academy of Pediatric Allergy and Respiratory Disease and the Korean Society of Pediatric Infectious Diseases proposed new guidelines for the treatment of MRMP pneumonia in the pediatric population in 2019[17,18]. According to the latest Korean guidelines[17,18], the pediatrician may choose alternative antibiotics, such as tetracycline and quinolone or additional immune modulators, potentially introducing regional differences in the choice of antibiotic medications within Korea. However, almost all studies of pediatric populations in Korea were performed at single centers based in one region and were conducted before 2019. Moreover, while coinfection of *M. pneumoniae* with other respiratory pathogens occurs routinely, potential regional trends for coinfections in Korea remain unknown[19].

Because of these limitations of the previous studies, the prevalence of *M. pneumoniae*, including MRMP and MSSP, and viral infection in children with CAP within various provinces in Korea has not been ascertained since 2019. Therefore, this study aimed at investigating the prevalence of *M. pneumoniae*, including MRMP and MSMP pneumonia, and respiratory virus co-infections in children with CAP in four provinces (Seoul-Gyeongin-Kangwon, Daejeon-Chungcheong, Gwangju-Honam, and Daegu-Busan-Yeungnam) by using the medical records obtained from 29 hospitals in 2018-2020. Additionally, the purpose of our study was to shed light on the relationship between four provinces and antibiotic treatment among residential regions.

Patients and Methods

Study Design

A prospective, multi-center study was conducted on children younger than 18 years from July 2018 to June 2020. A cooperative hospital monitoring network (Seoul-Gyeongin-Kangwon; Daejeon-Chungcheong; Gwangju-Honam; Daegu-Busan-Yeungnam) was established in Korea: 13 hospitals from Seoul-Gyeongin-Kangwon, 5 hospitals from Daejeon-Chungcheong, 5 hospitals from Gwangju-Honam, and 6 hospitals from Daegu-Busan-Yeungnam (Figure 1).

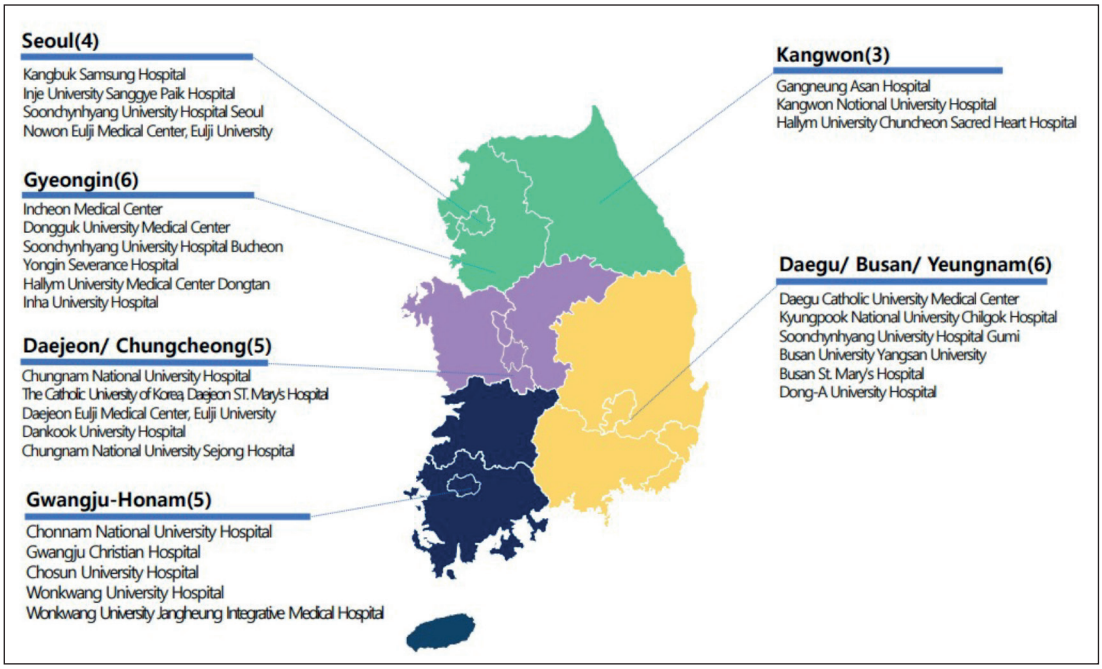

Figure 1. Study population.

Table I. Demographic characteristics of children with M. pneumoniae pneumonia in four provinces of Korea (n=454).

