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Surveillance of Macrolide-Resistant *Mycoplasma pneumoniae* in Beijing, China, from 2008 to 2012

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Macrolide resistance rates of *Mycoplasma pneumoniae* in the Beijing population were as high as 68.9%, 90.0%, 98.4%, 95.4%, and 97.0% in the years 2008 to 2012, respectively. Common macrolide-resistant mobile genetic elements were not detected with any isolate. These macrolide-resistant isolates came from multiple clones rather than the same clone. No massive aggregation of a particular clone was found in a specific period.

Mycoplasma pneumoniae is one of the important pathogens causing human respiratory tract infection, especially in community-acquired pneumonia (1, 2). The major clinical treatment for *M. pneumoniae* infection is the use of macrolide antibiotics (ML). With the widespread use of the drug, ML-resistant isolates have been reported worldwide (3–5). The resistance mechanism has been identified as a point mutation in the 23S rRNA gene. Other mechanisms of macrolide resistance cannot be excluded and have not been identified. In recent years, ML-resistant *M. pneumoniae* has become very serious in Asia (6, 7) and has attracted the attention of scientists. Studies on ML-resistant *M. pneumoniae* in China have only recently been conducted, and the limited reports have been mainly ML resistance analyses of a small number of strains isolated during a few months and from specific populations, such as children or adults (8–11). These reports are lacking continuous full-population surveillance data of *M. pneumoniae* drug resistance. In view of the above-mentioned information, we have studied drug resistance of 309 *M. pneumoniae* isolates from a whole population of strains isolated from people with respiratory infections in Beijing, China, from 2008 to 2012, a study which will help us to understand the status of drug-resistant *M. pneumoniae* in Beijing in recent years.

***M. pneumoniae* strains.** A total of 309 *M. pneumoniae* strains were isolated from 1,183 respiratory infection specimens from Beijing Chao-Yang Hospital, Beijing Children's Hospital, and Beijing Centers for Diseases Control and Prevention. One hundred fifty-six isolates were from 388 pediatric specimens of patients <14 years of age, and the remaining 153 isolates were collected from 795 adolescent and adult specimens. All 309 isolates were purified, cultured, and identified with a real-time PCR method (12).

Detection of macrolide resistance at the gene level. Genomic DNA of 309 *M. pneumoniae* isolates was extracted using the QIAamp DNA minikit (Qiagen). The extracts were distributed into aliquots and saved at −20°C. The domain V region of the 23S rRNA gene was amplified by PCR methods described previously (6). The amplification products were sequenced by the Beijing Genomics Institute (BGI). The results showed that there were existing point mutations in domain V of the 23S rRNA gene region of 280 strains in the 309 *M. pneumoniae* isolates. In 272 of the 280 isolates (97.1%), the mutation was identified as A2063G. Seven of the 280 isolates (2.5%) had the A2064G mutation, one of the 280 isolates (0.4%) had an A2063T mutation, and the remaining 29 isolates did not have a detectable mutation in this region. The genomic DNA of 309 *M. pneumoniae* isolates was examined for two erythromycin ribosome methylation (*erm*) genes (*ermA* and *ermB*) and one efflux pump gene (*mefA*) by previously described methods (13). No positive result was detected.

Antimicrobial susceptibility testing. A total of 180 isolates, including 29 isolates without domain V region mutations, 7 isolates with the A2064G mutation, 1 isolate with the A2063T mutation, and 143 isolates with the A2063G mutation, were selected. The MICs of 7 antibiotics were determined by broth microdilution methods with SP4 broth (Remel). *M. pneumoniae* reference strain M129 (ATCC 29342) was tested as an antibiotic-sensitive control. Susceptibility results showed that 143 isolates with the A2063G mutation and 7 isolates with the A2064G mutation displayed high-level resistance to erythromycin and clarithromycin (64 to >256 μg/ml). The MIC values of the isolate with the A2063T mutation to erythromycin, clarithromycin, and azithromycin were 32 μg/ml, 16 μg/ml, and 0.064 μg/ml, respectively. Twenty-nine isolates without a domain V regional mutation were sensitive to macrolides. All *M. pneumoniae* isolates, including M129, were sensitive to fluoroquinolones and tetracycline (Table 1).

Detection of VNTR numbers in the p1 gene of *M. pneumoniae* strains. The variable-number tandem-repeat (VNTR) region in the p1 gene of 309 *M. pneumoniae* isolates was amplified by PCR using our previously reported method (11). The sequencing

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TABLE 1 MIC range of seven antimicrobial agents against 180 *M. pneumoniae* clinical strains and M129

Mutation in the 23S rRNA gene	No. of strains with the mutation	MIC (μg/ml)[a]						
		ERY	CLR	AZM	TET	CIP	LVX	GAT
A2063G	143	128 to >256	64 to >256	2–64	0.032–0.5	0.125–2	0.125–2	0.016–0.125
A2064G	7	256 to >256	256 to >256	4–32	0.125–0.25	0. 5–1	0. 25–1	0.016–0.125
A2063T	1	32	16	0.064	0.25	0.5	0.25	0.064
None	29	0.008–0.016	<0.008–0.008	<0.008–0.008	0.016–0.5	0.008–1	0.008–1	0.008–0.125
None (M129 reference strain)	1	0.016	<0.008	<0.008	0.125	1	1	0.125

[a] MICs were determined by microdilution methods using SP4 broth. The MIC of each agent was defined as the lowest concentration of each antibiotic preventing the color change. ERY, erythromycin; CLR, clarithromycin; AZM, azithromycin; TET, tetracycline; CIP, ciprofloxacin; LVX, levofloxacin; GAT, gatifloxacin.

results showed that the VNTR number of the p1 gene from 309 isolates ranged from 4 to 14 (Table 2).

