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Macrolide-Resistance of *Mycoplasma pneumoniae* in Several Regions of China from 2013 to 2019

Yue Jiang, Haiwei Dou, Bo Xu, Baoping Xu, Wei Zhou, Hong Wang, Lixia Ge, Yinghui Hu, Xiaohua Han, Xuanguang Qin, Jing Li, Leping Ye, Liqun Wu, Huimin Zuo, Qi Zhang, Ling Liu, Wenjuan Hu, Junyan Shao, Qiaomian Yin, Lina Han, Xiaoyan Fu, Xiaopei Dong, Yan Dong, Yulin Fu, Mengmeng Zhao, Qing Sun, Jingwei Huo, Die Liu, Wenkao Liu, Yunjuan Li, Yang Wang, Deli Xin*, Kunling Shen*
*Correspondence: Deli Xin, xindl48@126.com; Kunling Shen, kunlingshen1717@163.com

Abstract

This paper retrospectively analyzed the prevalence of macrolide-resistant *M. pneumoniae* (MRMP) in some parts of China. Between January 2013 to December 2019, respiratory samples and clinical information were collected from children with respiratory tract infections in outpatient or inpatient departments. All the samples were performed PCR testing of *M. pneumoniae* and sequenced the domain V of 23S rRNA region. Minimum inhibition concentration (MIC) of macrolides and other antibiotics were tested. We collected 4145 respiratory samples, including pharyngeal swabs and alveolar lavage fluid. The highest PCR-positive rate of *M. pneumoniae* was 74.5% in Beijing, and the highest resistance rate was 100% in Shanghai, Gansu was the lowest with 20%. The highest PCR positive rate of *M. pneumoniae* was 74.5% in 2013, the highest MRMP was 97.4% in 2019; the PCR positive rate of *M. pneumoniae* for adults in Beijing was 17.9% and MRMP was 10.48%, which was significantly lower than that of children. The rate of *M. pneumoniae* positive samples and of macrolide-resistant strains is higher in school-age children than that in toddlers and preschool-age ones. The PCR and macrolide-resistant rates of *M. pneumoniae* in children admitted to the hospital were both higher than outpatients. Among the children diagnosed with CAP, the PCR-positive and macrolide-resistance rates of *M. pneumoniae* were both higher in the severe ones. A2063G in domain V of 23S rRNA was the major macrolide-resistance mutation, accounting for more than 90%. The MIC values of all MRMP to erythromycin and azithromycin were ≥64 μg/ml, and the MICs of tetracycline and levlevofloxacin were ≤0.5 μg/ml and ≤1 μg/ml respectively. The macrolide resistance varied in different regions and years. *M. pneumoniae* and macrolide-resistant rate was

higher in school-aged children. Among inpatients, the macrolide-resistant rate was higher in severe pneumonia. A2063G was the common mutation and we found no resistance to tetracycline and levlevofloxacin.
Key words: *M. pneumoniae,* macrolide, drug-resistant, epidemic, clinical features

Introduction

Mycoplasma pneumoniae (*M. pneumoniae*) is a common pathogen of respiratory tract infections in children, with an epidemic occurring every 3-7 years(1, 2), mostly in schools, military units and other places with relatively high population concentrations, counting for about 10-40% in children with community-acquired pneumonia(CAP)(3, 4). Children and adults are susceptible to *M. pneumoniae*, school-aged and adolescent children have a higher incidence and are more likely to develop pneumonia or severe disease(5). Macrolide-resistant *M. pneumoniae* (MRMP) was first isolated from clinical patients in Japan in 2000, after which the emergence of clinically resistant strains was reported in Asia, Europe and North America, with different rates of resistance in different regions. The resistance rate of *M. pneumoniae* in China is at a high level, reaching more than 90% in specific regions, and is higher in children than in adults, which making clinical treatment difficult.(6)

Our laboratory has conducted multicenter *M. pneumoniae* prevalence and macrolide- resistance surveillance since 2013. In the study, we conducted a retrospective study of *M. pneumoniae* clinical surveillance programs in selected regions of China between 2013 and 2019, statistically analyze macrolide-resistance in age, gender, outpatient and ward, and severe/common pneumonia. What's more, we conducted Minimum inhibition concentration (MIC) of commonly used antibiotics. This paper aims to increase the knowledge of *M. pneumoniae* and MRMP infection status, which is significant for the rational application of antibiotics.

