Tropical Journal of Pharmaceutical Research April 2023; 22 (4): 841-846
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v22i4.17

Original Research Article

Significance of the determination of DNA load of drug-resistant mycoplasma pneumoniae and 23sRNA gene mutation locus in children

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Sent for review: 2 December 2022 *Revised accepted: 30 March 2023*

Abstract

Purpose: *To determine the significance of the detection of Mycoplasma pneumoniae (MP)-DNA load and 23sRNA gene mutation locus in children with drug-resistant MP pneumonia.*
Methods: *A total of 158 children with MP pneumonia received drug sensitivity tests. The patients were divided into resistance group and non-resistance group. The MP-DNA load index (MPLI) and mutation rate of 23sRNA gene at 2063 locus were assessed and compared between the two groups: the MPLI-negative group and the MPLI-positive group, based on whether MPLI was greater than 6.12. The association of MPLI of all the patients and the 23sRNA gene mutation at 2063 locus in the resistance group, as well as clinical indicators were analyzed.*
Results: *The MPLI of the resistance group was lower than that of the non-resistance group. In the MPLI-positive group, the duration of disease, defervescence time, disappearance time of cough and expectoration, disappearance time of chest opacity, and length of stay were all longer than those of the MPLI-negative group, while the proportion of cases with extrapulmonary complications and the white blood cell (WBC) count were higher than those of the MPLI-negative group. The mutation rate of 23sRNA gene at 2063 locus in the resistance group was higher than that in non-resistance group ($p < 0.05$). The defervescence time, disappearance time of cough and expectoration, disappearance time of chest opacity and length of stay were longer in the mutation-positive group than those in the mutation-negative group ($p < 0.05$).*
Conclusion: *The mutation rate of 23sRNA gene at 2063 locus is higher in children with drug-resistant MP pneumonia. Furthermore, low MPLI and 23sRNA gene mutations at 2063 locus are associated with the duration of disease, disappearance time of clinical symptoms and other clinical indicators.*

Keywords: *Drug-resistant Mycoplasma pneumoniae pneumonia, MP-DNA load, 23sRNA gene mutation locus*

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INTRODUCTION

Mycoplasma pneumonias (MP) mainly causes respiratory tract infection, which leads to up to 40 % of community-acquired pneumonia (CAP) in all age groups, and is also the cause of regional epidemics. MP is generally susceptible to all groups, but the harm to children is more serious [1]. At present, MP pneumonia is often treated with macrolide antibiotics, and the 23sRNA gene

at 2063 locus has been verified to be their target [2,3]. MP quantitative detection is an important indicator for diagnosing MP pneumonia and evaluating antibiotic resistance, which is mainly regulated by MP-DNA. Therefore, it is of great significance to investigate the MP-DNA load used for evaluating antibiotic resistance. In this study, the MP-DNA load and genotype of 23sRNA gene at 2063 locus in children with drug-resistant MP pneumonia were analyzed to determine their associations with the clinical characteristics of pediatric patients.

METHODS

Clinical profile of patients

Children (158) with MP pneumonia treated in our hospital from December 2017 to December 2019 were selected, including 86 males and 72 females aged 2 - 14 years, with a mean of (7.27 ± 1.51) years. This study was approved by the ethical committee of Affiliated Hospital 2 of Nantong University (approval no.2021KT112). The study was conducted by following the Declaration of Helsinki.

Inclusion criteria

1) Pediatric patients meeting the diagnostic criteria for MP pneumonia [4]; 2) those approved by the Ethics Committee of Children's Hospital of Soochow University [5], and 3) those aged ≤ 14 years.

Exclusion criteria

1) Pediatric patients with severe heart, liver or kidney dysfunction; 2) those accompanied by other types of lung diseases such as bronchial asthma; 3) those infected with viruses, bacteria or other pathogens; 4) those with severe immune diseases; or 5) those treated with glucocorticoids within 1 week before the start of this study.

