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Molecular characterization and analysis of *Mycoplasma pneumoniae* among patients of all ages with community-acquired pneumonia during an epidemic in China

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ABSTRACT

Background: Analysis of the molecular characteristics of isolates is very important for clinical and epidemiological study of community-acquired pneumonia (CAP) caused by *Mycoplasma pneumoniae*.
Methods: Between 2010 and 2012, an epidemic period, *M. pneumoniae* was isolated from oropharyngeal swabs of consecutive CAP patients. Minimum inhibitory concentrations of macrolides, 23S rRNA gene sequencing, P1 gene and multilocus variable-number tandem-repeat analysis (MLVA) genotyping was conducted.
Results: 88.3% (181/205) of the isolates were macrolide-resistant *M. pneumoniae* (MRMP) and all harbored an A2063 G mutation. The strains were clustered into 7 MLVA types, and P1 type 1 and type 2 lineages were co-circulated (86.3% and 13.7%). Compared with adults, no specific MLVA type contributed to higher *M. pneumoniae* infection in children ($p = 0.14$). Similar macrolide profile and genotypes of *M. pneumoniae* was found between outpatients and inpatients. Significant differences in proportion of P1 types and two main MLVA types 4/5/7/2 and 3/5/6/2 were observed between MRMP and macrolide-sensitive *M. pneumoniae* (MSMP) ($p < 0.001$).
Conclusions: This study demonstrates a comprehensive profile of *M. pneumoniae* molecular characterization among CAP patients of all age, and provides more evidences on a correlation between MLVA type 4/5/7/2 and macrolide resistance in the setting of high incidence of MRMP.

Introduction

Mycoplasma pneumoniae (*M. pneumoniae*) is a common pathogen of respiratory diseases in humans of all age, especially in children and adolescents. *M. pneumoniae* infections vary clinically from mild, self-limiting upper respiratory symptoms to radiographically confirmed pneumonia requiring hospitalization, accounting for 10%–30% of community-acquired pneumonia (CAP) cases (Waites and Talkington, 2004; Cao et al., 2010; Chalker et al., 2011; Eibach et al., 2012). Epidemics of *M. pneumoniae* infections occur worldwide with an interval of 3–7 years (Lenglet et al., 2012). Due to the improved sensitivity and specificity compared with culture and serology, real-time polymerase chain reaction (PCR) has emerged as the primary technique for detection of *M. pneumoniae* in surveillance, and also in clinical practice in China (Qu et al., 2013). The molecular diagnosis methods help physicians and clinical microbiologists treat patients infected with *M. pneumoniae* appropriately and monitor epidemiological trends.

Macrolides are the first line antimicrobials against *M. pneumoniae* infections in the clinical practice. However, excessive or inappropriate antibiotic use provides selective pressure for the development of antimicrobial resistance. Macrolide-resistant *M. pneumoniae* (MRMP) has been spreading for 10 years all over the world, with prevalence ranging from ≤10% in Europe and the United States (Pereyre et al., 2007; Diaz et al., 2015a; Dumke et al.,

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2015), approximately 30% in Israel (Pereyre et al., 2012) and 69% to 100% in Japan and China (Cao et al., 2010; Zhou et al., 2015; Kawai et al., 2013). Several studies indicate that, compared to macrolide-sensitive *M. pneumoniae* (MSMP) infections, MRMP infections have been associated with increased febrile period, increased duration of persistent cough, and extended antibiotic therapy (Cao et al., 2010; Suzuki et al., 2006; Matsubara et al., 2009; Cardinale et al., 2013).

Analysis of the molecular characteristics of clinical isolates is very important for clinical and epidemiological study of *M. pneumoniae* infections and its outbreak. Two methods for molecular typing of *M. pneumoniae* isolates have been well documented, including classification as type 1 and type 2 lineages based on sequence of the P1 gene (Pereyre et al., 2007; Dumke et al., 2003), and more precise categorization of isolates based on the variable copy numbers of tandem repeated sequences at four to five loci, called MLVA typing (Degrange et al., 2009; Benitez et al., 2019; Sun et al., 2013). Although limited to fewer possible types, the modified four loci scheme appears to provide a more accurate classification of *M. pneumoniae* isolates (Sun et al., 2013).