***M. pneumoniae* ML resistance monitoring.** Of 309 isolates of *M. pneumoniae*, 61 were isolated in 2008, 20 were isolated in 2009, 64 were isolated in 2010, 131 were isolated in 2011, and 33 were isolated in 2012. The proportion of ML-resistant isolates was 68.9% in 2008, and the proportion of ML-resistant isolates was more than 90% in the last 4 years (Table 3). The ML resistance rate of *M. pneumoniae* strains isolated from cases of children below the age of 14 was 98.1% (153/156), and the rate was 83.0% (127/153) for strains isolated from cases of adolescents and adults.

ML resistance of *M. pneumoniae* is very common all over the world. In this study, the resistance mechanism is clearly a point mutation in the specific locus of the 23S rRNA gene, especially in loci 2063 and 2064 (6, 14). Other macrolide-resistant mechanisms were not identified in this study. We found that in recent years, based on surveillance of *M. pneumoniae* isolated from cases of respiratory tract infections from 2008 to 2012, ML resistance rates in *M. pneumoniae* in Beijing, China, are much higher than those of other countries. The reasons for the extremely high ML resistance rates of *M. pneumoniae* in Beijing may be the inability to provide surveillance results and also the overuse of antibiotics. Because of the lack of strains isolated from years prior to those observed in this study, we were unable to know the earlier data of ML resistance of *M. pneumoniae* in Beijing. Until now, dozens of *erm* and *mef* genes borne by transposons or plasmids have been reported in pneumococci and enterobacteria (13) but not in *M. pneumoniae*. In this study, these three most common ML-resistant genes were not detected with any *M. pneumoniae* isolate. The ability to take up mobile genetic elements by *M. pneumoniae* isolates was not able to be demonstrated. This might be due to the reduced genome of the unique bacterium. ML-resistant isolates in Beijing from 2008 to 2012 were usually isolates with mutations in the domain V region loci 2063 (97.1%) and 2064 (2.5%) of the 23S rRNA gene. We found no isolates with low ML resistance with the locus 2617 mutation (6) and only one isolate with the A2063T mutation. This situation of high ML resistance for first-line treatment drugs had caused great difficulties in the clinical treatment of *M. pneumoniae*, especially in pediatric infections. The data of the susceptibility test showed that all *M. pneumoniae* isolates were sensitive to fluoroquinolones *in vitro*, especially the new fluoroquinolone gatifloxacin (0.008 to 0.125 μg/ml). In the situation of high ML resistance in China, such antibiotics have the potential to become alternative medicines for treating *M. pneumoniae* infection in adults, but these drugs have strict limitations of use for children.

The stable VNTR (11, 15) sequencing results of the p1 gene in 309 isolates indicated that ML-resistant isolates and ML-sensitive isolates of *M. pneumoniae* in Beijing were not from a single clone but were from multiple clones, and the aggregation phenomena of a certain number of VNTR clones were not found during the years of this study. In addition, the multilocus VNTR analysis (MLVA) typing results of the 201 *M. pneumoniae* isolates (16) also supported the above-mentioned conclusion. The use of the MLVA method or single VNTR locus detection of *M. pneumoniae* may be useful for molecular epidemiology studies.

In summary, ML resistance of *M. pneumoniae* in the Beijing population was at a high rate of more than 90% from 2008 to 2012. A total of 280 isolates with ML resistance were isolated from multiple clones instead of the same clone, since the large aggregation phenomenon of a particular clone was not found within a specific

TABLE 2 VNTR numbers in the p1 gene of 280 ML-resistant and 29 ML-susceptible *M. pneumoniae* strains

No. of AGT VNTR	No. of detected strains with each no. of VNTR	
	ML resistant	ML susceptible
4	4	0
5	18	2
6	32	8
7	77	13
8	62	5
9	45	0
10	30	1
11	9	0
12	2	0
14	1	0

TABLE 3 ML resistance in *M. pneumoniae* surveillance data from 2008 to 2012 in Beijing, China

Yr	No. of strains		ML resistance rate (%)
	ML susceptible (no mutation in the 23S rRNA gene)	ML resistant (A2063G, A2064G, or A2063T mutation)	
2008	19	42	68.9
2009	2	18	90.0
2010	1	63	98.4
2011	6	125	95.4
2012	1	32	97.0
Total	29	280	90.6

time period. In the future, it will be necessary to establish a greater scope and scale for long-term monitoring of *M. pneumoniae* to guide domestic clinical treatment of *M. pneumoniae* infection in China.

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