Materials and methods

Enrolled Patients

We collected specimens from seven regions, including Beijing, Shenyang, Changsha, Changchun, Shanghai, Wuhan, Gansu, during January 2013 to December 2019. Both outpatients and inpatients are included, the inclusion criteria were as follows: (a) fever; (b) cough, sore throat, or other symptoms of respiratory tract infections; (c) the course of illness lasts 1 to 7 days; (d) white blood cell count is 4.0-12.0 × 109/L. The exclusion criteria were as follows: (a)

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parent or child who refused to be tested, and (b) bacterial or viral infection with a precise etiological diagnosis. The clinical pneumonia diagnostic criteria were (1) abnormal pulmonary signs: 1) new cough or aggravation of existing respiratory disease symptoms with or without sputum, chest pain, dyspnea, or hemoptysis.; 2) fever; 3) solid lung changes or wet rales with or without radiographic evidence; (2) chest radiograph showing new patchy infiltrative shadows, lobar or segmental solid changes, hyaline of hairy glass, or interstitial changes with or without pleural effusion. Severe pneumonia (Severe pneumonia) was diagnosed if there were signs of severe pneumonia such as ultra-high or persistent fever, dyspnea, lung lesions involving >2/3 of the lung tissue, and combined complications(7), such as pleural effusion, myocardial or liver damage. For all cases, age, sex, and clinical diagnosis of the patients was collected, and the year and month of collection were recorded. Oral consent from the parents or guardians of the children in this study was approved by the Ethics Committee of Beijing Friendship Hospital (No. 2019-P2-176-02).

Specimen processing and DNA extraction

Respiratory samples were collected from all children, including some alveolar lavage fluid from hospitalized children, most are pharyngeal swabs. All the samples from adult patients are pharyngeal swabs. For some children who included both pharyngeal swabs or alveolar lavage fluid, alveolar lavage fluid was used as the primary sample, duplicate sample information was excluded and counted as one case. The collected respiratory samples were immediately placed in 2 ml of PPLO transfer medium (as described before(8)) and stored at -20~-80℃ until transfer. The transfer was carried out by cold chain transport to Beijing Tropical Medicine Research Institute, Beijing Friendship Hospital in Beijing, China, where it was uniformly processed by professional staff. The DNA extraction was performed on the original fluid portion after thawing, using the universal genomic DNA Kit (CoWin Biosciences, Jiangsu, China) following the manufacturer's instructions, and the rest was cultured.

Detection of *M. pneumoniae* and MRMP by PCR sequencing

They were tested by PCR and sequenced for the detection of macrolide resistance genes in domain Ⅴof 23S rRNA, the primer was as before (9-11). If the sequence was successful, this strain was defined as PCR positive, then comparing the sequencing results with the reference strain amplification of *M. pneumoniae* (FH, ATCC 15531, recorded on the National Center for Biotechnology Information) using BioEdit software.

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Culture and detection of MIC

The throat swab and bronchoalveolar lavage fluid specimens were inoculated in *M. pneumoniae* liquid medium, mixed evenly, and placed in an incubator at 37 ° C with 5% CO_2 for culture, *M. pneumoniae* growth caused a decrease in the medium's pH that was indicated by a color change (from red to yellow)(8). If there was no color change for more than one month, the culture was defined as negative. The preparation method was as described in reference 10. Antimicrobial susceptibility testing was carried out using a broth microdilution method according to standard operating procedures from the Clinical and Laboratory Standards Institute (CLSI document M43A), when the growth control well first showed a color change, the lowest concentration of antibiotic in wells without color change was defined as MIC (12). Erythromycin, azithromycin, tetracycline, and levofloxacin were used (9); *M. pneumoniae* reference strain FH (ATCC 15531) was used as a drug-susceptible control strain, all tests were performed in triplicate. According to the latest version of CSLI M43-A(12)(12)(12)[12](12)(Waites et al. 2011)[12](Waites et al., 2011)(12)[12](12)(12)(12)(12)(12)(12), MIC of erythromycin and azithromycin ≤ 0.5 μg/ml is defined as sensitive, tetracycline is ≤2 μg/ml and levofloxacin is ≤ 1 μg/ml. The resistant MIC of erythromycin and azithromycin is ≥1 μg/ml. There is no definition for tetracycline and fluoroquinolones resistance.

Data management and statistical analysis

Statistical analysis was performed using IBM SPSS software, version 25.0, for Windows (SPSS, Chicago, IL, USA). Continuous data were compared using the ManneWhitney U test or Student's t-test. The differences in the categorical variables were assessed using the Chi-squared or Fisher's exact test. A P-value of less than 0.05 was considered statistically significant.