Due to the significant regional differences in macrolide-resistant (MR) rate, period, patient age and disease type among the studies (Pereyre et al., 2007; Cardinale et al., 2013; Diaz et al., 2015b; Ho et al., 2015; Kogoj et al., 2015), to date there are limited investigations on the distribution of *M. pneumoniae* types between adult and pediatric populations. Additionally, based on the finding from different regions (Diaz et al., 2015a,b; Ho et al., 2015; Kogoj et al., 2015; Sun et al., 2017; Yan et al., 2015), the correlation between macrolide resistance and *M. pneumoniae* genotype is still controversial.

In this study, we employed P1 gene and MLVA genotyping to 205 clinical *M. pneumoniae* isolates from a cohort of both children and adult patients with CAP in a surveillance conducted during a *M. pneumoniae* epidemic in China from 1st August 2010 to 29th February 2012. The molecular characterizations of *M. pneumoniae* between adults and children, inpatients and outpatients, as well as MRMP and MSMP infections, had been profiled.

Materials and methods

CAP patients

From 1st August 2010 to 29th February 2012, a total of 1229 consecutive CAP patients from Beijing Chao-Yang Hospital in Beijing, China, were prospectively enrolled in a surveillance study. Oropharyngeal swabs were obtained from all patients for molecular detection of respiratory viruses and *M. pneumoniae* (Qu et al., 2015). CAP was defined as an acute infiltrate on chest radiography and at least one of the following (Mandell et al., 2007): (a) new or escalating cough, sputum production, purulent sputum, chest pain; (b) body temperature > 37.8 °C, or body temperature < 35.6 °C; (c) white blood cell count: $>10 \times 10^9$/L or $<4 \times 10^9$/L. None of the patients were immune-compromised: patients with HIV infection, neutropenia, or receiving immunosuppressive chemotherapy were excluded. In addition, pregnant or lactating mothers, patients from nursing homes, patients whose onset time was longer than 7 days or patients who had been admitted to a hospital longer than 2 days within the last 90 days were also excluded. The study was approved by the Institutional Review Board of Beijing Chao-Yang Hospital (project approval number 10-KE-49). Written informed consent was provided by all adults and the legal representatives of patients aged <18 years.

M. pneumoniae *culture and antimicrobial susceptibility test*

Culture of *M. pneumoniae* was performed as described previously by Waites et al. (2001). Two hundred and five *M. pneumoniae* isolates were obtained from oropharyngeal swabs (each specimen collected from one patient). All the isolates were identified by PCR assay.

Minimum inhibitory concentrations (MICs) were determined by broth micro-dilution methods with SP4 broth (Remel) (Waites et al., 2001). Susceptibility tests were performed in triplicate. *M. pneumoniae* reference strain FH (ATCC 15531) was also included. According to CLSI guideline (M43-A, 31(19), 2011) for antimicrobial susceptibility for human mycoplasmas including *M. pneumoniae*, susceptible strains have a MIC of erythromycin and azithromycin $\leq$0.5 μg/ml and resistant strains have a MIC $\geq$ 1 μg/ml. The total length of the 23S ribosomal RNA (rRNA) gene of each *M. pneumoniae* isolate was amplified and sequenced by the method described previously (Morozumi et al., 2005). The resistance related mutations of 23S rRNA were determined by alignment with the sequence of FH.

