Comparison of Molecular Characteristics of *Mycoplasma pneumoniae* Specimens Collected from the United States and China

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***Mycoplasma pneumoniae*-positive clinical specimens obtained from the United States and China during the same period were studied for their molecular characteristics. We found much more diverse genotypes and a lower prevalence of macrolide resistance in the U.S. specimens. Data from the study also showed an association of the resistance with certain genotypes.**

Mycoplasma pneumoniae is one of the most important pathogens causing community-acquired pneumonia (CAP) in children and adults (1). Worldwide epidemics have been reported every 3 to 7 years, and they usually last up to 2 years each time (2, 15). The latest global epidemic was from late 2010 to 2012 (2–9). Decreasing herd immunity and different *M. pneumoniae* genotypes circulating in the human population are believed to be the main causes of epidemics (10). Macrolide-resistant *M. pneumoniae* was first isolated in Asia and has emerged worldwide over the past 15 years, possibly as a result of the widespread use of macrolide antibiotics. Therefore, monitoring the progress of epidemics, identifying outbreaks, and surveying for macrolide resistance have become increasingly important.

Currently, P1 restriction fragment length polymorphisms (RFLP) and multiple-locus variable-number tandem repeat (VNTR) analysis (MLVA) are the most widely used methods for strain typing for this pathogen (2–12). We improved the MLVA method by amplifying four VNTRs (without Mpn1) in one tube for epidemic and outbreak analyses (11). In this study, we used MLVA, P1 RFLP and macrolide resistance-associated mutations testing data to analyze and directly compare the molecular characteristics of *M. pneumoniae* specimens collected from the United States and China.

***M. pneumoniae* specimens.** Fifty-nine U.S. *M. pneumoniae* specimens included in this study were derived as a subset of samples from a previous study (13). These clinical specimens were collected in four different geographical locations in the United States (Kansas City, MO; Seattle, WA; New York, NY; and Chicago, IL) from August 2012 and January 2014.

For comparison purposes, we analyzed the data of 198 *M. pneumoniae*-positive clinical specimens consecutively collected in the Affiliated Children's Hospital of the Capital Institute of Pediatrics in Beijing, China during the same time period. Among them, 127 were previously described (11), and the other 71 are being reported for the first time. Positive specimens were identified using real-time PCR as described previously (3).

Specimens collected from the two countries were divided into four age groups: 0 to 3 years, 4 to 6 years, 7 to 12 years, and adults.

MLVA typing. MLVA typing was performed based on a system that included four VNTR loci, Mpn13, Mpn14, Mpn15, and Mpn16, as described in our previous publication (11). The following six distinct MLVA types were identified in the 59 specimens

from the United States: M4-5-7-2, M3-5-6-2, M3-6-6-2, M3-5-6-1, M4-5-7-3, and M5-5-7-2. Among them, type M3-5-6-1 was detected for the first time. Compared with the specimens from the United States, those from China belonged to nine distinct MLVA types. While the predominant MLVA types of M4-5-7-2, M3-5-6-2, M4-5-7-3, and M5-5-7-2 were the same as those found in the United States, the remaining 5 MLVA types, M4-5-6-2, M3-5-7-2, M4-4-7-2, M4-5-4-2, and M4-5-5-2 were only found in the specimens from China (Table 1).

P1 gene typing. The P1 gene typing was performed by P1 RFLP as described previously (7). Twenty-nine U.S. specimens were P1 type 1, 9 were type 2, 18 were type 2c, and 3 were type 2a. All M3-5-6-1, M3-5-6-2, and M3-6-6-2 were P1 type 2 and variants, and all M4-5-7-2, M4-5-7-3, and M5-5-7-2 were P1 type 1. Compared with the 49.2% P1 type 1 specimens from the United States, the Chinese specimens were 99.5% (197/198) type 1 and 0.50% type 2c (1/198).

Detection of macrolide resistance. Macrolide resistance-associated mutations in domain V of the 23S rRNA gene were detected using PCR melting curve analysis, DNA sequencing, and MIC testing for U.S. samples (13), and the nested PCR-linked capillary electrophoresis and single-strand conformation polymorphisms (nPCR-CE-SSCP) were used for Chinese specimens (14). The 7 macrolide-resistant specimens (7/59, 11.9%) of the U.S. specimens (with the mutation A2063G) were all MLVA type M4-5-7-2 (Fig. 1) and P1 type 1. All had erythromycin MICs of >256 µg/ml (13). Among the Chinese specimens, 193 (97.5%) contained an A2063G mutation in the 23S rRNA gene conferring macrolide resistance, and no mutation was found in domain V of the 23S rRNA for the remaining 5 specimens (2.53%). Whereas all 7 U.S.