Results

Specimen summary

A total of 4145 respiratory samples were collected in this study, including 3916 samples from children (<18 years old) and 229 samples from adults, 3580 were pharyngeal swab samples and 565 were alveolar lavage fluid. A total of 19 Hospitals from 7 regions of China participated. The following were the hospitals from Beijing: Beijing Children's Hospital Affiliated to Capital Medical University, Civil Aviation General Hospital, Peking University Third Hospital,

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China Mei-tan General Hospital, Beijing Chaoyang Hospital, Changping District Integrated Traditional Chinese and Western Medicine Hospital, New Century International Children's Hospital, Peking University First Hospital, Dongfang Affiliated Hospital of Beijing University of Chinese Medicine, The First Hospital of Tsinghua University, China-Japan Friendship Hospital, Beijing Friendship Hospital, Xiyuan Hospital of CACMS. Here were the hospitals from other regions: Shengjing Hospital of China Medical University, Shenyang, Liaoning, the Second People's Hospital of Hunan Province, Changsha, Hunan, The First Hospital of Jilin University, Changchun, Jilin, Shanghai Children's Medical Center, Shanghai, Hubei Provincial Hospital of Traditional Chinese Medicine, Wuhan, Hubei. The respiratory samples of outpatients were all pharyngeal swabs, and some inpatients had two samples of pharyngeal swabs and alveolar lavage fluid, and the results of alveolar lavage fluid were selected and counted as one case. Individual children with incomplete information on age and sex were not included in the statistics of the corresponding results.

Prevalence of *M. pneumoniae* and MRMP in different regions over different years

The results of PCR testing and drug resistance statistics of 789 children's respiratory samples from different regions in 2013 showed that the highest positive rate of MP testing was in Beijing with 74.5%, followed by Shanghai and Liaoning with 50%, followed by Jilin 41.3%, Hubei 41.2%, Gansu 38.5% and Hunan 19.6% in that order. The results of the MRMP testing showed that Shanghai had the highest resistance rate of 100%, followed by Liaoning 84.2%, Hunan 83.3%, Beijing 75.8%, Jilin 73.7%, Hubei 71.4%, and Gansu 20%. (Table 1). 23S rRNA region V sequencing results were seen for A2063G and A2064G point mutations, or both mutations, with A2063G accounting for 96% of all mutated strains. Annual analysis of 3543 childhood respiratory infection samples collected in Beijing from January 2013 to December 2019 showed that the highest positive *M. pneumoniae* detection rate was 74.5% in the year of 2013, the lowest detection rate was 32.9% in 2017, and the remaining years were 41.7% in 2014, 47.8% in 2015, 44.4% in 2016, 53.1% in 2018, and 58.1% in 2019, respectively. MRMP results showed the lowest total drug resistance detection rate was 70.2% in 2014, the highest was 97.4% in 2019, and the remaining years were 75.8% in 2013, 71.2% in 2015, 74.3% in 2016, 84.8% in 2017, and 94% in 2018(Table2). All adult samples were collected from 2016, 103 males and 126 females, and the *M. pneumoniae* test results for all samples showed 41 positive samples, with a positive test rate of 17.9%, including 24 cases containing the A2063G point mutation and 17 cases without drug-resistant mutations, with a resistance rate of 10.48%, and the positive test rate and resistance rate were lower than those of children.

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Prevalence of *M. pneumoniae* and MRMP in different age groups

The enrolled children were divided into three groups: toddlers (0-3 years old), pre-school children (4-6 years old), and school-age children (over 7 years old) for comparative analysis of *M. pneumoniae* and MRMP in different age groups. The results showed that the positive rate of *M. pneumoniae* in school-age children was 66.8%, which was higher than 31.1% in the toddlers and 47.6% in the pre-school children, the difference was statistically significant ($P < 0.01$). The macrolide-resistant rate in the school-age group was 86.9%, higher than 73.5% in the toddlers and 79.5% in the pre-school group, and the difference was statistically significant ($P < 0.01$).

Characteristics of *M. pneumoniae* and MRMP by genders

The positive rate of *M. pneumoniae* in children between genders showed that 48.2% of males tested positive and 42.1% of females, with no significant difference ($P=0.236$); the macrolide-resistant rate of *M. pneumoniae* showed 79.9% of males and 98.3% of females, also with no significant difference ($P=0.067$).

Prevalence Characteristics of *M. pneumoniae* and MRMP between inpatient and outpatient

The analysis of *M. pneumoniae* and MRMP between outpatient and inpatient was conducted in cases from Beijing and Shenyang, a total of 3543 samples were included, and the overall *M. pneumoniae* positive rate was 48.9%, of which a total of 1677 outpatient medical records with a PCR positive rate of 34.5% and a total of 1866 inpatient samples with a PCR positive rate of 61.9%, the PCR positive rate of hospitalized children was significantly higher than that of the outpatient ones, and the difference was significant ($P < 0.01$). Among *M. pneumoniae* positive samples, there were 1734 cases of MRMP with a total resistance rate of 81.8%, among which 382 cases from outpatient with a macrolide-resistant rate of 66% and 1036 cases from inpatient with a macrolide-resistant rate of 89.7%. MRMP was more prevalence in patient who were admitted to hospital, the difference was significant ($P < 0.01$). (table 2, figure 1)