P1 gene and MLVA typing

P1 gene typing on *M. pneumoniae* isolates was performed using a multiplex PCR as described by Kenri et al. (2008). MLVA typing on *M. pneumoniae* isolates was performed using PCRs with fluorescently labeled primers targeting four VNTR loci (the Mpn1 locus was excluded due to documented instability (Sun et al., 2013), followed by capillary electrophoresis as previously described (Degrange et al., 2009; Benitez et al., 2019). One reference strain of FH, was included as the positive control with P1 type 2 and MLVA type 3/6/6/2. Two peaks of molecular weight signal in Mpn13 and Mpn15 were found in one sample and two samples, respectively, and the numbers of VNTR were finally determined by sequencing of PCR products.

Statistical analysis

Categorical variables were described with counts and percentages. Continuous variables were presented as mean (SD) or median (IQR) where appropriate. The χ^2 or Fisher's exact test was used as appropriate in the comparisons of characteristics between adults and children, outpatients and inpatients, as well as MRMP and MSMP. Statistical analyses were performed with SPSS Statistics (v21, IBM Corp., USA). *P* value <0.05 was considered significant.

Results

Characteristics of CAP patients positive with M. pneumoniae

Among the CAP patients, 26.4% (325/1229) were children, and 16.7% (205/1229) were positive in *M. pneumoniae* culture and PCR assay (Table 1). Compared with adults, *M. pneumoniae* infections were more common in children CAP patients (27.1% vs. 12.9%, $p <$ 0.001; Table 1).

The main characteristics of CAP patients positive with *M. pneumoniae* were summarized in Table 2. 57.1% (117/205) were obtained from adults and 42.9% (88/205) were from children, and the most affected age groups were young adults (accounting for 82.0% of adults patients), and preschool and school children

Table 1
The *M. pneumoniae* detection of the whole cohort in the surveillance. Data were represented as n (%).

	Total	*M. pneumoniae* Negative	Detection Positive	*P* value
Children	325 (26.4)	237 (73.1)	88 (27.1)	< 0.001
Adults	904 (73.6)	787 (87.1)	117 (12.9)	
Total	1229 (100)	1024 (83.8)	205 (16.7)	

Table 2
Characteristics of *M. pneumoniae* positive CAP patients. Data were represented as n (%).

Characteristic	Total (205)	Adults (117)	Children (88)	*P* value
Age, median (IQR)	23 (22)	30 (15)	9 (8)	NA
Age group				NA
0–4 years	16 (7.8)		16 (18.2)	
5–9 years	34 (16.6)		34 (38.6)	
10–17 years	38 (18.5)		38 (43.2)	
18–44 years	96 (46.8)	96 (82.0)		
45–64 years	18 (8.8)	18 (15.4)		
≥65 years	3 (1.5)	3 (2.6)		
Gender				0.397
Female	110 (53.7)	66 (56.4)	44 (50.0)	
Male	95 (46.3)	51 (43.6)	44 (50.0)	
Site of care				0.008
Outpatients	70 (34.1)	49 (41.9)	21 (23.9)	
Inpatients	135 (65.9)	68 (58.1)	67 (76.1)	

NA: not available.

(accounting for 38.6% and 43.2% of children patients, respectively). Among the patients, 53.7% (110/205) were female, 65.9% (135/205) were treated in wards of internal medicine. The admission rate of children and adults was 76.1% and 58.1%, respectively. None of the patients required invasive mechanical ventilation, or treatment in intensive care unit (ICU).

Analysis on macrolide susceptibility of clinical isolates

In vitro activities of nine antimicrobials are listed in supplementary Table 1. 88.3% (181/205) were resistant to macrolide with MIC ≥ 32 μg/ml to erythromycin. These erythromycin-resistant isolates also showed cross-resistance to clarithromycin and azithromycin. All isolates were sensitive to tetracyclines and fluoroquinolones. Moxifloxacin was more active than ciprofloxacin and levofloxacin. The MIC$_{50}$ and MIC$_{90}$ of moxifloxacin were 0.064 μg/ml and 0.064 μg/ml, respectively, much lower than those of ciprofloxacin and levofloxacin (0.5 μg/ml and 1 μg/ml). Further, 83 (94.3%) MR isolates were detected in 88 children, and 98 (83.8%) were found in 117 adults ($p = 0.027$; Table 3). Results of 23S rRNA gene sequencing indicated that all MR isolates harbored an A2063G mutation, and all sensitive isolates did not have such a mutation (Table 5).