	Seoul-Gyeongin -Kangwon	Daejeon-Chungcheong	Gwangju-Honam	Daegu-Busan-Yeungnam	*p*-value
Number (%)	173 (100)	94 (100)	44 (100)	143 (100)	0.074
MRMP	141 (81.5)	67 (71.3)	39 (88.6)	109 (76.2)	
MSMP	32 (18.5)	27 (28.7)	5 (11.4)	34 (23.8)	
Sex (male/ female)	89/84	49/45	19/25	64/79	0.495
Mean age (years)	7.16±3.53	6.54±3.73	6.25±2.80	7.00±4.30	0.489
Age (years)					0.009
≤2	13 (7.5)	19 (20.2)	4 (9.1)	26 (18.2)	
3-5	47 (27.2)	16 (17.0)	13 (29.5)	29 (20.3)	
6-11	92 (53.2)	52 (55.3)	26 (59.1)	67 (46.9)	
≤12	21 (12.14)	7 (7.4)	1 (2.3)	21 (14.7)	
Incident season					0.009
Spring (Mar, Apr, May)	13 (7.5)	19 (20.2)	4 (9.1)	26 (18.2)	
Summer (Jun, Jul, Aug)	47 (27.2)	16 (17.0)	13 (29.5)	29 (20.3)	
Autumn (Sep, Oct, Nov)	92 (53.2)	52 (55.3)	26 (59.1)	67 (46.9)	
Winter (Dec, Jan, Feb)	21 (12.14)	7 (7.4)	1 (2.3)	21 (14.7)	
Total febrile duration before visiting (days)	5.25±2.99	5.02±3.61	4.77±2.46	4.64±3.20	0.221
Daily center or kindergarten, n (%)	144 (83.2)	76 (80.9)	32 (72.7)	97 (67.8)	0.008
Sibling, n (%)	111 (64.2)	55 (58.5)	26 (59.1)	92 (64.3)	0.737
Pulmonary infiltration, n (%)					0.242
Bronchopneumonia	59 (34.1)	22 (23.4)	14 (31.8)	50 (35.0)	
Segmental/lobar pneumonia	113 (65.3)	67 (71.3)	35 (79.5)	86 (60.1)	
Pleural effusion	10 (5.8)	8 (8.5)	6 (13.6)	9 (6.3)	
Respiratory virus co-infection, n (%)	73 (42.2)	54 (57.4)	15 (34.1)	64 (44.8)	**0.036**
Rhinovirus	35 (20.2)	32 (34.0)	7 (15.9)	27 (18.9)	
Adenovirus	17 (9.8)	10 (10.6)	6 (13.6)	6 (4.2)	
Respiratory syncytial virus	14 (8.1)	17 (18.1)	1 (2.3)	19 (13.3)	
Prescribed antibiotics					
Initial antibiotics, n (%)	162 (93.6)	88 (93.6)	44 (100)	132 (92.3)	0.323
Macrolide, n (%)	149 (86.1)	56 (59.6)	41 (93.2)	77 (53.8)	**<0.001**
Macrolide alone	83 (48.0)	28 (29.8)	5 (11.4)	13 (9.1)	**<0.001**
Macrolide + cephalosporin	23 (13.3)	8 (8.5)	13 (29.5)	53 (37.1)	**<0.001**
Macrolide + β-lactams	24 (13.9)	15 (16.0)	15 (34.1)	10 (7.0)	**<0.001**
Tetracycline or quinolone, n (%)	17 (9.8)	21 (22.3)	3 (6.8)	42 (24.3)	**<0.001**

Values are presented as numbers (%) and mean ± standard deviation. Numbers in bold indicate significant differences ($p < 0.05$). Abbreviations: MRMP, macrolide-resistant *M. pneumoniae*; MSMP, macrolide-susceptible *M. pneumoniae*; MRMP*, macrolide-resistant *M. pneumonia*e with admission; MSMP*, macrolide-susceptible *M. pneumoniae* with admission; NA, not evaluated.
Scale variables were analyzed using the Chi-squared test or Fisher exact test, and continuous variables were analyzed using Student's *t*-test or Mann-Whitney U test.

The diagnosis of pneumonia was based on the findings of both physical examination and radiologic assessments performed in each hospital by respiratory and allergy specialists. Among 1,063 children with CAP, 454 eligible patients showed positive results for *M. pneumonia* in a polymerase chain reaction (PCR) assay. In the present study, we identified MRMP by a positive PCR result with mutations at residues 2063 or 2064 (n = 356) and MSMP by a positive PCR result with no mutations at residues 2063 or 2064 (n = 98). Respiratory and allergy specialists from 29 hospitals reviewed the medical records at each hospital to collect general information about the children, including their sex, birth date, height, weight, family history of allergic disease, clinical and demographic characteristics, and chest radiograph findings.

Collection of Samples and Detection of Pathogens

Sputum, bronchoalveolar lavage, nasopharyngeal aspiration, or nasopharyngeal swab samples were obtained within 24 hours after enrollment. The AllplexTMPneumoBacter Assay (Seegene, Seoul, Korea) was performed according to the manufacturer's instructions to detect *M. pneumoniae*. Respiratory syncytial virus (RSV) A and B, influenza virus A and B, parainfluenza virus 1, 2,

3, and 4, adenovirus, human rhinovirus, human metapneumovirus, coronavirus 229E, NL63, and OC43, and bocavirus were examined using multiplex PCR method. The detailed method had been described in a previous study[20].