Clinical Characteristics of *M. pneumoniae* and MRMP

Among all children hospitalized for CAP, the PCR positive rate of *M. pneumoniae* was higher in children with severe pneumonia than in common pneumonia, 76.8% vs. 55.2%, respectively, with significant difference ($P < 0.01$). Besides,

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the macrolide-resistant rate was significantly higher in severe pneumonia than in common pneumonia, 93.9% vs. 88.1%, respectively, with significant differences ($P < 0.01$). (table 3)

MICs of antibiotics

All the collected respiratory tract samples were cultured, a total of 481 *M. pneumoniae* clinical strains were isolated, 45 clinical isolates were selected for MIC detection of four antibiotics as follows: Erythromycin, azithromycin, tetracycline, and levofloxacin. The MIC values of all MRMP to erythromycin and azithromycin were ≥64 μg/ml and ≥32 μg/ml respectively. The MICs of tetracycline and levlevofloxacin were ≤0.5 μg/ml and ≤1 μg/ml respectively. (supplemental file)

Discussion

M. pneumoniae is an important pathogen of respiratory infections in children and adult, accounting for about 10-40% of community acquired pneumonia in children, with an epidemic every 4-7 years(4, 13). *M. pneumoniae* is mostly droplet-borne and is more likely to spread in relatively closed population centers such as schools and military units(14). People of all ages are susceptible, and clinical manifestations vary, can be self-limiting or can develop into severe cases, causing multiple complications within and outside the respiratory system. *M. pneumoniae* lacks cell-wall, which makes it naturally resistant to antibiotics that act on the cell wall. Considering children are in a special period of growth and development period, macrolide antibiotics are the first-line antibiotics for clinical treatment of *M. pneumoniae* infection in childhood, tetracyclines and quinolones antibiotics are restricted in use in children(15). With the use of antibiotics, *M. pneumoniae* faces tremendous pressure for drug selection. Since the first report of macrolide-resistant *M. pneumoniae* clinical isolates in Japan from 2000, countries have reported successively. However, the resistance rate varies widely among different countries and regions, and the resistance rate is generally high in China. Point mutations in domain V of the 23S rRNA of *M. pneumoniae* cause drug resistance by affecting the action site of macrolide antibiotics, which is the main resistance mechanism, no other mechanisms related to drug resistance have been found(16).

In our study, analysis of the prevalence and macrolide-resistance of *M. pneumoniae* in some domestic regions in 2013 showed that the highest positive PCR testing rate was in Beijing (74.5%), followed by Shanghai, Shenyang (50%), Changchun (41.3%), Wuhan (41.2%), Xiahe, (38.5%) and Changsha (19.6%), while the macrolide-resistance showed that Shanghai had the highest resistance rate with 100%, while Gansu has the lowest resistance rate of 20%. Macrolide-resistance

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varied among different regions, Xue et al. reported that the resistant rate was 86.7% in Beijing,81.8% in Shanghai, 74.3% in Kunming, 66.7% in Harbin, 80% in Urumqi, 20% in Nanjing(17), but number of samples from Nanjing is only ten. Xu et al. reported macrolide-resistant rate of *M. pneumoniae* is 92.39% in children, from 2014 to 2016, in Nanjing(18). Jiang et al reported the *M. pneumoniae* epidemic in Qingdao, with a positive *M. pneumoniae* test rate of 59% for respiratory tract infections in children, of which the macrolide-resistance rate was as high as 100%(19), and the PCR positive rate of *M. pneumoniae* in children reported in Weihai during 2019 was 88.1% and the macrolide-resistance rate was 98.78%(20). These reports are basically consistent with those in our paper. This study is the first to report on Hunan, Hubei, and Gansu regions. The prevalence and drug resistance of *M. pneumoniae* varies greatly in different regions and years. *M. pneumoniae* is a respiratory pathogen that is transmitted by droplet transmission, correlates with urban population density and population mobility, and is influenced by temperature, humidity, and other climatic factors. Xiahe, as a small county in Gansu, China, has a sparsely populated area and a dry climate, which may have contributed to the low rate of *M. pneumoniae* infection and drug resistance. Although the *M. pneumoniae* positive rate and MRMP rate in Kunming are at a high level, *M. pneumoniae* is not a common pathogen in the southern areas and has rarely been reported in Guangdong, Guangxi, and Fujian provinces. As a first-tier city in China, there's no report of *M. pneumoniae* outbreak in children in Guangzhou, but in Beijing, *M. pneumoniae* is one of the major pathogens of respiratory infections in children, especially in fall and winter. There is a regional disparity of *M. pneumoniae* infection and drug resistance rates. With limited medical resources, it is of great significance to increase the surveillance of *M. pneumoniae* and MRMP in specific areas to provide early warning for clinics.

