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Mutation analysis of Mycoplasma pneumoniae A2063G and A2064G in children infected with Mycoplasma pneumoniae in Tai'an area

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Abstract: *Mycoplasma pneumoniae infection is a self-limiting disease, but if the infection is not treated in time, it can easily develop into severe pneumonia, bronchitis, asthma and community-acquired pneumonia, which seriously affects the health of the infected person. Macrolide antibiotics are the first choice for the treatment of Mycoplasma pneumoniae infection, and macrolide-resistant strains of Mycoplasma pneumoniae have been isolated in clinical practice. Patients infected with drug-resistant strains of Mycoplasma pneumoniae had significantly longer fever duration, hospitalisation time and fever duration after drug administration than sensitive strains. The aim of this study was to analyse the mutation of A2063G and A2064G drug-resistant gene loci in children with Mycoplasma pneumoniae infections admitted to Tai'an Maternal and Child Healthcare Hospital in the past two years, and to explore the epidemiological characteristics of these strains, so as to provide a basis for the prevention and treatment of Mycoplasma pneumoniae in children.*

Keywords: *Mycoplasma pneumoniae, Resistance genes, Mutations, Epidemiological features*

1. Introduction

Mycoplasma pneumoniae (MP) is one of the smallest microorganisms that can survive on its own, with strong vitality, and humans are the only host, the main means of transmission is droplets, aerosols, but also through direct contact. Children are more susceptible to infection because of their underdeveloped immune system.MP infections can occur throughout the year, with variability in seasonal distribution depending on climatic conditions. Mycoplasma pneumoniae is the main causative agent of community ac-quired pneumonia (CAP) and bronchiolitis, accounting for 10%-40% of all CAP [1]. Mycoplasma pneumoniae has no cell wall, so it is not sensitive to penicillin and cephalosporin antibiotics that act on the cell wall, and macrolides are the first choice of therapeutic drugs for it, but in recent years, with the widespread use of macrolides, or even the abuse of macrolides, resulting in the phenomenon of organismal resistance is becoming more and more serious. The binding site of macrolides is located in the 23SrRNA structural domain V, and nucleotide point mutations occurring in this region will reduce the affinity between antibiotics and ribosomes, thus making Mycoplasma pneumoniae resistant [2]. The mutation sites identified so far include 2063, 2034, 2067 and 2617 [2].The mutation rates of sites A2063 and A2064 in the V region of the 23S rRNA structural domain are the highest, and they produce the strongest resistance, which is the main marker of MP resistance and the focus of current research[3].Only point mutations at loci 2063 and 2064 have been found in China [3].

In this study, 721 patients with mycoplasma pneumoniae infection in Tai'an Maternal and Child Health Hospital from 2023 to 2024 were selected to detect A2063G and A2064G resistance gene loci. According to the test results, the patients were divided into two groups: mutant and non-mutant. The epidemiological characteristics of the disease are further analysed in terms of sex, age and seasonal detection, with a view to providing a basis for the prevention and treatment of pneumococcal infection in children and reducing the harm caused by pneumococcail infection to children's health.

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2. Materials and methods

2.1. Materials

2.1.1. Research Objectives

A retrospective study of 721 patients with positive nucleic acid test for Mycoplasma pneumoniae who attended the Department of Paediatric Internal Medicine of Tai'an Maternal and Child Health Hospital from 1 January 2023 to 31 December 2024, including 379 males and 342 females, was conducted.

2.1.2. Instruments and reagents

MP test reagent manufacturer for Mycoplasma pneumoniae and drug-resistant mutation site detection kit developed by Jiangsu Merak Bio-technology Co.

2.2. Methodology

2.2.1. MP detection

Oropharyngeal swabs were collected within 24h after admission to the hospital, 1.0ml of sterilised saline was added to the sampling tube, shaking well, squeezing the swabs dry, pouring the liquid into a sterile test tube, centrifuging it immediately, and 200ml of liquid from the bottom of the test tube was added to the fully automated nucleic acid extraction kit for nucleic acid extraction. After extraction, 5ml of nucleic acid was added into the reaction tube of MP Nucleic Acid Detection Reagent for PCR amplification. The PCR reaction conditions were as follows: 93℃→2 minutes, 93℃ for 45 seconds→55℃ for 60 seconds→10 cycles, 93℃ for 30 seconds→55℃ for 45 seconds→30 cycles.

