Mycoplasma Pneumoniae Infection and Macrolide Resistance in Outpatients and Inpatients Children with Respiratory Tract Infection

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Introduction. To analyze the infection status of Mycoplasma pneumoniae (MP) in children with respiratory tract infections in outpatients or inpatients, as well as their resistance to macrolide drugs.
Method. 160 pediatric patients with respiratory tract infections in outpatient and inpatient were selected, and blood and sputum samples were collected for serum MP IgM antibody detection, MP isolation and culture, and analysis of MP macrolide resistance. Statistics on MP infection was conducted and the differences was analyzed in gender, age, and seasonal distribution of infections. Evaluation of its resistance to macrolide drugs was also analyzed.
Result. Among 160 children with respiratory tract infections, 27 cases were detected as MP infections, with an infection rate of 16.88%. In terms of MP infection, the infection rate of female children was 23.29% higher than that of male children, which was 11.49%, and the difference was statistically significant (χ^2=3.935, P=0.047). As age increased, the MP infection rate of outpatient and inpatient with respiratory tract infection children also increased, but there was no statistically significant difference in MP infection between different age groups and genders of children (P>0.05). The incidence of MP infection in summer and autumn is higher than that in spring and winter, and the difference is statistically significant (P<0.05). 27 strains of MP infected bacteria were isolated and cultured, and a total of 11 different resistant strains were cultured. MP showed severe resistance to macrolide antibiotics.
Conclusion. The incidence of MP infection is high among outpatient and inpatient with respiratory tract infections, and the incidence is higher in females than males. MP infection is particularly resistant to macrolide drugs.
Keywords. Respiratory Infection, Children, Mycoplasma Pneumoniae, Macrolide Drugs, Drug Resistance

INTRODUCTION

Mycoplasma pneumoniae (MP) is an important pathogen that causes respiratory infections, especially community-acquired pneumonia, especially in children [1]. MP infection usually presents with mild to moderate respiratory symptoms, but in some cases, it may also lead to severe pneumonia. In recent years, with the changes in antibiotic use patterns and the increasing problem of drug resistance, the management of MP infection has become increasingly challenging [2]. Macrolide antibiotics are usually used to treat MP infection, mainly because these drugs have good tissue penetration and fewer side effects. However, research and clinical observations in recent years have revealed the severeness of the drug resistance, especially the resistance rate of macrolide drugs has increased significantly [3]. Drug resistance

reduces the effectiveness of traditional treatment strategies, leading to treatment failure and persistence of infection, increasing medical burden and health risks. In response to this phenomenon, strengthening the understanding of infection and taking reasonable preventive intervention measures has become a hot topic at present. [4]. In this study, an in-depth analysis of Mycoplasma pneumoniae infection and its resistance to macrolide drugs in outpatient and inpatient children with respiratory tract infections was conducted to provide a reasonable reference for clinical practice. The report is as follows。

1 MATERIALS AND METHODS

1.1 Basic Information

A total of 160 children with respiratory tract infections admitted to the outpatient clinic or hospital from January 2022 to March 2024 were selected. Inclusion criteria: All patients were admitted to the outpatient clinic or hospital for treatment and suffered from respiratory tract infections. Children were aged 1 to 12 years old and had complete clinical data. Family members were aware of the study and signed the informed consent. Exclusion criteria: Children with blood diseases or autoimmune diseases. Children with liver damage.

1.2 Methods

1.2.1 MP Infection Examination

All children had 1-2 ml of venous blood collected within 24 hours of admission. The collected blood was sent for serum MP IgM antibody testing as soon as possible. The method is as follows: Take 10 μL of serum, dilute it with sample buffer at a ratio of 1:101, and after thorough mixing, add the diluent to the microwells of the antigen-coated microplate, and add 100 μL of standard serum and control serum to the corresponding microwells. Incubate at room temperature (18-25°C) for 30 minutes, then wash the microplate 3 times with washing buffer, add 100 μL of peroxidase-labeled anti-human IgM (sheep) antibody to each microwell, and incubate again at room temperature (18-25°C) for 30 minutes, pour out the liquid in the microwell, and wash the microplate 3 times. Add 100 μL of chromogen substrate solution to each microwell, incubating in the dark for 15 minutes, and add 100 μL of stop solution to each microwell at the same speed and order. After terminating the reaction, use an enzyme reader to measure the absorbance (D value) at a wavelength of 450 nm, calculate the ratio of the D value of the sample to the D value of the standard, and make a semi-quantitative judgment based on the ratio: a ratio of >1.1 is positive, a ratio between 0.8-1.1 is suspicious, and a ratio of <0.8 is negative. Recheck the serum upon discharge. If the MP IgM value increases by 1.5 times or more compared to the first test at admission, it is diagnosed as acute infection with MP.