Table 3
Molecular characteristics of 205 *M. pneumoniae* clinical isolates between children and adults. Data were represented as n (%).

Characteristic	Total (205)	Adults (117)	Children (88)	*P* value[a]
Macrolide profile				0.027
Resistant	181 (88.3)	98 (83.8)	83 (94.3)	
Sensitive	24 (11.7)	19 (16.2)	5 (5.7)	
P1 genotype				0.042
Type 1	177 (86.3)	96 (82.1)	81 (92.0)	
Type 2 lineages	28 (13.7)	21 (17.9)	7 (8.0)	
MLVA type				0.14
4/5/7/2	165[b] (80.5)	89 (76.1)	76 (86.4)	
3/5/6/2	30[c] (14.6)	22 (18.8)	8 (9.1)	
Other	10[d] (4.9)	6 (5.1)	4 (4.5)	
4/5/7/3	3 (1.5)	2 (1.7)	1 (1.1)	
3/5/7/2	3 (1.5)	1 (0.9)	2 (2.2)	
4/5/6/2	2 (1.0)	2 (1.7)	0 (0)	
4/4/7/2	1 (0.5)	1 (0.9)	0 (0)	
4/5/7/1	1 (0.5)	0 (0)	1 (1.1)	

[a] The χ^2 test or Fisher's exact test was performed in comparison between children with adults.
[b] 165 strains included 164 P1 type 1 and one type 2 lineage.
[c] 30 strains included four P1 type 1 and 26 type 2 lineage.
[d] 10 strains included nine P1 type 1 and one type 2 lineage (4/5/7/3).

Molecular typing of M. pneumoniae *isolates*

Type 1 and type 2 lineages, the main P1 types of *M. pneumoniae*, accounted for 86.3% and 13.7% of 205 clinical isolates, respectively (Table 3). Type 1 accounted for the majority of the isolates from all ages, including 82.1% of the detections in adults and 92% in children, whereas type 2 lineages accounted for 17.9% in adults and only 8.0% in children ($p = 0.042$; Table 3). Additionally, 205 *M. pneumoniae* clinical isolates were divided into seven MLVA types (Table 3). The 88 and 117 isolates from children and adult patients were clustered into 6 and 5 MLVA types. The majority (80.5%) of the detections were type 4/5/7/2, including 76.1% of adults and 86.4% of children. Type 3/5/6/2 was the second (14.6%) most commonly identified MLVA type in both adults (18.8%) and children (9.1%). Two MLVA types, 4/5/6/2 (n = 2) and 4/4/7/2 (n = 1), were identified only in adults, whereas type 4/5/7/1 was identified in a single child specimen (Table 3). Similar distribution of MLVA types have been found between children and adults ($p = 0.14$). The proportions of MLVA types 4/5/7/2, 3/5/6/2, and other types were shown in Figure 1. And there was a peak of infection during November 2010 to March 2011.

Molecular characterization

According to the site of care, the patients were divided into inpatients and outpatients. The distribution of MRMP, P1 types and MLVA types were nearly equal between the two groups ($p = 0.929$, 0.833 and 0.953; Table 4).

The admission rates of patients infected with MSMP and MRMP were both around 66% (Table 5). Although the MRMP rate of female patients was higher than that of male patients, there was no significant difference ($p = 0.274$). All MSMP isolates were determined as P1 type 2 lineages. Oppositely, the majority of P1 type of MRMP isolates were type 1 (97.8%), only 4 isolates were detected as type 2 lineages (2.2%). There was a significant difference in the distribution of P1 types between MSMP and MRMP ($p < 0.001$). Similarly, the proportions of predominant MLVA types were significantly different between MSMP (95.8% type 3/5/6/2, 4.2% type 4/5/6/2 and no type 4/5/7/2) and MRMP (91.2% type 4/5/7/2, 3.9% type 3/5/6/2 and 4.9% other types) ($p < 0.001$; Tables 5).