2.2.2. Detection of resistance gene mutation sites

For the samples with positive PCR amplification of Mycoplasma pneumoniae, take 5ml of nucleic acid extract and add it into the drug resistance mutation site detection kit, the PCR reaction system is as follows: 6.0ul of buffer, 2.0ul of primer probe, 0.5ul of enzyme, and 11.5ul of water, and the PCR reaction conditions are as follows: 50℃ for 2min and 1 cycle, 95℃ for 2min and 1 cycle, 91℃ for 15sec→64℃ for 1min and 40 cycles, and the data collection is set at 64℃ to collect the fluorescence signal. 1min 40 cycles, and the data acquisition was set at 64℃ to collect the fluorescence signal.

2.2.3. Statistical analysis

Statistical analyses were performed using SPSS 25 software, and the count data were expressed as percentages, and comparisons between groups were made using X2 (Pearson), with $P<0.05$ as the statistically significant difference in the data.

3. Results

3.1. General information

Among the 721 patients with positive MP nucleic acid test who sent test samples, the number of samples with mutations in the A2063G and A2064G drug resistance gene loci was 633, and the mutation positivity rate was 87.8%. Among the 84 samples sent for testing in 2023, 69 had mutations, with a mutation rate of 82.1%; among the 637 samples sent for testing in 2024, 564 had mutations, with a mutation rate of 88.54%. The difference between the two years was not statistically significant (P value > 0.05) .

3.2. Gender distribution

In 2023-2024, there were 330 male and 303 female children with mutations in the A2063G and A2064G resistance loci in patients with Mycoplasma pneumoniae infections, and the mutation positivity rates were 87.1% (330/379) and 88.6% (88.6%), respectively, and the differences between the two were not statistically significant ($X^2=0.390$, $P=0.532$, $P>0.05$). 0.05). Details are shown in Table 1.

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Table 1 Gender distribution of mutations at the A2063G A2064G drug resistance locus in patients with Mycoplasma pneumoniae infection

distinguishing between the sexes	A2063G A2064G No mutation at the drug resistance locus		A2063G A2064G Mutations at the drug resistance locus	
	Number(persons)	Proportion(%)	Number(persons)	Proportion(%)
Male	9	12.9	330	379
Female	39	11.4	303	342
Comparison between groups	X^2=0.390 P=0.532			

3.3. Age distribution

Patients infected with Mycoplasma pneumoniae in 2023-2024 were divided into the following five groups according to their ages:<1 year old (including 1 year old), 1~3 years old (including 3 years old), 3~5 years old (including 5 years old), 5~8 years old (including 8 years old), and 8-12 years old (including 12 years old), and the mutations of drug-resistant genes loci occurred in each group as follows:87.55% (7/8), 94.2% (49/52), 79.9%(115/144),88.9%(367/413),91.3%(95/104).Among them,the mutation rate of patients in the groups of 1~3 years old and 8~12 years old was significantly higher than that of the other groups.Comparison between the groups,X^2=10.116,P=0.039 (p<0.05),and the difference was statistically significant. Details are shown in Table 2.

Table 2 Age distribution of mutations at the A2063G A2064G drug resistance locus in patients with Mycoplasma pneumoniae infection

(a person) age	A2063G A2064G No mutations at the drug resistance locus		A2063G A2064G Mutations at the drug resistance locus	
	Number(persons)	Proportion(%)	Number(persons)	Proportion(%)
<1 year	1	12.5	7	87.5
1~3years	3	5.8	49	94.2
3~5years	29	20.1	115	79.9
5~8years	46	11.1	367	88.9
8~12 years	9	8.7	95	91.3
Comparison between groups	X^2=10.116 P=0.039			

3.4. Detection in different seasons

In 2023-2024, the number of patients with Mycoplasma pneumoniae infections with mutations at the A2063G A2064G resistance locus in spring, summer, autumn and winter was 35, 48, 239 and 311, respectively, with positive mutation rates of 79.5% (35/44), 65.8% (48/73), 91.2% (239/262) and 90.9% (311/342), among which, the mutation positive rate in autumn and winter was significantly higher than that in spring and summer.), of which, the mutation positive rate was significantly higher in autumn and winter than in spring and summer, and the difference between the groups was statistically significant by using X^2 test, X^2=41.91, P<0.01 (P<0.05). Details are shown in Table 3.