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics (version 23.0, IBM Corp., Armonk, NY, USA) and R version 2.8.134 (Vienna, Austria). The experimental results are presented as mean ± standard deviation values, while categorical data are reported as numbers (percentages). Inter-group comparisons were performed using the Mann-Whitney test or Kruskal-Wallis' test for continuous variables and the Chi-squared test or Fisher's exact test for categorical variables. Seasonal Mann-Kendall tests, a type of non-parametric statistical analyses, were used to detect mono-tonic trends in monthly data with an annual seasonal pattern. Statistical significance was defined as a p-value below 0.05.

Results

Characteristics of the Children with M. Pneumoniae Pneumonia in the Four Provinces

All participating children (n = 454 [100%]; mean age = 6.89 ± 3.77 years) with positive *M. pneumoniae* PCR results were divided into two groups: MRMP (n = 356, 78.4%) and MSSP (n = 98, 21.6%) pneumonia. All isolates in the MRMP pneumonia group (n = 356) had A2063G point mutations. Of the 454 children, females (233, 51.3%) were more likely to have MRMP pneumonia than males (221, 48.37%).

The characteristics of children with *M. pneumoniae* pneumonia categorized by region are shown in Table I. The 454 children with *M. pneumoniae* included 173 from Seoul-Gyeongin-Kangwon, 94 from Daejeon-Chungcheong, 44 from Gwangju-Honam, and 143 from Daegu-Busan-Yeungnam. A total of 356 children had MRMP pneumonia, including 141 from Seoul-Gyeongin-Kangwon, 67 from Daejeon-Chungcheong, 39 from Gwangju-Honam, and 109 from Daegu-Busan-Yeungnam. Gwangju-Honam exhibited the highest prevalence of MRMP pneumonia among the four provinces, whereas Daejeon-Chungcheong exhibited the lowest. However, there was no significant difference in the prevalence of MRMP and MSMP pneumonia among the four provinces ($p = 0.074$) (Table I). The four provinces showed statistically significant differences in the age distribution, incident season, and attendance for daily center or kindergarten ($p < 0.05$). However, no significant differences were observed in sex, mean age, and total febrile duration before visiting the hospital among the four provinces ($p > 0.05$) (Table I).

The prevalence of *M. pneumoniae* pneumonia with respiratory virus co-infection was the highest in Daejeon-Chungcheong (57.4%) and the lowest in Gwangju-Honam (34.1%), and the four provinces showed statistically significant differences in the prevalence of respiratory virus co-infection ($p = 0.036$). Rhinovirus co-infections were observed in 101 children (22.2%) and were the most frequent respiratory virus co-infections in the four provinces (15.0-34.0%). RSV infections were the second-most common respiratory virus co-infections and occurred in 51 children (11.2%), with the co-infection rates in the four provinces ranging between 2.3-18.1% (Table I).

No regional differences were observed in the initial antibiotic treatment regimens ($p = 0.323$). However, the rate of macrolide treatment was the highest in Gwangju-Honam (93.2%) and the lowest in Daegu-Busan-Yeungnam (53.8%), with statistically significant differences among the four provinces ($p < 0.001$). Additionally, the rate of tetracycline or quinolone treatment was the lowest in Gwangju-Honam (6.8%) and the highest in Daegu-Busan-Yeungnam (53.8%), with statistically significant differences among the four provinces ($p < 0.001$) (Table I).

Annual Patterns of M. Pneumoniae Pneumonia and Respiratory Viruses in Four Regions

Two epidemics of *M. pneumoniae* pneumonia occurred during the study period (November 2018 and from October 2019 to February 2020). The number of children with *M. pneumoniae* pneumonia was the highest between November and December 2019 (Figure 2A). In analyses based on the regions, the highest number of children with *M. pneumoniae* pneumonia in three regions (Seoul-Gyeongin-Kangwon; Gwangju-Honam; Daegu-Busan-Yeungnam) was reported in November 2019, and the highest number in the fourth region (Daejeon-Chungcheong) was reported in January 2020 (Figure 2B).

Concurrently, the number of children with MRMP pneumonia in three regions (Seoul-Gyeongin-Kangwon; Gwangju-Honam; Daegu-Busan-Yeungnam)