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TABLE 1 Molecular characteristics of specimens collected in the United States and China

Characteristic	Total no. (%) ^a	No. collected in the United States (%) ^b	No. collected in China (%) ^c
MLVA type			
M3-5-6-1	1 (0.39)	1 (1.69)	0 (0)
M3-5-6-2	19 (7.39)	18 (30.5)	1 (0.51)
M3-5-7-2	1 (0.39)	0 (0)	1 (0.51)
M3-6-6-2	11 (4.28)	11 (18.6)	0 (0)
M4-4-7-2	1 (0.39)	0 (0)	1 (0.51)
M4-5-4-2	1 (0.39)	0 (0)	1 (0.51)
M4-5-5-2	1 (0.39)	0 (0)	1 (0.51)
M4-5-6-2	5 (1.95)	0 (0)	5 (2.53)
M4-5-7-2	213 (8.29)	27 (45.8)	186 (93.9)
M4-5-7-3	2 (0.78)	1 (1.69)	1 (0.51)
M5-5-7-2	2 (0.78)	1 (1.69)	1 (0.51)
P1 genotype			
Type1	226 (87.9)	29 (49.2)	197 (99.5)
Type2	9 (3.50)	9 (15.3)	0 (0)
Type 2a	3 (1.17)	3 (5.08)	0 (0)
Type 2c	19 (7.39)	18 (30.5)	1 (0.51)
Macrolide sensitive	57 (22.2)	52 (88.1)	5 (2.53)
Macrolide resistant	200 (77.8)	7 (11.9)	193 (97.5)

^a A total of 257 specimens from the United States and China were studied.
^b A total of 59 specimens were collected in the United States.
^c A total of 198 specimens were collected in China.

macrolide-resistant specimens belonged to MLVA type M4-5-7-2, 184 (95.3%) of the 193 macrolide-resistant specimens from China were also M4-5-7-2 ($P < 0.05$; significantly associated with macrolide resistance) (Fig. 1). The MLVA types of the other 9 resistant

specimens were M4-5-6-2 ($n = 3$), M3-5-6-2 ($n = 1$), M3-5-7-2 ($n = 1$), M4-4-7-2 ($n = 1$), M4-5-5-2 ($n = 1$), M4-5-7-2 ($n = 1$), and M5-5-7-2 ($n = 1$).

No association was found between MLVA type, P1 genotype, macrolide resistance, and specimen collection time or patient age in the two countries. Additionally, there was no statistically significant difference in the distribution of MLVA type, P1 genotype, and macrolide resistance for specimens obtained in the four different geographic regions of the United States ($P > 0.05$).

Previously, we reported the obvious differences in molecular characteristics of *M. pneumoniae*-positive specimens collected in Australia and China from 2008 to 2012 (9). To investigate further the molecular characteristics between different regions in this study, a comparison was conducted on specimens obtained from the United States and China. The predominant MLVA type of M4-5-7-2 (93.9%) and P1 genotype of type 1 (99.5%) in specimens from China in this study were the same as those found in earlier Chinese studies (14). In contrast, 3 predominant genotypes (M4-5-7-2, M3-5-6-2, and M3-6-6-2) were found in the specimens from the United States collected during the same period of time.

During the past 15 years, macrolide resistance for *M. pneumoniae* has been reported in many countries in Europe, Asia, and Africa and in the United States (2–13). Similar to previous reports (6, 8), the prevalence of mutation of the macrolide resistance-associated gene in this study was high in China. Interestingly, all 7 macrolide-resistant specimens from the United States were MLVA type M4-5-7-2, and 184 of 193 (95.3%) macrolide-resistant specimens from China were also M4-5-7-2. Therefore, we have observed a clear association between the M4-5-7-2 type and macrolide resistance ($P < 0.005$). Although it was concluded that

FIG 1 The relationship between MLVA types and macrolide resistance in the United States and China. Each column represents a unique MLVA type in the two countries. The colors (red, pink, blue, and green) of the columns mark the resistance or susceptibility of Mp in different countries.

there was no obvious association between MLVA type and macrolide resistance in previous studies by Dégrange et al., Benitez et al., and Zhao et al. (2, 5, 6), Pereyre et al. reported a possible correlation between MLVA type M7-4-5-7-2 (Z) and macrolide resistance in France (4). A possible reason for these conflicting data is the result of inclusion of the *Mpn1* locus in the analysis since this locus is known to be unstable and it affects effective type analysis (7). Qu et al. reported an association between macrolide susceptibility and MLVA type M3-5-6-2 in China (8). More recently, Diaz et al. (12) reviewed 17 investigations of cases, clusters, and outbreaks collected from 2006 to 2013 in the United States and found a 10% macrolide resistance rate. However, there was no apparent association between this resistance and P1 or MLVA type. The reason for this difference between the study of Diaz et al. (12) and our present study regarding macrolide association with MLVA type is unclear. Possibilities may include differences in sample collection, the geographic locations of patients, the time of collection, and the smaller sample size of our current study. This underscores the need for surveillance. Our results show that the high rate of macrolide resistance in China may be the results of dissemination of the MLVA type M4-5-7-2 clone.

This is the first study comparing the molecular characteristics of *M. pneumoniae* specimens from China and the United States collected during the same period. There are clear differences in MLVA types and P1 genotypes between the two countries, and there is some relationship between MLVA type M4-5-7-2 and macrolide resistance in the two countries. Molecular typing may help better understand the epidemiology and emergence of antibiotic resistance of *M. pneumoniae* infections.

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