1.2.2 MP Isolated Culture

The sample extracted from the nasopharyngeal secretion or throat swab of the child is inoculated into the culture medium for Mycoplasma pneumoniae culturing, mixed evenly, and incubated in a 37°C incubator. The isolated mycoplasma is identified. First, its morphology is observed under a microscope, and then the polymerase chain reaction (PCR) technology is used for genetic testing. In the PCR

test, DNA is first extracted from the isolate. The specific steps are to centrifuge the isolate at high speed, discard the supernatant, and then resuspend the precipitate with the treatment solution. Then, the sample is heated in 100°C water for 10 minutes, and the resulting solution is used as a DNA template for later use. dNTPs (the basic component for constructing DNA), primers, buffer, sample DNA and Taq DNA polymerase are added to the reaction tube, mixed and sealed with liquid paraffin. And then 35 temperature cycles of PCR reaction are performed. Each experiment has negative and positive controls. The results of PCR are detected by electrophoresis technology. Run under an electric field for 5 to 10 minutes. Then a UV detector was used to observe the electrophoresis results and take pictures for recording purpose.

1.2.3 Drug Resistance Analysis

The lab prepared PPLO basal medium and then added 15% newborn calf serum, 10% yeast extract, an appropriate amount of phenol red indicator, 1% glucose and a certain amount of penicillin. The preserved mycoplasma standard strain was cultured continuously for 3 generations using the liquid culture method. And the third-generation bacterial solution was taken for the experiment. In order to measure the concentration of mycoplasma, the color change unit (CCU/mL) method was used. Then the measured bacterial solution was packaged and preserved as seed solution for subsequent experiments. When conducting drug testing, the MP bacterial solution first was diluted to a certain concentration. And then use liquid culture medium to dilute erythromycin, azithromycin and josamycin to different concentrations, adding an equal amount of mycoplasma bacterial solution to each test tube, then it was mixed evenly, and cultured in a 37°C incubator. The results were observed during the experiment every day for 1-2 weeks. To ensure the accuracy of the experiment, a control was set up. Mycoplasma solution was added to the culture medium of the positive control. If the mycoplasma grows well, the color of the culture medium will change from red to yellow. Mycoplasma was not added to the negative control, so the color of the culture medium will not change. If there is no mycoplasma growth in the test tube of a certain drug, it means that the drug at this concentration can inhibit the growth of mycoplasma. This concentration is the minimum inhibitory concentration (MIC) of the drug. It Referred to the 2001 standard of the National Committee for Clinical Laboratory Standards (NCCLS) of the United States to determine whether mycoplasma is sensitive to macrolide antibiotics. In order to ensure the reliability of the results, the experiment was repeated 3 times.。

1.3 Observation Indicators

(1) Data collection for patients, specifically gender and age data. (2) Statistics on MP infection in children, and statistics on the distribution of infection by gender, age, and season. (3) Statistics on MP isolation and culture and resistance to macrolide drugs (MIC value).

1.4 Statistical Analysis

SPSS21.0 software was used for data processing. The count data were tested by χ^2test, expressed as [n(%)].The difference of $P<0.05$ was considered as statistically significant.

2 RESULTS

2.1 Basic Information of Patients

Among the 160 children, 87 were male, accounting for 54.38%. There were 73 females, accounting for 45.62%. The age of the children ranged from 1 to 12 years old, with an average age of (6.02±0.77) years old. Among them, 51 children were 1 to 4 years old (31.88%), 50 children were >4 to 7 years old (31.25%), 42 children were >7 to 10 years old (26.25%), and 17 children were >10 years old (10.62%).

2.2 MP Examination Result

Among 160 children with respiratory tract infection who were outpatients or inpatients, 27 were found to be infected with MP, with a detection rate of 16.88%. Among them, 10 were detected in males, accounting for 11.49%, and 17 were detected in females, accounting for 23.29%. The detection rate of MP infection in females was higher than that in males (P<0.05). With the increase of age, the MP infection rate continued to increase in both males and females. There was no statistically significant difference in the detection of MP in children of different age groups and different genders (P>0.05), Seeing Table 1 for details.