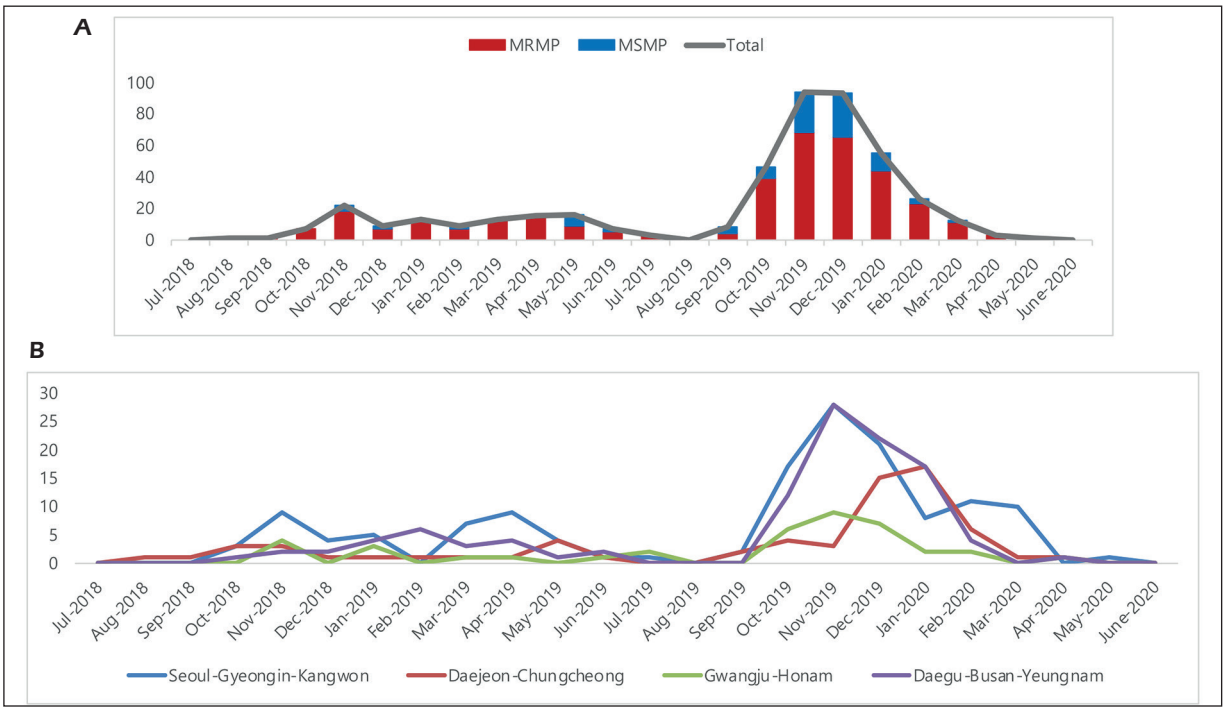

Figure 2. Number of *M. pneumoniae* pneumonia from July 2018 to June 2020. **A**, Children with MRMP and MSMP pneumonia; **B**, *M. pneumoniae* pneumonia in four provinces (Seoul-Gyeongin-Kangwon, Daejeon-Chungcheong, Gwangju-Honam, Daegu-Busan-Yeungnam).

was the highest in November 2019 and that in the fourth region (Daejeon-Chungcheong) was the highest in January 2020 (Figure 3A). The number of children with MRMP pneumonia and viral co-infection showed the same region-wise trends (Figure 3C). Meanwhile, the number of children with MSMP pneumonia in two regions (Seoul-Gyeongin-Kangwon; Daegu-Busan-Yeungnam) was the highest in November 2019, and the corresponding number in the other two regions (Daejeon-Chungcheong; Gwangju-Honam) was the highest in December 2019 (Figure 3B). The number of children with MSMP pneumonia and virus co-infection was the highest in one region (Daegu-Busan-Yeungnam) in November 2019 and in three regions (Seoul-Gyeongin-Kangwon; Daejeon Chungcheong; Gwangju-Honam) in December 2019 (Figure 3D).

Time-Dependent Changes in the Rate of MRMP/Total M. Pneumoniae Pneumonia and the Tetracycline or Quinolone Antibiotic Prescriptions in the Four Regions

The seasonal Mann-Kendall tests showed that the monthly rate of MRMP/total *M. pneumoniae* pneumonia was 0.0-100.0%, and it changed dramatically during the study period. However, the region-wise mean monthly rates of MRMP/total *M. pneumoniae* pneumonia were as follows: Seoul-Gyeongin-Kangwon, 66.94%; Daejeon Chungcheong, 75.37%; Gwangju-Honam, 51.26%; and Daegu-Busan-Yeungnam, 54.14%, with statistically significant differences among regions ($p = 0.001$) (Figure 4A, 4B, 4C, and 4D).

The monthly rates of MRMP/total *M. pneumoniae* pneumonia cases showed an increasing trend in three regions (Seoul-Gyeongin-Kangwon, slope = 0.680; Daegu-Busan-Yeungnam, slope = 0.119; Gwangju-Honam, slope = 2.09) and a decreasing trend in one region (Daejeon Chungcheong, Sen's slope = -1.979). However, no significant differences were observed among the monthly rates in the four regions: Seoul-Gyeongin-Kangwon, trend $p = 0.977$; Daejeon Chungcheong, trend $p = 0.094$; Daegu-Busan-Yeungnam, trend $p = 0.906$; Gwangju-Honam, trend $p = 0.231$, respectively (Figure 4A, 4B, 4C, and 4D).