In this paper, the age of children ranged from 1 month to 18 years old, but the number of children younger than 1 year old and older than 14 years old were both small, less than 10 cases. All cases were divided into three groups: toddler group, pre-school group, and school-age group according to different age stages, and the prevalence and infection of *M. pneumoniae* were analyzed and studied, the results showed that school-age children had a higher PCR-positive rate of *M. pneumoniae* and macrolide resistance than the other two groups of children in both outpatient and inpatient children, and the difference was significant. There was no significant correlation between *M. pneumoniae* infection and drug resistance rates in children of different genders, independent of disease severity susceptibility, which is consistent with previous reports(21, 22).

Comparative analysis of medical records collected from outpatient and inpatient showed that the rate of positive PCR tests was significantly higher in ward children than in outpatient children, 61.9% vs 34.5%, and the rate of drug resistance was also higher than in outpatient clinics, 89.7% vs 66%, both significantly different. Comparing with common pneumonia, the severe ones had higher positive rate of testing and drug resistance. The results of our *M.*

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pneumoniae resistance testing of inpatients with severe and general pneumonia in 2019 showed that the fever course, duration of antibiotic treatment, and length of hospital stay may be longer for infections with drug-resistant strains. The pathogenic mechanism of *M. pneumoniae* is complex and includes multiple aspects such as direct damage, toxin damage, and immune damage(23). Although there are no studies showing that drug-resistant strains are directly correlated to clinical manifestation, but it is shown that the rapid and massive multiplication of *M. pneumoniae* in the alveoli could cause direct tissue damage and blood exposure, which stimulates the body's immune response, contributing to neutrophils and cytokines expression, which may in return exacerbate the disease(24, 25). Children infected with resistant strains transferring antibiotics to tetracyclines or fluoroquinolones could significantly shortened the length of hospitalization and fever duration(26, 27), and it is important to use glucocorticoids promptly to modulate immunity(28). However, analysis of the clinical characteristics of MRMP infections in the United States showed no statistical difference(29). Studies with multicenter and adequate clinical samples are needed.

The sequencing results of drug-resistant strains showed that A2063G was the main drug-resistant mutation, accounting for 93.7%, and A2064G accounted for 4.5%, 1.8% combined with A2063G+A2064G mutation. The MIC results of commonly used antibiotics on clinical isolates showed that the resistant strains generally had a high level of resistance to erythromycin and azithromycin of macrolides (MIC≥256 μg/ml), and no resistance to tetracycline and levlevofloxacin (MICs were less than 1 μg/ml). The mutant-free strain was < 1 μg/ml for erythromycin and azithromycin MIC. This is the same as reported in previous studies. The development of resistance in *M. pneumoniae* may be related to the pressure of antibiotics. There are different types of macrolides, containing different metacyclic rings, the loci may be slightly different. It has been reported in Japan that the MIC resistance value of the A2064G point mutant strain to erythromycin is lower than that caused by A2063G(30). But strains containing mutations at the position 2063 and/or 2064 has not been successfully isolated. As shown in Figure 2, sequencing of DNA extracted directly from pharyngeal swabs showed that in vivo, susceptible and resistant strains may coexist(31), it may be associated with exposure to different antibiotics. However, since we did not isolate a strain containing both mutations after purification, whether this is a single strain with both mutations or a mixed infection is unknown and requires further study.

Limitations of this article: The article only collected samples and data from some regions of the country in 2013, and the samples were mainly from Beijing and Shenyang, with little data from the central and western regions, which has an impact on our understanding of the current situation of infection in the central and western regions, and most of the data in this article came from the provincial capitals of the regions, which is insufficient for understanding the infection and prevalence at the grassroots level. We should go

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further to promote the surveillance situation of *M. pneumoniae* and MRMP in multicenter and multidisciplinary centers nationwide, and promote the clinical routine testing of MRMP, especially in the eastern regions where MRMP is prevalent, which is significant for clinical early warning work.

Data availability statement

The datasets generated and/or analyzed in the current study are not publicly available due to them containing personal data but will be made available by the corresponding author upon reasonable request provided the data is anonymized according to the Norwegian Data Protection Authority guide on anonymization of personal data.

Ethics statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References:

(1) Jain S, Williams DJ, Arnold SR, et al. Community-acquired pneumonia requiring hospitalization among U.S. children. The New England Journal of Medicine 2015; 372(9): 835-45.

(2) Jain S, Self WH, Wunderink RG, et al. Community-Acquired Pneumonia Requiring Hospitalization among U.S. Adults. The New England Journal of Medicine 2015; 373(5): 415-27.

(3) Xiao L, Ratliff AE, Crabb DM, et al. Molecular Characterization of Mycoplasma pneumoniae Isolates in the United States from 2012 to 2018. Journal of Clinical Microbiology 2020; 58(10).

(4) Waites KB, Atkinson TP. The role of Mycoplasma in upper respiratory infections. Current Infectious Disease Reports 2009; 11(3): 198-206.