Drug sensitivity test

Pharyngeal swab specimens were collected within 2 h after admission, added into the drug-sensitive plate with MP medium in the negative wells, and cultured in an incubator at 37 °C for 24 h, followed by analysis of drug resistance. If the medium in the control well turned yellow compared with that in the negative control well, the specimens were resistant to antibiotics. In contrast, if the medium in the control well had no change in color compared with that in the negative control well, the specimens were sensitive and non-resistant to antibiotics.

MP-DNA load assay

DNA extraction

Pharyngeal swab specimens were rinsed with normal saline, and centrifuged using a TGL-16GB high-speed centrifuge (Shanghai Anting Scientific Instrument Factory) at 13,000 r/min for 10 min. Then the lower-layer precipitate was harvested, added with 50 μL of DNA lysis buffer, placed in a metal bath and centrifuged again. The supernatant was harvested for later use.

Determination of DNA load

The content of MP-DNA was detected by fluorescence quantitative polymerase chain reaction (PCR). PCR amplification (PE5700 gene detection system for data processing) was performed with the kit provided by Anlong Gene Technology Co. Ltd and the following conditions: pre-denaturation at 94 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C for 30 s and extension at 72 °C for 1 min; final extension at 72 °C for 5 min. Besides, with H actin-DNA content as an internal control, PCR was conducted as follows: pre-denaturation at 94 °C for 4 min, followed by 40 cycles of denaturation at 93 °C for 30 s and annealing at 60 °C for 30 s. The primers and probe sequences are shown in Table 1.

Calculation of MP-DNA load index (MPLI)

MPLI was computed as in Eq 1.

$$\text{MPLI} = -\lg\frac{\text{MP-DNA copy number}}{\text{H actin-DNA copy number}} \quad \text{..... (1)}$$

Based on the technical standard for application of *in vitro* diagnostic reagents [6], MPLI > 6.12 was considered negative, and MPLI ≤ 6.12 positive.

Genotype detection of 23sRNA gene at 2063 locus

The 23sRNA gene amplification was carried out using the primer sequences shown in Table 1. The polymerase chain reaction (PCR) conditions were as follows: pre-denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 15 s, annealing at 52 °C for 30 s and extension at 72 °C for 30 s; final extension at 72 °C for 10 min. The PCR product was stored at 4 °C and detected with 1 % agarose gel electrophoresis. Then, the single product band was recovered, purified and sequenced. The sequencing result was compared with the reference sequence in the gene bank [7], and the

genotype at 2063 locus was determined: A as wild type, and C, G and T as mutant type.

Table 1: Gene primer sequences

Target gene	Primer	Sequence
β-actin	β-actin-F	GTGTGGCTCCCGAGGAG
	β-actin-R	CACTCACCTGGGTCATCTTC
	β-actin-Probe	FAM-CCGTGCTGCTGACCGAGGC-BHQ
MP 23sRNA	MP-SENSE	TACCTTCAGTACGGTTGGAAATC
	MP-ANTI	TTCTCACCTGTTCGACCTGTAT
	MP-PROBE	FAM-TCTCACAGTCAAGCACCCTTACACCA-BHQ

Evaluation of indices/indicators

(1) Based on the results of the drug sensitivity test, the patients were divided into resistance group and non-resistance group. (2) The MPLI and mutation rate of 23sRNA gene at 2063 locus were compared between the two groups. (3) The clinical data of all patients were collected, and the associations of MPLI and 23sRNA gene mutation at 2063 locus as well as clinical indicators were analyzed.

Statistical analysis

SPSS 17.0 software was used for data processing. Enumeration data was described by percentage (%) and compared by χ^2 test between the two groups. After the normality test, measurement data was presented as mean ± standard deviation (SD) and compared by *t*-test between the two groups. $P < 0.05$ was considered statistically significant.