Meanwhile, during the study period, the mean monthly rate of tetracycline or quinolone prescriptions was the highest in Daejeon-Chungcheong (23.81%) and the lowest in Gwangju-Honam (3.54%): Seoul-Gyeongin-Kangwon, 6.14%; Dae-

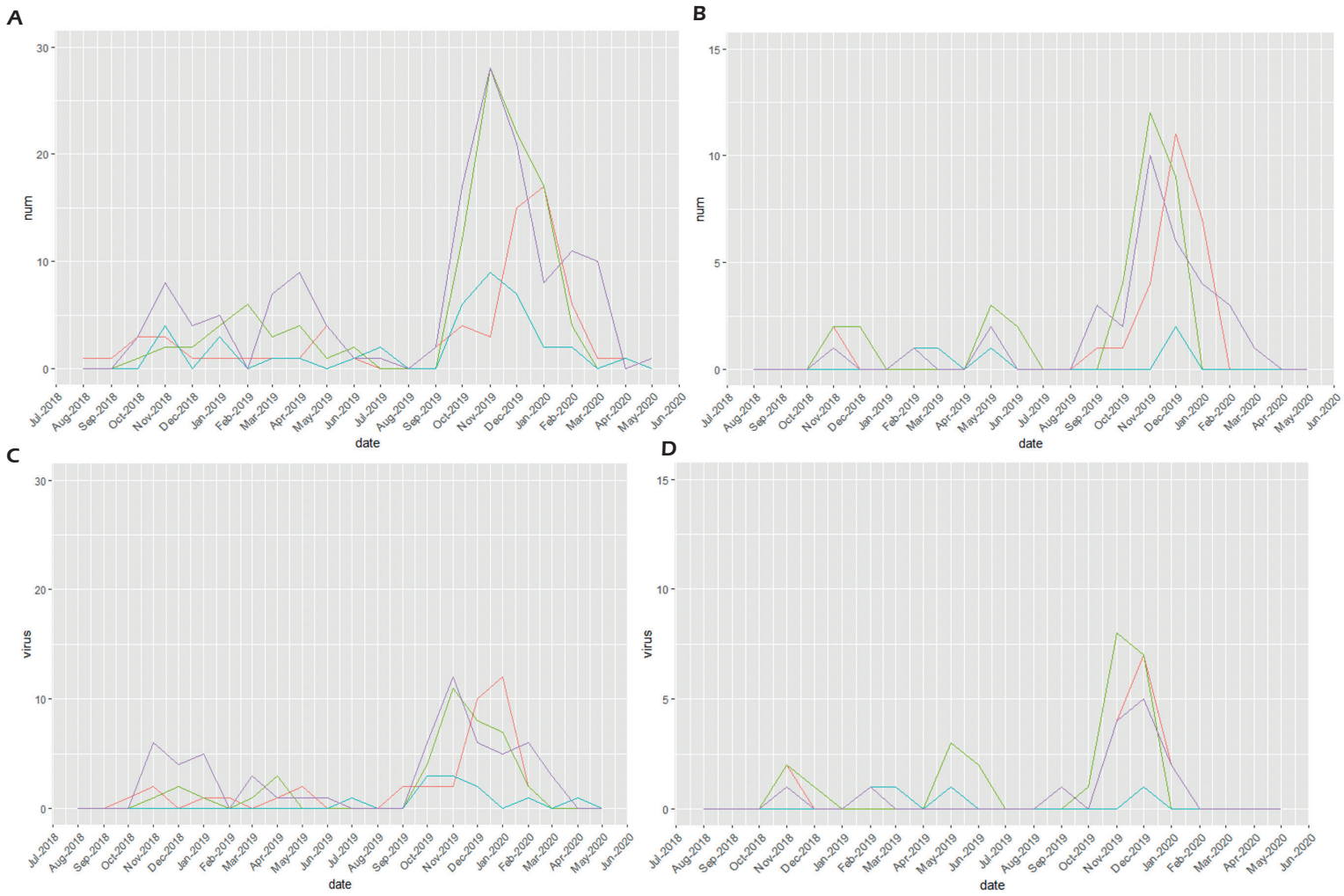

Figure 3. The number of children with MRMP or MSMP pneumonia and respiratory virus co-infection between 2018 and 2020 in four provinces (Seoul- Gyeongin-Kangwon, Daejeon-Chungcheong, Gwangju-Honam, Daegu-Busan- Yeungnam) in Korea. **A**, MRMP; **B**, MSMP; **C**, MRMP with co-respiratory virus infection; **D**, MSMP with co-respiratory virus infection.
Abbreviations: MRMP, macrolide-resistant M. pneumoniae; MSMP, macrolide-susceptible M. pneumoniae.