Table 1 Statistics of MP Infection in Children of Different Genders and Ages

Age (year)	Male		Female		χ^2	P
	Cases	MP ratio[n（%）]	Cases	MP ratio[n（%）]		
1～4	27	0（0）	24	1（4.17）	1.147	0.284
＞4～7	28	1（3.57）	22	4（18.18）	2.922	0.087
＞7～10	24	3（12.50）	18	6（33.33）	2.651	0.103
＞10	8	6（75.00）	9	6（66.67）	0.141	0.707
Total	87	10（11.49）	73	17（23.29）	3.935	0.047

2.2 MP Infection in Different Seasons

There are differences in MP infection in children in different seasons. The incidence of MP infection in summer and autumn is higher than that in spring and winter, and the difference is statistically significant (P<0.05), seeing table 2.

Table 2 MP Detection in Children with Respiratory Tract Infection in Inpatients and Outpatients in Different Seasons

Seasons	Cases	MP Detection Ratio[n（%）]
Spring	48	2（4.17）
Summer	41	12（29.27）
Automn	39	10（25.64）
Winter	32	3（9.38）
Total	160	27（16.88）

2.3 MIC Values of MP Infection Resistance to Macrolides

27 MP infection strains were isolated and cultured by MP, and a total of 11

different resistant strains were cultured. The resistant strains were resistant to azithromycin and clarithromycin at the same time. According to the results in Table 3, MP was judged to be severely resistant to macrolide antibiotics based on the MIC values.

Table 3 MIC Values of MP International Standard Strains and Clinical Isolates for Macrolide Resistance

	MIC Value		
	Erythrocin	Azithromycin	Clarithromycin
International Standard Strains	0.25	0.5	0.5
isolate*1	16	8	4
isolate*16	32	16	16
isolate*19	16	8	8
isolate*20	32	16	16
isolate*23	64	32	32
isolate*45	32	16	16
isolate*B13	64	32	32
isolate*B75	32	16	16
isolate*B101	64	32	32
isolate*M29	0.01	0.001	0.1
isolate*M30	0.01	0.001	0.1

3 CONCLUSION

Pediatric respiratory tract infection is one of the most common diseases in children, mainly caused by pathogenic microorganisms such as viruses and bacteria. These infections include symptoms as colds, laryngitis, coughs, bronchitis, etc. And in severe cases, it may cause complications such as pneumonia and respiratory failure. Because children's immune systems are relatively immature, they are more susceptible to these pathogens [5]. The symptoms of childhood respiratory tract infection are diverse, and common symptoms include fever, cough, runny nose, sore throat, etc. In addition, some children may also experience symptoms such as shortness of breath and hoarseness, which have a huge impact on children's health. It is crucial to take reasonable preventive intervention measures [6]. Studies have shown that the common pathogens that cause childhood respiratory tract infections are usually MP, which can be transmitted through droplets and the infections caused do not differ by season. Understanding the MP infection status of children with respiratory tract infections can provide a reference for intervention and medication guidance for children.

In this study, statistics were collected on the MP infection of children with respiratory tract infections in outpatients and inpatients. The results showed that among 160 cases of pediatric respiratory tract infections, 27 cases of MP infection were detected, with an infection rate of 16.88%. Further studies have shown that with the increase of age, the MP infection rate of children with respiratory tract infections

also increases. In addition, the MP infection rate of females is higher than that of males. For children's respiratory tract infections, the MP infection rate in children's respiratory tract infections also increases with age. This is mainly because that the immune system of young children is not yet fully mature and their defense against pathogens such as MP is weak. With they age, the immune system gradually matures, but the susceptibility to MP infection still exists. In addition, with they age, the type of respiratory infection in children will also change, and MP infection is more obvious in older age groups [7]. As for the gender difference in MP infection, the reason why female children have a higher rate of MP infection is usually related to their living environment or social behavior. For example, girls have more opportunities to come into contact with MP in certain environments, or social behavior puts girls at a higher risk of MP infection. In terms of the seasonal distribution of MP infection, the infection rate in summer and autumn is higher than that in spring and winter. This is mainly because the temperature and humidity are usually higher in summer and autumn, which is conducive to the survival and spread of MP. MP is more easily transmitted in a warm and humid environment because the concentration of microorganisms in the air increases [8]. Children participate in more outdoor activities and social activities in summer and autumn, which increase the chance of exposure to pathogens, especially in the autumn after school starts.