RESULTS

MPLI data

According to the results of the drug sensitivity test, the patients were divided into a resistance group (n = 112) and a non-resistance group (n = 46). The MPLI in the resistance group (5.03 ± 1.27) was lower than that of the non-resistance group (6.03 ± 1.32); ($t = 4.373$, $p = 0.000$).

Association between MPLI and clinical indicators

In the MPLI-positive group, the duration of disease, defervescence time, disappearance time of cough and expectoration, disappearance time of chest opacity and length of stay were all longer than those in the MPLI-negative group, and the proportion of cases with extrapulmonary complications and the white blood cell (WBC) count were higher than those in the MPLI-negative group ($p < 0.05$; Table 2).

Genotype of 23sRNA gene at 2063 locus in the two groups

The mutation rate of 23sRNA gene at 2063 locus in the resistance group was higher than that in the non-resistance group ($p < 0.05$) (Table 3).

Table 2: Association between MPLI and clinical indicators

Clinical indicator	MPLI-negative group (n = 41)	MPLI-positive group (n = 117)	χ^2/t	*P*-value
Age (years)	7.19 ± 1.38	7.32 ± 1.67	0.447	0.655
Duration of disease (days)	7.64 ± 1.07	8.29 ± 1.18	3.107	0.002
Defervescence time (days)	3.38 ± 1.13	4.96 ± 1.09	8.227	0.000
Disappearance time of cough and expectoration (days)	4.59 ± 1.38	5.82 ± 1.51	4.586	0.000
Disappearance time of chest opacity (days)	10.24 ± 1.96	12.85 ± 2.19	6.741	0.000
Length of stay (days)	14.76 ± 2.17	17.95 ± 2.31	7.726	0.000
Extrapulmonary complications				
Yes	9	47	4.405	0.036
No	32	70		
WBC ($\times 10^9$/L)	8.61 ± 1.79	7.88 ± 1.62	2.415	0.017
CRP (mg/L)	13.18 ± 2.25	12.55 ± 2.17	1.585	0.115

Table 3: Genotype of 23sRNA gene at 2063 locus in the two groups [n (%)]

Group	n	A	C	G	T	Mutation rate
Resistance group	112	15	12	76	9	97 (86.61)
Non-resistance group	46	33	3	9	1	13 (28.26)
χ^2	52.484					
P-value	<0.05					

Table 4: Association between 23sRNA gene mutation at 2063 locus and clinical indicators in resistance group

Clinical indicator	Wild type (n = 15)	Mutant type (n = 97)	χ^2/t	*P*-value
Age (year)	7.20 ± 1.42	7.29 ± 1.61	0.204	0.838
Duration of disease (day)	7.86 ± 1.09	8.13 ± 1.12	0.872	0.385
Defervescence time (day)	3.43 ± 0.89	4.68 ± 1.13	4.087	0.000
Disappearance time of cough and expectoration (day)	4.65 ± 1.41	5.71 ± 1.49	2.581	0.011
Disappearance time of chest opacity (day)	10.33 ± 2.01	12.56 ± 2.15	3.769	0.000
Length of stay (day)	15.76 ± 2.12	17.13 ± 2.29	2.176	0.032
Extrapulmonary complications				
Yes	5	19	1.458	0.227
No	10	78		
WBC ($\times 10^9$/L)	8.29 ± 1.75	8.13 ± 1.68	0.341	0.733
CRP (mg/L)	13.01 ± 2.20	12.52 ± 2.18	0.809	0.420

Association between 23sRNA gene mutation at 2063 locus and clinical indicators in resistance group

The defervescence time, disappearance time of cough and expectoration, disappearance time of chest opacity and length of stay were longer in the mutation-positive group than in the mutation-negative group ($p < 0.05$; Table 4).