Additional pathogens

In most patients *M. pneumoniae* was identified as the sole pathogen. Additional pathogens were detected in 25 patients (12.2%; Supplemental Table 2), including 20 inpatients and 5 outpatients. Among them, there were 19 cases of MLVA type 4/5/7/2 (76%), 5 cases of 3/5/6/2 (20%) and 1 case of 4/5/6/2 (4%), which was consistent with the distribution of MLVA types. The common additional pathogens included: influenza virus A, respiratory syncytial virus, human rhinovirus, parainfluenza viruses, bacteria and other respiratory viruses. Moreover, triple and quadruple infections were found in two cases and one case. One 73-year old male patient was co-infected with *M. pneumoniae* of MLVA type 4/5/7/2, and both subtypes of influenza virus A (H1N1 pandemic 2009 and seasonal H3N2).

Discussion

This study was conducted simultaneously with worldwide reported epidemics from 2010 to 2012 (Pereyre et al., 2007; Ho et al., 2015; Nir-Paz et al., 2012; Okada et al., 2012; Kim et al., 2015). The age of *M. pneumoniae* positive cases was 23 (22) years, 53.7% were female and 65.9% were inpatients. Compared with adult CAP patients, higher positivity and MR rate of *M. pneumoniae* had been found in children ($p < 0.001$ and $p = 0.027$). A significantly higher

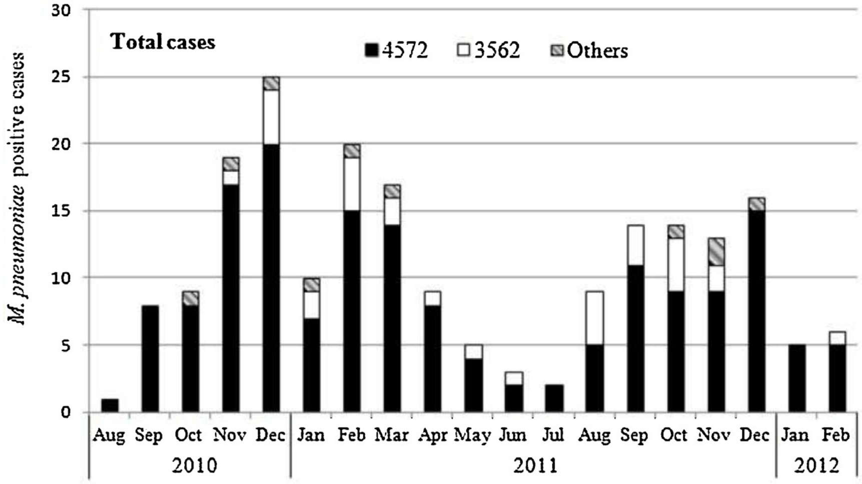

Figure 1. Number of *M. pneumoniae* detections of the two predominant MLVA types, 4/5/7/2 (black bars) and 3/5/6/2 (white bars), and other types (hashed bars) in total cases during August 2011 to February 2012. The x-axis indicated the year-month.

Table 4
Molecular characteristics of 205 *M. pneumoniae* clinical isolates by site of care. Data were represented as n (%).

Characteristic	Total (205)	Inpatients (135)	Outpatients (70)	*P* value[a]
Macrolide profile				0.929
Resistant	181 (88.3)	119 (88.1)	62 (88.6)	
Sensitive	24 (11.7)	16 (11.9)	8 (11.4)	
P1 genotype				0.833
Type 1	177 (86.3)	117 (86.7)	60 (85.7)	
Type 2 lineages	28 (13.7)	18 (13.3)	10 (14.3)	
MLVA type				0.953
4/5/7/2	165 (80.5)	108 (80.0)	57 (81.4)	
3/5/6/2	30 (14.6)	20 (14.8)	10 (14.3)	
Other	10 (4.9)	7 (5.2)	3 (4.3)	
4/5/7/3	3 (1.5)	2 (1.5)	1 (1.4)	
3/5/7/2	3 (1.5)	2 (1.5)	1 (1.4)	
4/5/6/2	2 (1.0)	2 (1.5)	0 (0)	
4/4/7/2	1 (0.5)	1 (0.7)	0 (0)	
4/5/7/1	1 (0.5)	0 (0)	1 (1.4)	