(5) Edelstein IA, Ivanova OV, Romashov OI, Kozlov RS. Course of Lower Respiratory Tract Infection in Young People Treated at the Military Hospital of Smolensk Garrison with Detected Mycoplasma pneumoniae Carrying a Macrolide-Resistant Mutation in 23S rRNA Gene. Pathogens 2023; 12(1).

(6) Akashi Y, Hayashi D, Suzuki H, et al. Clinical features and seasonal variations in the prevalence of macrolide-resistant Mycoplasma pneumoniae. Journal of General and Family Medicine 2018; 19(6): 191-7.

(7) [Guidelines for management of community acquired pneumonia in children(the revised edition of 2013) (II)]. Zhonghua Er Ke Za Zhi 2013; 51(11): 856-62.

(8) Liu X, Jiang Y, Chen X, Li J, Shi D, Xin D. Drug resistance mechanisms of Mycoplasma pneumoniae to macrolide antibiotics. Biomed Research International 2014; 2014: 320801.

(9) Dou HW, Tian XJ, Xin L, et al. Mycoplasma pneumoniae Macrolide Resistance and MLVA Typing in Children in Beijing, China, in 2016: Is It Relevant? Biomedical and Environmental Sciences 2020; 33(12): 916-24.

(10) Guo D, Hu W, Xu B, et al. Allele-specific real-time PCR testing for minor macrolide-resistant Mycoplasma Pneumoniae. BMC Infectious Diseases 2019; 19(1): 616.

(11) Guo D, Hu W, Xu B, et al. Allele-specific real-time PCR testing for minor macrolide-resistant Mycoplasma Pneumoniae. BMC Infectious Diseases 2019; 19(1): 616.

(12) Waites KB, Bade DJ, Bebear C, et al. Methods for Antimicrobial Susceptibility Testing for Human Mycoplasmas; Approved Guideline. Wayne (PA): Clinical and Laboratory Standards Institute, 2011.

(13) Kuo CY, Tsai WC, Lee HF, et al. The epidemiology, clinical characteristics, and macrolide susceptibility of Mycoplasma pneumoniae pneumonia in children in Southern Taiwan, 2019-2020. Journal of Microbiology, Immunology, and Infection 2021.

(14) Bebear C. Editorial commentary: infections due to macrolide-resistant Mycoplasma pneumoniae: now what? Clinical Infectious Diseases 2012; 55(12): 1650-1.

(15) Atkinson TP, Balish MF, Waites KB. Epidemiology, clinical manifestations, pathogenesis and laboratory detection of Mycoplasma pneumoniae infections. FEMS Microbiology Reviews 2008; 32(6): 956-73.

https://doi.org/10.1017/S0950268824000323 Published online by Cambridge University Press

(16) Jacobs E, Ehrhardt I, Dumke R. New insights in the outbreak pattern of Mycoplasma pneumoniae. International Journal of Medical Microbiology 2015; 305(7): 705-8.
(17) Xue G, Li M, Wang N, et al. Comparison of the molecular characteristics of Mycoplasma pneumoniae from children across different regions of China. PLoS One 2018; 13(8): e198557.
(18) Xu C, Deng H, Zhang J, et al. Mutations in domain V of Mycoplasma pneumoniae 23S rRNA and clinical characteristics of pediatric M. pneumoniae pneumonia in Nanjing, China. Journal of International Medical Research 2021; 49(6): 675889992.
(19) Jiang FC, Wang RF, Chen P, et al. Genotype and mutation patterns of macrolide resistance genes of Mycoplasma pneumoniae from children with pneumonia in Qingdao, China, in 2019. Journal of Global Antimicrobial Resistance 2021; 27: 273-8.
(20) Guo Z, Liu L, Gong J, et al. Molecular features and antimicrobial susceptibility of Mycoplasma pneumoniae isolates from paediatric inpatients in Weihai, China: Characteristics of M. pneumoniae In Weihai. Journal of Global Antimicrobial Resistance 2022; 28: 180-4.
(21) Yang D, Chen L, Chen Z. The timing of azithromycin treatment is not associated with the clinical prognosis of childhood Mycoplasma pneumoniae pneumonia in high macrolide-resistant prevalence settings. PLoS One 2018; 13(1): e191951.
(22) Shah SS. Mycoplasma pneumoniae as a Cause of Community-Acquired Pneumonia in Children. Clinical Infectious Diseases 2019; 68(1): 13-4.
(23) He J, Liu M, Ye Z, et al. Insights into the pathogenesis of Mycoplasma pneumoniae (Review). Molecular Medicine Reports 2016; 14(5): 4030-6.
(24) Zhang Z, Dou H, Tu P, et al. Serum cytokine profiling reveals different immune response patterns during general and severe Mycoplasma pneumoniae pneumonia. Frontiers in Immunology 2022; 13: 1088725.
(25) Lee YC, Chang CH, Lee WJ, et al. Altered chemokine profile in Refractory Mycoplasma pneumoniae pneumonia infected children. Journal of Microbiology, Immunology, and Infection 2021; 54(4): 673-9.
(26) Chen J, Qi X, Yin Y, Zhang L, Zhang J, Yuan S. Effects of minocycline on macrolide-unresponsive Mycoplasma pneumoniae pneumonia in children: a single-center retrospective study. Translational Pediatrics 2021; 10(11): 2997-3004.
(27) Ahn JG, Cho HK, Li D, et al. Efficacy of tetracyclines and fluoroquinolones for the treatment of macrolide-refractory Mycoplasma pneumoniae pneumonia in children: a systematic review and meta-analysis. BMC Infectious Diseases 2021; 21(1): 1003.
(28) Tsai TA, Tsai CK, Kuo KC, Yu HR. Rational stepwise approach for Mycoplasma pneumoniae pneumonia in children. Journal of Microbiology, Immunology, and Infection 2021; 54(4): 557-65.
(29) Kutty PK, Jain S, Taylor TH, et al. Mycoplasma pneumoniae Among Children Hospitalized With Community-acquired Pneumonia. Clinical Infectious Diseases 2019; 68(1): 5-12.
(30) Waites KB, Ratliff A, Crabb DM, et al. Macrolide-Resistant Mycoplasma pneumoniae in the United States as Determined from a National Surveillance Program. Journal of Clinical Microbiology 2019; 57(11).
(31) Li J, Liu L, He L, Meng F, Zhang J, Zhao F. First report of macrolide-resistant and -susceptible Mycoplasma pneumoniae clinical strains isolated from a single case. Journal of Global Antimicrobial Resistance 2021; 24: 228-32.