Table 3 Seasonal distribution of mutations at the A2063G A2064G resistance locus in patients with Mycoplasma pneumoniae infection

pneumococcal	A2063G A2064G No mutations at the drug resistance locus		A2063G A2064G Mutations at the drug resistance locus	
	Number(persons)	Proportion(%)	Number(persons)	Proportion(%)
Spring	9	20.5	35	79.5
Summer	25	34.2	48	65.8
Autumn	23	8.8	239	91.2
Winter	31	9.1	311	90.9
X2	41.91			
P	<0.01			

Note: February~April is spring, May~July is summer, August~October is autumn, and November~January is winter.

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4. Discussion

MP was isolated in 1944 and officially recognised as a new pathogen in 1963. It is a group of smallest prokaryotic organisms with no cell wall, only cell membrane, no fixed morphology and reproduces in a dicotyledonous or emergent manner [4]. It is one of the main pathogens causing Community Acquired Pneumonia (CAP) infections in China, with regional epidemics occurring every 4 to 7 years, lasting 1 to 2 years, and the number of patients in an epidemic year is several times that of a non-epidemic year. [5] Children and adolescents are susceptible to infection, especially in densely populated areas such as childcare facilities and schools, where the probability of concentrated infection is high. Although the prevalence of MP infection is seldom affected by season and climate, the epidemiological trend is not exactly the same in different regions. In China, the incidence is significantly higher in autumn and winter than in spring and summer, whereas in the United States, outbreaks are more likely to occur in late summer and early autumn.

In China, one of the leading causes of death in children under 5 years of age is pneumonia, and the World Health Organization has reported that pneumonia was the cause of 920,000 deaths in children under 5 years of age in 2016, of which developing countries accounted for 98% [6].

Since Mycoplasma pneumoniae has no cell wall, so it is not sensitive to penicillin and cephalosporin antibiotics acting on the cell wall, and macrolides are the first choice of therapeutic drugs for it, but in recent years, with the widespread use of macrolides, or even the abuse of macrolides, it causes more and more serious phenomenon of resistance in the organism. In recent years, drug resistance of Mycoplasma pneumoniae has been reported all over the world, and the Asian region is one of the most serious drug-resistant regions. Although the incidence of drug resistance varies among different countries, it was shown that the mutation rate of 23SrRNA structural domain V is the highest at sites 2063 and 2064.

Among a total of 721 positive mycoplasma pneumoniae samples sent for testing in 2023 and 2024, a total of 634 people were positive for A2063G and A2064G drug resistance gene site mutation, with a positive rate of 87.8%. Among them, 70 of the 84 samples sent for testing in 2023 were positive for drug-resistant gene site mutation. , the positive rate is 83.3%. Among the 637 samples sent for testing in 2024, 564 were positive for drug-resistant gene site mutation, with a positive rate of 88.54%. The detection rate of mycoplasma pneumoniae A2063G and A2064G drug-resistant gene mutations are 35/44 (79.5%), 48/73 (65.8%), 239/262 (91.2%) and 311/342 (90) in spring, summer, autumn and winter, respectively. .9%); The positive rate of mutation in each age group of <1 year old, 1~year old, 3~year old, 5~year old, 8~12 years old is 7/8(87.5%), 49/52(94.2%), 115/144(79.9%), 367/413(88.9%), 95/10 4 (91.3%); The positive rate of mutations in different genders of men and women is 330/379 (87.1%) and 303/342 (88.6%), respectively.