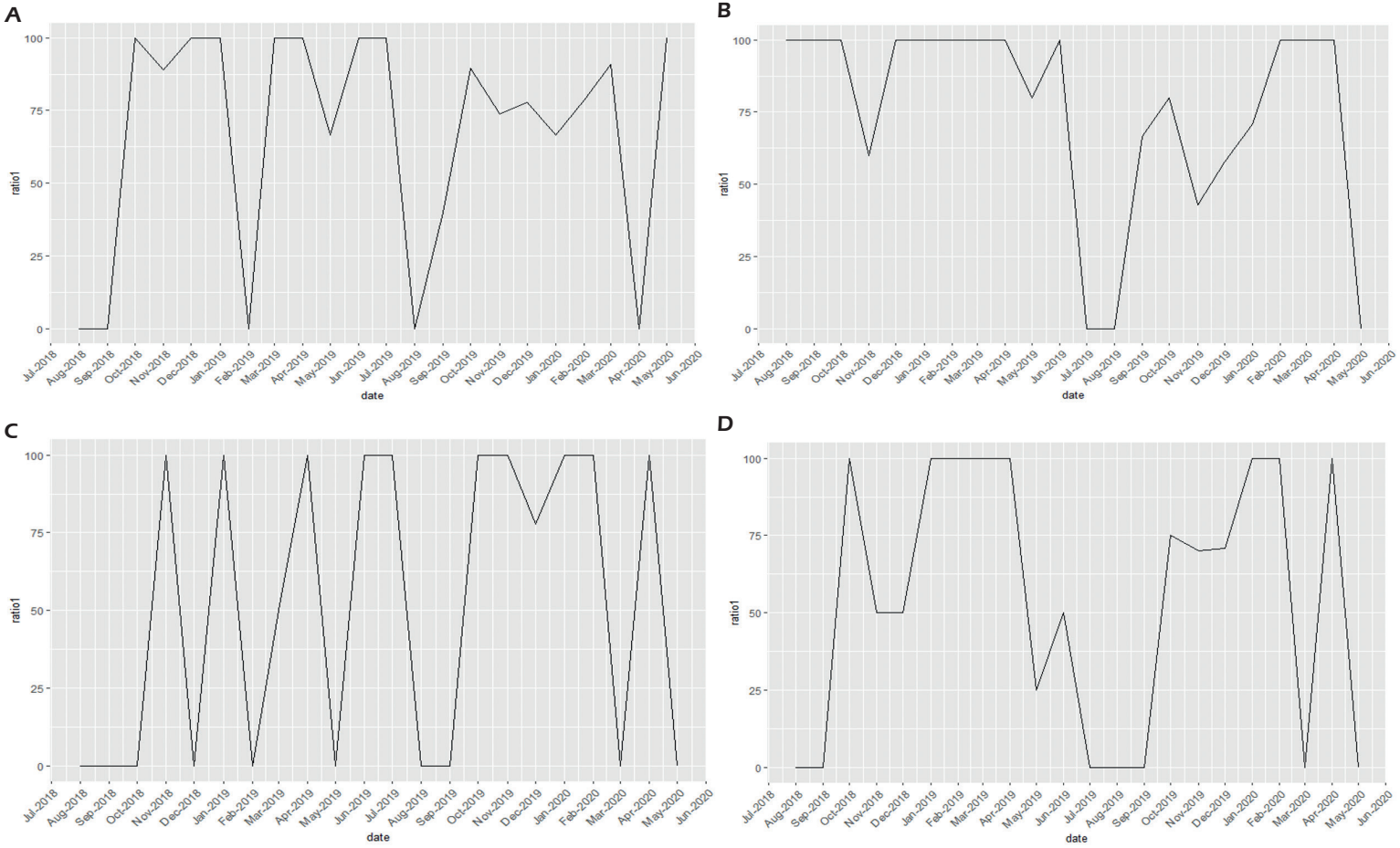

Figure 4. Time-dependent changes in the ratios of MRMP pneumonia/total *M. pneumoniae* pneumonia in the four provinces in Korea. **A**, Seoul- Gyeongin-Kangwon; **B**, Daejeon-Chungcheong; **C**, Gwangju-Honam; **D**, Daegu-Busan- Yeungnam. *Abbreviations:* M. pneumoniae, Mycoplasma pneumoniae; MRMP, macrolide-resistant M. pneumoniae; ratio1, MRMP pneumonia/total cases of M. pneumoniae pneumonia.

jeon Chungcheong, 23.81%; Gwangju-Honam, 3.54%; and Daegu-Busan-Yeungnam, 12.46%. The prescription rates showed statistically significant differences among regions ($p < 0.001$) (Figure 5A, 5B, 5C, and 5D).

The monthly rate of tetracycline or quinolone prescriptions increased in all four regions (Seoul-Gyeongin-Kangwon, slope = 0.129; Daejeon Chungcheong, slope = 0.074; Daegu-Busan-Yeungnam, slope = 0.060; and Gwangju-Honam, slope = 0.502, respectively). However, the results for the four regions did not show significant differences: Seoul-Gyeongin-Kangwon, trend $p = 0.129$; Daejeon Chungcheong, trend $p = 0.074$; Daegu-Busan-Yeungnam, trend $p = 0.060$; Gwangju-Honam, trend $p = 0.139$ (Figure 5A, 5B, 5C, and 5D).

Discussion

This multicenter and prospective study characterized the annual and regional patterns of respiratory etiologies of pediatric *M. pneumoniae* pneumonia between July 2018 and June 2020 in Korea. Among the children with *M. pneumoniae* pneumonia during 2018-2020 in Korea in the present study, 78.4% had the A2063G mutation in domain V of 23S rRNA, consistent with the findings of a previous study[12] conducted between 2018-2020. The number of children with *M. pneumoniae* pneumonia was the highest from October 2019 to February 2020 during this study period and then dropped dramatically since the first outbreak of COVID-19 in February 2020. Specifically, the prevalence of MRMP pneumonia peaked in November 2019 in three regions (Seoul-Gyeongin-Kangwon, Daegu-Busan-Yeungnam, and Gwangju-Honam), but Daejeon-Chungcheong showed a regional difference in the peak time.