[a] The χ^2 test or Fisher's exact test was performed in comparison between inpatients with outpatients.

Table 5
Comparison of characteristics between MRMP and MSMP. Data were represented as n (%).

	MSMP	MRMP	*P* value
Site of care			0.563
Outpatients	8 (33.3)	62 (34.3)	
Inpatients	16 (66.7)	119 (65.7)	
Gender			0.274
Female	11 (45.8)	99 (54.7)	
Male	13 (54.2)	82 (45.3)	
P1 genotype			< 0.001
Type 1	0 (0)	177 (97.8)	
Type 2 lineages	24 (100.0)	4 (2.2)	
MLVA type			< 0.001
3562	23 (95.8)	7 (3.9)	
4572	0 (0)	165 (91.2)	
Other[a]	1 (4.2)	9 (4.9)	
23S rRNA genotype			NA
A2063G	0 (0)	181 (100)	

MSMP: macrolide-sensitive *M. pneumoniae*; MRMP: macrolide-resistant *M. pneumoniae*; NA: not available.

[a] The MLVA type of the only MSMP is 4/5/6/2.

rate of admission had also been observed, which reflected that there were more severe cases in children (58.1% vs. 76.1%, $p = 0.008$). Two investigations on hospitalized patients with *M. pneumoniae* pneumonia reported that the rates of ICU admission were as high as 10% and 16.3% (Diaz et al., 2015a; Khoury et al., 2016). However, none of 135 hospitalized patients in our study were treated in the ICU. The underestimated ICU admission rate could be possibly due to, (1) relatively small amount of inpatients; (2) the severe pediatric patients were transferred to Children's Hospital.

Co-circulation of 2 main P1 types, as well as MLVA types 4/5/7/2 and 3/5/6/2 was observed during the study period. The pattern of monthly distributions of MLVA types coincided with the prevalence of *M. pneumoniae*. An association between the 2 main typing methods has previously been reported: type 1 and type2 lineage strains are mainly, but not exclusively, MLVA type 4/5/7/2 and 3/5/6/2 (Benitez et al., 2019; Diaz et al., 2015b; Sun et al., 2017). A similar correlation was also observed in the current study, 164 (99.4%) of 165 strains with MLVA type 4/5/7/2 were type 1, 26 (86.7%) of 30 type 3/5/6/2 strains were P1 type 2 lineages. Therefore, MLVA typing may have greater utility because it affords the ability to further distinguish strains while still predicting P1 type. However, there are also nine strains that show irregular combination of the P1 and MLVA types, including two type 2 lineage strains that exhibited MLVA 4/5/7/2 and 4/5/7/3, four type 1 strains that exhibited MLVA 3/5/6/2 and three type 1 strains that exhibited MLVA 3/5/7/2.