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Figure 1

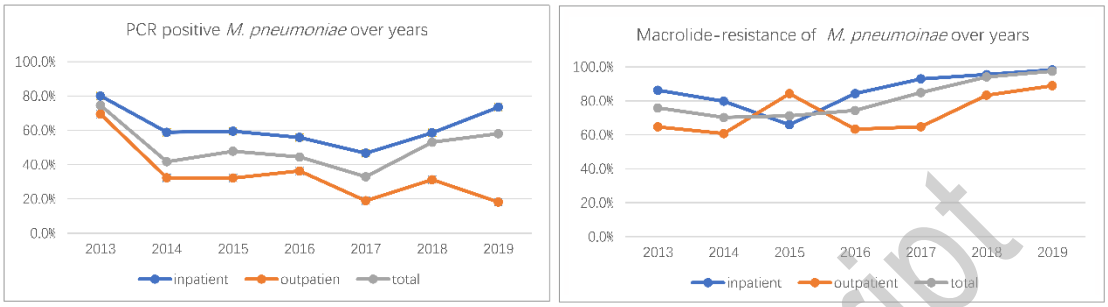

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Figure 2

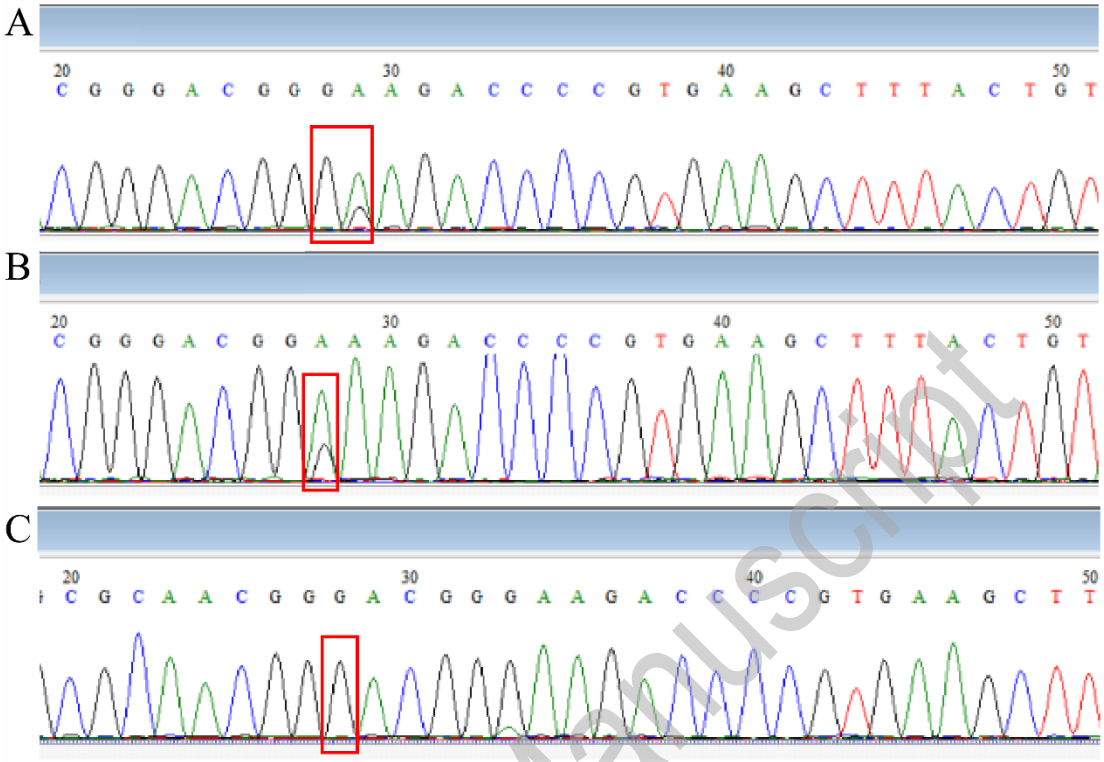

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Table 1 Prevalence of *M. pneumoniae* and MRMP in different regions, in the year 2013

	PCR Positive Rate				Macrolide-resistance Rate*			
	Positive	Negative	Total	Positive rate	MRMP	MSMP	Total	Resistant rate
Changchun, Jilin	19	27	46	41.3%	14	5	19	73.7%
Xiahe, Gansu	5	8	13	38.5%	1	4	5	20.0%
Shanghai	22	22	44	50.0%	22	0	22	100.0%
Wuhan, Hubei	7	10	17	41.2%	5	2	7	71.4%
Changsha, Hunan	10	33	51	19.6%	15	3	18	83.3%
Shenyang, Liaoning	101	101	202	50.0%	85	16	101	84.2%
Beijing	310	106	416	74.5%	235	75	310	75.8%
Total	474	307	789	60.1%	377	105	482	78.2%

*note: The data shown in this table are the PCR sequencing results of DNA extracted directly from the original respiratory samples.

MRMP=macrolide-resistant *M. pneumoniae*; MSMP=macrolide-sensitive *M. pneumoniae*;

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Table2 Prevalence Characteristics of *M. pneumoniae* and MRMP between Inpatient and outpatient over years

Year		PCR positive rate					Macrolide-resistance rate				
		positive(total)	Positive rate	Positive in all	χ^2	*P* value	MR(total)	Resistant rate	Resistant in all	χ^2	*P* value
2013	inpatient	160(200)	80.00%	74.50%	6.094	0.014	138(160)	86.30%	75.80%	19.664	< 0.01
	outpatient	150(216)	69.40%				97(150)	64.70%			
2014	inpatient	163(277)	58.80%	41.70%	51.94	< 0.01	130(163)	79.80%	70.20%	14.104	< 0.01
	outpatient	163(505)	32.30%				99(163)	60.70%			