This study also analyzed the resistance of MP infection to macrolide drugs, showing that MP is severely resistant to macrolide antibiotics. Macrolide drugs inhibit protein synthesis by binding to the 50S subunit of the bacterial ribosome, thereby inhibiting bacterial growth. However, MP develops resistance to these drugs through a variety of mechanisms. Among them, the drug efflux pump mechanism enables MP to excrete drugs from the body and reduce the effective concentration of drugs in the cell [9]. Secondly, target site changes are another important resistance mechanism. MP can reduce the binding ability of drugs by changing the binding site of the ribosome, further reducing the antibacterial effect of the drug. Although the production of enzymes is relatively rare in MP, it still destroys antibiotics by producing enzymes. Therefore, when facing MP infection, the efficacy of macrolide drugs alone may not be sufficient, and it is necessary to consider the use of other types of antibiotics or combination therapy. In addition, with the increase in MP resistance, regular resistance monitoring and sensitivity testing have become particularly important [10]. Through sensitivity testing, effective treatment options can be selected more accurately, avoiding the use of drugs with known resistance thereby improving the success rate of treatment.

In summary, a high proportion of children with respiratory tract infections who are outpatients or inpatients have MP infection. And there are gender and seasonal differences in the occurrence of infection. The incidence of MP infection in female children with respiratory tract infections is higher than that in males, and the MP infection rate in summer and autumn is higher than that in spring and winter. MP is widely resistant to macrolide drugs (erythromycin, azithromycin, clarithromycin). In clinical practice, it is necessary to strengthen the monitoring of MP resistance and explore more effective treatment methods to cope with this increasingly severe public

health challenge.

REFERENCES

[1] Chen Pei, Liu Wenmei. Analysis of risk factors for macrolide resistance in children with mycoplasma pneumonia and construction of risk assessment model[J]. *Journal of Clinical Drug Therapy*, 2023, 21(8):65-70.

[2] Zhang Yingtao. Observation on the correlation between allergic cough and Mycoplasma pneumoniae infection in children and the efficacy of macrolide drugs[J]. *Chinese Community Physician*, 2022, 38(6):70-72.

[3] Zhang Lijun, An Shuhua, Wang Yanyan, et al. Clinical characteristics of children with macrolide-resistant Mycoplasma pneumoniae pneumonia and pulmonary consolidation[J]. *Clinical Misdiagnosis and Treatment*, 2021, 34(4):54-58.

[4] Perumal D, Heamchandsaravanan AR, Shanmugam K.Dhamodharan S. Nandan J.Dhandapani P.Detection of Macrolide Resistant Mycoplasma pneumoniae in Children with Lower Respiratory Tract Infection by Sanger Sequencing Targeting Domain V Region of 23S rRNA Gene[J]. *Journal of Pure & Applied Microbiology*, 2023, 17(1):338-344.

[5] MA Jiangang. Correlation between Mycoplasma pneumoniae infection and variant cough in children and analysis of the efficacy of macrolide drugs[J]. *Systems Medicine*, 2019, 4(21):115-117.

[6] Yuan Benquan. Efficacy of macrolides in the treatment of allergic cough induced by Mycoplasma pneumoniae infection in children[J]. *Chinese Medical Guide*, 2018, 16(23):22-23.

[7] Zhang Binbao, Xing Haijian, Jiang Rui. Clinical characteristics and drug sensitivity analysis of Mycoplasma pneumoniae infection in children with community-acquired pneumonia[J]. *Clinical Medical Research and Practice*, 2019, 4(16):98-100.

[8] Hayashi D, Akashi Y, Suzuki H, et al. Implementation of Point-of-Care Molecular Diagnostics for Mycoplasma pneumoniae Ensures the Correct Antimicrobial Prescription for Pediatric Pneumonia Patients[J]. *The Tohoku Journal of Experimental Medicine*, 2018, 246(4):225-231.

[9] Gui Qiaodi, Shi Ruijie, Zhang Xiangyang, et al. Investigation of Mycoplasma pneumoniae infection and drug resistance in 2837 children in Xi'an[J]. *Shaanxi Medical Journal*, 2018, 47(2):265-267.

[10] Lu Yan, Wu Ming, Zhang Ailian, et al. Clinical characteristics and drug resistance gene mutations in children with severe Mycoplasma pneumoniae pneumonia[J]. *Zhejiang Medical Education*, 2018, 17(1):52-54.

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