Compared with adults, *M. pneumoniae* infections were more common in children, and most studies focused on children population. Herein, the molecular characterizations of *M. pneumoniae* were compared between children and adults. P1 type 1 was more common in children than adults (92% vs. 82.1%, $p = 0.042$). But the distribution of MLVA types did not differ between adults and children ($p = 0.14$; Table 3). These findings were partly consistent with the Diaz report that the distributions of both P1 and MLVA types differed between adult and pediatric populations ($p = 0.02$ and $p < 0.01$) (Diaz et al., 2015a). The much higher proportion of MLVA type 4/5/7/2 in our study could possibly contribute to this inconsistency. Moreover, most investigations focused on hospitalized patients (Diaz et al., 2015a; Ho et al., 2015), we firstly analyzed the molecular characterization of *M. pneumoniae* isolates between CAP outpatients and inpatients. Interestingly, neither the macrolide resistance nor the P1 or MLVA types were different between the two groups ($p = 0.929$, $p = 0.833$, $p = 0.953$). Considering the symptoms of outpatients were clinically milder than those of inpatients, we speculated that macrolide resistance or genotype of *M. pneumoniae* would not affect the severity of infected patients. Similarly, Nilsson et al. previously compared the clinical characteristics between 24 inpatients with *M. pneumoniae* pneumonia and 21 outpatients with *M. pneumoniae* mild infections, and reported that clinical severity did not correlated with genotype ($p = 0.62$) but with bacterial load ($p = 0.039$) in oropharyngeal secretions (Nilsson et al., 2010). Diaz et al. also reported no significant differences in clinical characteristics identified among patients infected with varied strain types (Diaz et al., 2015a). Therefore, more investigations from different regions and periods are warranted to give a more accurate conclusion on molecular characterization between children and adults, as well as the association between genotypes and clinical severity, which is also helpful to understand the biological and epidemiological reasons that contribute to this phenomenon.

The A2063 G transition was the only mutation identified, which was the most important molecular mechanism of MRMP. However, the correlation between macrolide resistance and a specific *M. pneumoniae* genotype is still controversial among studies conducted in different geographic areas, whether during epidemics or non-epidemics. In the USA and Europe with incidence of MRMP ≤ 10%, although P1 type 1 and MLVA type 4/5/7/2 were more common in MRMP, no association of an individual strain type with the resistant genotype had been found (Diaz et al., 2015a,b; Kogoj et al., 2015). A limited amount of MRMP isolates and sporadic occurrence of other genotypes could be responsible for this phenomenon, whereas opposite results were found in areas with a higher incidence of MRMP. A correlation between increasing macrolide resistance and MLVA type 4/5/7/2 had been found in Hong Kong based on four years of data during 2011–2014 (Ho et al., 2015), as well as in the specimens collected from the United States and China (Sun et al., 2017; Yan et al., 2015). In the current study, similarly, all *M. pneumoniae* strains with P1 type 1 or MLVA type 4/5/7/2 were resistant to macrolide, however, those with type 2 lineages or type 3/5/6/2 could be probably sensitive to macrolide. This suggests that the higher incidence of MRMP infections is likely a result of accumulated transmission of P1 type 1 or MLVA type 4/5/7/2 rather than the introduction of new types into the community.

All of the specimens were obtained from patients with *M. pneumoniae* pneumonia. No patients required invasive mechanical ventilation or treatment in ICU. No patients died within four weeks. Since *M. pneumoniae* infections commonly occurred in winter and spring, most pathogens of co-infections were respiratory viruses, and there are no associations between those pathogens and *M. pneumoniae* genotypes.

A major limitation is that the type 2 lineages in this study had not been distinguished more precisely, which may contain type 2 (although type 2 is very rare (Sun et al., 2017; Suzuki et al., 2017)), 2a, 2b and 2c. In the reports (Sun et al., 2017; Suzuki et al., 2017), the MLVA 3/5/6/2 is associated with more type 2a and 2c, type 2b is more associated with MLVA 3/5/5/2. We speculated that the type 2 lineages here were mainly type 2a and 2c, since the mostly related MLVA type was found to be 3/5/6/2.

This study provides a comprehensive characterization of *M. pneumoniae* molecular epidemiology and macrolide resistance among CAP patients of all age during an epidemic, as well as more evidences on a correlation between MLVA type 4/5/7/2 and macrolide resistance in the setting of a high incidence of MRMP.

Conflicts of interest

None.

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Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional Review Board of Beijing Chao-Yang Hospital (project approval number 10-KE-49) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all adults and the legal representatives of patients aged <18 years included in the study.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2019.03.028.

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