Nevertheless, this nationwide study demonstrated that the MRMP/MSMP pneumonia rates showed no regional differences among the four provinces within Korea. The monthly rates of MRMP pneumonia showed no increasing trends in the four regions during the study period because the study period of 24 months was not sufficiently long to show the trend of MRMP pneumonia and the COVID-19 epidemic. On the basis of these results, we assumed that the peak time of *M. pneumoniae* pneumonia could differ among different regions within the same country. Moreover, we recognized that respiratory co-infections of *M. pneumoniae* and virus showed a strong association with the high level of mask-wearing and social distancing in Korea.

Viral coinfection is known to be common in the pediatric population with *M. pneumoniae* pneumonia, especially in children under 5 years of age[21,22]. The present study identified viral co-infections in 45.4% of the patients with *M. pneumoniae* pneumonia, and rhinovirus infections (22.2%) were the most commonly identified co-infections. In another Korean study, 32.4% of the patients showed *M. pneumoniae* and respiratory virus co-infections and rhinovirus (44.4%) was the most common virus[21], consistent with the results of the present study. However, in another Korean study[22], the most common causes of respiratory virus co-infections were RSV (20.3%) and rhinovirus (15.5%), and RSV was the most common cause of CAP requiring hospitalization in children aged under two years. In comparison with that study[22], our study had a higher average patient age (6.89 ± 3.77 years).

Our findings also showed regional differences in the incidence of *M. pneumoniae* pneumonia with respiratory virus co-infection. We found that respiratory virus co-infection in children with *M. pneumoniae* pneumonia was higher in cases of MSMP pneumonia, in agreement with the results of previous studies[16,20,21]. To the best of our knowledge, this study may be the first investigation of regional differences in the prevalence of pediatric cases of *M. pneumoniae* pneumonia in Korea from 2018 to 2020. Most of the previous studies[15,16,21] had been conducted at single centers and included fewer participants than in our study, which was conducted across 29 centers from four provinces and included 456 participants with *M. pneumoniae* pneumonia across Korea.

One interesting result from this study is that the initial antibiotic regimens showed no regional differences among the four provinces in Korea. However, the usage of macrolide antibiotics and tetracycline or quinolone showed regional differences. The monthly rates of prescription of tetracycline or quinolone also showed regional differences. We postulated that the new guidelines for the treatment of MRMP pneumonia in the pediatric population in 2019 may have dramatically changed the antibiotic prescription patterns. However, the monthly rates of antibiotic prescriptions showed no significant increasing trends in the four regions during the study period, which could be attributed to the fact that a 24-month period is insufficient to show such trends and that only 67 children received tetracycline or quinolone. According to a previous Korean report[12], only 3.9% of the children admitted for *M. pneumoniae* pneu-