DISCUSSION

With the wide use of macrolides in clinical practice, the drug resistance rate has been increasing. The mechanism of MP resistance to macrolides is mainly related to the mutation of the target locus. The base point mutation in 23sRNA domain directly binding to macrolides can lead to a decrease in affinity between antibiotics and ribosomes, resulting in drug resistance [8]. With the use of antibiotics, the proliferation of resistant strains is inhibited, and the DNA copy number declines, so their MPLI is different from that of the sensitive strains. In this study, the MPLI in the resistance group was lower than that in the non-resistance group, suggesting that the MP-DNA copy number in children with drug-resistant MP pneumonia is high, which is consistent with the result of the relevant study [9]. It can be seen that the antibiotic resistance of MP is related to the MP-DNA load, and antibiotic resistance in child patients can be judged by detecting the MP-DNA load, and whether to give antibiotic therapy can then be determined based on the detection results.

The decrease in MPLI is primarily related to the fact that the division and proliferation of resistant strains are not restricted by therapeutic drugs during treatment. The higher the MP-DNA load, the longer the retention time of MP in the respiratory tract. As a result, persistent damage will be caused to the bronchial mucosa, and the patients become less sensitive to drugs, weakening the efficacy and delaying rehabilitation [10, 11]. In this study, it was found that in MPLI-positive group, the duration of disease, defervescence time, disappearance time of cough and expectoration, disappearance time of chest opacity and length of stay were all longer than those in MPLI-negative group, and the proportion of cases with extrapulmonary complications and the WBC count were higher than those in MPLI-negative group. The above results demonstrated that the efficacy can be judged by determining the MP-DNA load.

Resistant locus mutation is the main cause of drug-resistant MP pneumonia in children. Zhou *et al*. [12,13] determined the genotype of 23sRNA gene at 2063 locus in MP-resistant and MP-sensitive child patients and found that the probability of genotype C, G and T of 23sRNA gene at 2063 locus in MP-resistant group is far higher than that in MP-sensitive group. In this study, it was further confirmed that the mutation rate of 23sRNA gene at 2063 locus in resistance group was higher than that in non-resistance group. The main reason is that 23sRNA gene at 2063 locus is the target of macrolide antibiotics, and its mutation will lead to MP resistance.

MP is the major pathogen causing community-acquired respiratory tract infections which frequently occur in preschool children. Recently, MP infections have been increasing, but the incidence of MP resistance has gradually emerged due to the wide use of antibiotics. MP resistance is the main cause of refractory conditions, which will affect the normal life and growth and development of children [14]. MP resistance in children is mainly attributed to the locus change of 23sRNA domain of the 50S ribosomal subunit [15]. Due to the nucleotide sequence change of 23sRNA domain of the 50S ribosomal subunit, the affinity between antibiotics and ribosomes is reduced, thus leading to antibiotic resistance. It has been found that

23sRNA gene mutation at 2063 locus corresponds to a higher probability of MP resistance [16]. In this study, the defervescence time, disappearance time of cough and expectoration, disappearance time of chest opacity and length of stay were longer in mutation-positive group than those in mutation-negative group, suggesting that the efficacy on child patients with mutant 23sRNA gene at 2063 locus is less significant than those with wild-type gene. The main reason is that 23sRNA gene mutation at 2063 locus may lead to MP resistance, thus weakening the treatment effect.

CONCLUSION

The mutation rate of 23sRNA gene at 2063 locus is higher in children with drug-resistant MP pneumonia. Moreover, low MPLI and 23sRNA gene mutation at 2063 locus are associated with prolonged disappearance time of clinical symptoms and length of stay. Therefore, antibiotic resistance in pediatric patients can be determined by evaluating the MP-DNA load and the genotype of 23sRNA gene at 2063 locus, and then the decision to give antibiotic therapy can be made based on the results.

DECLARATIONS

Acknowledgements

This work was supported by Social and People's Livelihood Science and Technology Plan Project of Nantong Science and Technology Bureau in 2022 (no. MSZ2022020).

Funding

None provided.

Ethical approval

This study was approved by the ethical committee of Affiliated Hospital 2 of Nantong University, China (approval no.2021KT112).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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