2015	inpatient	47(79)	59.50%	47.80%	10.081	0.01	31(47)	66.00%	71.20%	2.199	0.138
	outpatient	19(59)	32.20%				16(19)	84.20%			
2016	inpatient	153(274)	55.80%	44.40%	12.117	< 0.01	129(153)	84.30%	74.30%	6.895	< 0.01
	outpatient	139(383)	36.30%				88(139)	63.30%			
2017	inpatient	127(272)	46.70%	32.90%	59.595	< 0.01	118(127)	92.90%	84.80%	14.188	< 0.01
	outpatient	51(269)	19.00%				33(51)	64.70%			
2018	inpatient	222(379)	58.60%	53.10%	23.218	< 0.01	212(222)	95.50%	94.00%	6.983	< 0.01
	outpatient	30(96)	31.30%				25(30)	83.30%			

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2019	inpatient	283(385)	73.50%	58.10%	135.324	< 0.01	278(283)	98.20%	97.40%	8.561	< 0.01
	outpatient	27(149)	18.10%				24(27)	88.90%			
total	inpatient	1155(1866)	61.90%	48.90%	360.21	< 0.01	1036(1155)	89.70%	81.80%	210.46	< 0.01
	outpatient	579(1677)	34.50%				382(579)	66.00%			

*note: The data shown in this table are the PCR sequencing results of DNA extracted directly from the original respiratory samples. The total samples were 3543, including 1866 from inpatients and 1677 from outpatients.

MRMP=macrolide-resistant *M. pneumoniae*; MSMP=macrolide-sensitive *M. pneumoniae*;

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Table 3 Clinical Characteristics of *M. pneumoniae* and MRMP

PCR Positive Rate					Macrolide-resistance Rate					
Common pneumonia		Severe pneumonia			Common pneumonia			Severe pneumonia			
Positive number (total number)	Positive rate	Positive number (total number)	Positive rate	*P* value	MRMP	MSMP	Resistant rate	MRMP	MSMP	Resistant rate	*P* value
573(746)	76.8%	1205(2183)	55.2%	< 0.01	538	35	93.9%	1062	143	88.1%	< 0.01

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