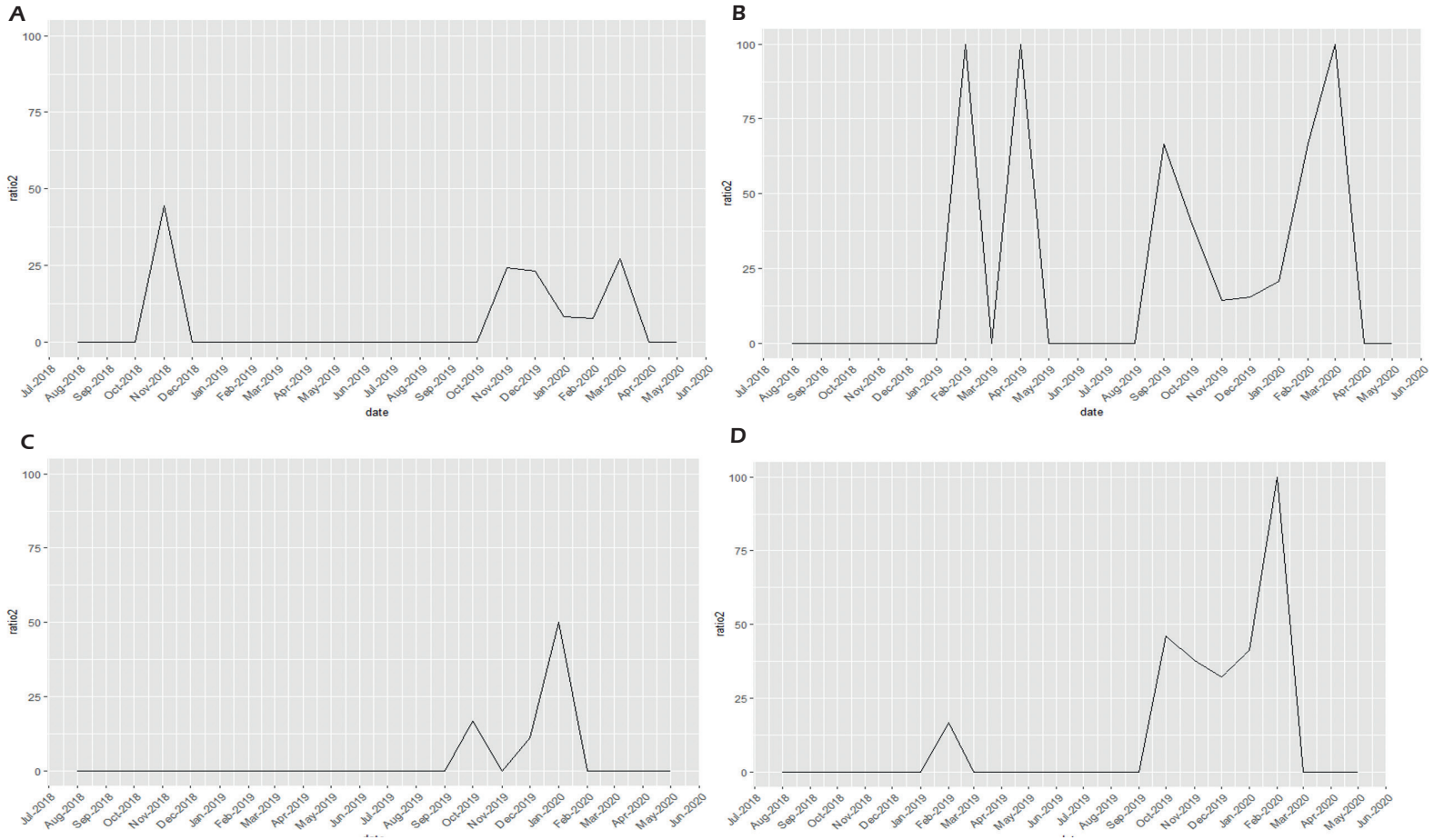

Figure 5. Time-dependent changes in the ratios of tetracycline or quinolone/ total antibiotic prescriptions in four provinces in Korea. **A**, Seoul- Gyeongin-Kangwon; **B**, Daejeon-Chungcheong; **C**, Gwangju-Honam; **D**, Daegu-Busan-Yeungnam.
Abbreviations: Ratio2, tetracycline or quinolone/ total antibiotic prescriptions.

monia received tetracycline and fluoroquinolone, which was lower than the corresponding prescription rates in the present study since macrolide-insensitive pneumonia was reported in only 37.8% of the participants in that study[12], while it was observed in 78.4% of the participants in the present study. Tetracycline has been shown to be more effective than fluoroquinolones in some recent studies[23-25], but the number of patients receiving tetracycline or quinolones in this study was insufficient to analyze this aspect. Meanwhile, systemic corticosteroid treatment is one of the most commonly administered regimens for *M. pneumoniae* pneumonia in Korea[12,15,16,22] and corticosteroids are preferred to secondary antibiotics as a front-line treatment for pediatric *M. pneumoniae* pneumonia[18]. However, unfortunately, we could not obtain medical records for steroid prescriptions. Therefore, these results should be generalized carefully, and additional data are required to obtain more definitive findings.

Limitations

This study had a few limitations. First, the investigation period was from July 2018 to June 2020; the number of samples decreased dramatically from February 2020, the beginning of the COVID-19 epidemic. Second, some regional differences were observed in the number of participants among the four provinces within Korea. Thus, further studies in other provinces and hospitals after the end of the COVID-19 epidemic will be necessary to address these limitations. Nevertheless, the strengths of the present study are that we analyzed data from 454 children after obtaining PCR results following viral tests and a medical review including radiological findings and prescribed antibiotics in four provinces within Korea over 24 months. Unlike previous literature on this topic, we were able to analyze the regional and annual differences in *M. pneumoniae* pneumonia and virus co-infections and prescribed antibiotics in children.

Conclusions

We found that the prevalence of *M. pneumoniae* and virus co-infection and the prescribed antibiotics may differ according to region, although the overall prevalence of MRMP showed no differences within Korea.

Conflicts of Interest
The authors declare no conflicts of interest.

Data Availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request for clinical research purposes.

Informed Consent
Written informed consent was obtained from the parents or guardians of all participants following a detailed explanation of the study.

Ethics Approval
The study protocol was approved by the Institutional Review Board and Ethics Committee of Chungnam National University Hospital (IRB No. 2019-07-037) and all participating medical centers.

Funding
This study was supported by a grant from the Korea Disease Control and Prevention Agency, Republic of Korea (Grant Number: 4800-4821-304).

Authors' Contributions
M.S and H.-J.C gave a substantial contribution to the clinical assessment of children, the analysis of data and the drafting of the paper. All the authors selected and recruited children and were also in charge of the traditional clinical follow-up. J.-Y. S and E.-H. C are responsible for the study design and the approval of the submitted version of the paper. All the authors had complete access to the study data of this work. The authors read and approved the final manuscript.

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