The A2063G and A2064G resistance sites were detected in 721 children with Mycoplasma pneumoniae infections who came to hospitals from 2023 to 2024, and the mutation rate was 87.8% in 633 cases, which is consistent with the drug sensitivity test conducted by GUO D X et al. in 2019 on MP samples from 12 hospitals in Beijing, which showed that the resistance rate was as high as 87.69%. It was found that in the past two years, the drug resistance of children infected with Mycoplasma pneumoniae in Tai'an was found throughout the year, but the incidence rate in autumn and winter was significantly higher than that in spring and summer, with statistically significant differences; the number of cases in boys was slightly higher than that in girls, but the difference between the two was not statistically significant; in terms of age groups, the mutation rate was lowest in infants, which is in line with the data reported by the various regions[7], which may be due to the simple living environment in infants, which may be the reason for the high drug resistance rate of 87.69%. This finding is consistent with the data reported in various regions[8], which may be due to the fact that infants live in a simple environment, have a low frequency of contact with other people, and take relatively few drugs. The highest number of mutations in the drug-resistant loci occurred in children aged 5-8 years old, which may be due to the fact that the number of children infected with MP in this age group was generally higher than that in other age groups because of their young age, imperfect immune system development, and being in the school crowded area for a long period of time; and preschoolers had the highest incidence of drug-resistant loci, which may be due to the fact that they were young and immune. The highest incidence of drug resistance in preschool children may be attributed to their young age, low immunity, and high incidence of respiratory tract infections, so they use more antibiotics, and macrolide antibiotics, as broad-spectrum antibiotics, are used more often, and thus are very prone to drug dependence. The high mutation rate of drug resistance in 8-12 years old may be due to the fact that children in the older age group take more drugs, which may induce drug resistance in the organism.

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The significant difference in the mutation status of A2063G and A2064G between the age groups also indicated that the overall resistance rate was age-related.

The study shows that once mycoplasma resistance occurs, conventional macrolide antibiotic treatment can not achieve good therapeutic effect, not only the treatment time is longer, the use of a variety of antibiotics in combination with the treatment plan, increase the toxicity of drugs, the probability of adverse reactions and other complications will be increased, and may even develop into refractory pneumonia, which will cause greater damage to the organism.

Although Mycoplasma pneumoniae infection is a self-limiting disease, timely diagnosis and effective treatment can significantly shorten the course of the disease and reduce the damage to the organism. However, MP resistance to macrolides has become a common problem worldwide. Therefore, screening for drug resistance in children with Mycoplasma pneumoniae infections should be performed in a timely manner to improve the efficiency of treatment and reduce unnecessary injuries.

In conclusion, the mutation of A2063G and A2064G in the drug-resistant gene locus of Mycoplasma pneumoniae in children infected with the disease in Taian from 2023 to 2024 occurred throughout the year, with a significantly higher incidence in autumn and winter, and the incidence rates of various age groups also differed significantly, which provided effective evidence for the diagnosis and treatment of the disease, and was conducive to the rational use of antibiotics to reduce the incidence of drug-resistant conditions and improve the treatment of the disease. The results of this study show that the incidence of antibiotic resistance in the disease was significantly different in all age groups.

5. Prospect

At present, 2063-spot and 2064-bit mutation detection are widely used in clinical practice for drug resistance monitoring of Mycoplasma pneumoniae, but the correlation between drug-resistant gene mutation and the clinical manifestations of infected people needs to be further studied. Some studies believe that the use of macrocyclic antibiotics may induce MP drug resistance in the body. The appearance, but it may also be the drug-resistant strains infected by the body itself, but it has not been detected due to the small number. Since then, the use of macrocyclic antibiotics has promoted the proliferation of drug-resistant strains, resulting in drug-resistant strains becoming dominant strains before they are detected. At the same time, it should be noted that there is a certain difference between the in vitro drug resistance test and the actual drug resistance in the body, and the mechanism of resistance of macrocyclic ester drugs is complex and changeable, which has not been fully clarified at present, so further research and discussion are needed. The emergence of drug-resistant strains has greatly increased the difficulty of clinical treatment and increased the burden on patients. In daily work, medical personnel should standardize the use of macrocyclic ester drugs to reduce the occurrence of drug resistance. They should also diagnose drug resistance as soon as possible, and adjust and optimize the treatment plan in time, so as to effectively improve children's pneumonia. The clinical effect of mycoplasma infection minimizes the harm caused by pathogen infection to children.

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