

# LABORATORY DIAGNOSIS AND TREATMENT OF MYCOPLASMA PNEUMONIAE INFECTION IN CHILDREN

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## Abstract

Mycoplasma pneumoniae (MP) is the cause of Mycoplasma pneumoniae pneumonia (MPP) in children and adolescents, with the clinical manifestations highlighted by intermittent irritating cough, accompanied by headache, fever and muscle pain. This paper aimed to study the research status and focal points in MP infection, especially the common laboratory diagnostic methods and clinical treatment of Mycoplasma pneumoniae. Laboratory diagnostic methods include molecular assay, serological antibody detection, rapid antigen detection and isolation and culture. Polymerase chain reaction (PCR) is the gold standard with high sensitivity and specificity. The serological antibody can detect various immune antibodies qualitatively or quantitatively in serum. Rapid antigen can be detected faster, with no equipment environment requirements, which can be used for the early diagnosis of MP infection. While the culture growth cycle is long and insensitive, not recommended for routine diagnosis.

**Keywords:** Mycoplasma pneumoniae, Mycoplasma pneumoniae pneumonia, laboratory diagnosis, treatment, children.

## Introduction

Mycoplasma pneumoniae (MP) is the smallest pathogenic microorganism, small prokaryotic cells without a rigid cell wall, which is between bacteria and viruses and can live independently, and the adhesion ability to host cells is positively correlated with virulence [1]. The genome size of MP is extremely small, about 816 kilo base-pairs [2]. 6 of the 16 species of human mycoplasma can cause diseases, and the most important and the most predominant pathogen is MP [3, 4]. The lack of a cell wall barrier in mycoplasma makes them insensitive to cell wall antimicrobials (such as beta-lactam), not stained by Gram staining, difficult to survive in dry environments, and also affects their appearance under the microscope [5]. MP attaches to ciliated cells within the respiratory epithelium via attachment organelles and produces an ADP-ribosyl transferase, also known as community-acquired respiratory distress syndrome toxin (CARDS toxin), which is responsible for entering host cells through clathrin-mediated endocytosis [6, 7].

MP can induce upper and lower respiratory tract infections, and cause Mycoplasma pneumoniae pneumonia (MPP), tracheobronchitis, etc., with headache, fever, muscle pain, sore throat, cough, dry cough or mucus-like sputum representing a predominant form of community-acquired pneumonia in pediatric populations, constituting a significant threat to children health [8, 9]. It can also cause various extrapulmonary manifestations, involving almost all organs, including skin and nerves, blood, cardiovascular, genitourinary system, musculoskeletal system,



and can cause pseudomembranous necrotizing laryngotracheobronchitis, myelin oligodendrocyte glycoprotein antibody-associated meningoencephalitis [10–13]. Infections often occur in summer or early autumn, as well as at any time of the year. The main route of transmission is fulminant, the incubation period is 2–3 weeks, and the incidence rate is the highest among children and adolescents [14]. MP re-infects over some time probably because it can hide in host cells to protect it from antibodies and antibiotics; the second is the lack of protective immunity due to some important factors such as variation and rearrangement of surface antigens [15]. Studies have shown that clinical signs, symptoms and laboratory findings are not sufficient to distinguish pneumonia caused by MPP and other pathogens, and correct etiological diagnosis, as well as drug treatment largely depend on accurate and rapid laboratory diagnosis.

### Materials and Methods

PCR is considered the new "gold standard" with the higher sensitivity, most assays can detect <100 CFU/mL; The specificity is strong and there is no cross-reactivity when appropriate target selection and amplification conditions are validated. Nucleic acid amplification techniques used to detect MP DNA or RNA differ in the selection of target genes used (e.g. P1 gene, 16S rDNA, 16S rRNA, ATPase operon gene, etc.) (PCR versus isothermal amplification techniques) and the form of detection (conventional versus real-time, single versus multiple) [17]. The most problematic issue with PCR is colonization or asymptomatic carriage.

It is extremely rare to compare the performance of PCR methods with different *Mycoplasma pneumoniae* target areas and primers. P1 adhesin gene primers were found to be more sensitive than 16S rRNA primers, which may be due to the presence of multiple copies of the P1 cell adhesion gene. Studies have compared three different PCR detection methods: the detection method initially described by Bernet, with and without additional hybridization steps for amplicon detection, and the newly developed nested PCR [18]. All three PCR methods are reliable in detecting MP in respiratory specimens, but nested PCR is the most sensitive [19]. Due to the differences in sample collection, transportation and extraction procedures, input sample size, target genes, primers, cycle parameters, and detection systems, the comparison of sensitivity data for different PCRs becomes complicated.

### Clinical Treatments

When MP infection is clearly defined, the main treatment method is drug treatment. Rational and standardized use of antibiotics can reduce symptoms and shorten the course of disease [37].

Mild MPP is more common in school-age children over 5 years old [38], with a course of 7–10 days, most patients have a good prognosis. The main clinical manifestations are fever and cough, wheezing and dyspnea can be detected in a small number of infants and young children. Imaging findings are bronchitis and bronchopneumonia; only a few patients can develop into severe [39]. Severe MPP refers to the severe condition of MPP, which conforms to any of the following manifestations: high fever  $\geq 5$  days or fever  $\geq 7$  days, or wheezing, shortness of breath, dyspnea, chest pain, hemoptysis and other symptoms. These manifestations are related to severe lesions, combined with plastic bronchitis, asthma attacks, pleural effusion and pulmonary embolism; extrapulmonary complications occurred, but did not meet the criteria for critical illness; finger pulse oxygen saturation  $\leq 93\%$  when breathing air at rest. The imaging findings were one of the following: large area of pulmonary consolidation; single lung diffuse or double lung multi-



leaf segmental bronchiolitis showed [40, 41]. Critically MPP refers to severe MPP with rapid progression, respiratory failure or life-threatening extrapulmonary complications that require life-support treatment [39].

### Antibiotics

MP lacks a cell wall and is resistant to all antimicrobials targeting the cell wall, susceptible to antibiotics that act on the bacterial ribosome and inhibit protein synthesis. Commonly used antibiotics include macrolides such as azithromycin, clarithromycin, roxithromycin, etc., new tetracycline antibiotics such as doxycycline, minocycline and omacycline, quinolones such as levofloxacin, ciprofloxacin, moxifloxacin. Tetracyclines can inhibit peptide chain lengthening of protein synthesis by acting on the 30 S subunit of MP ribosomes. The treatment time is generally 10 ~ 14 days, and some severe patients can be extended to about 3 weeks [40].

Macrolide antibiotics, represented by azithromycin and erythromycin, are the preferred drugs and have been widely used in children with MPP in recent years, while the drug resistance rate is also increasing, marked by point mutations in the 23S rRNA gene [41]. Given the increasing prevalence of macrolide resistance worldwide, especially in East Asian countries such as Japan, China and South Korea, the search for alternative antibiotics to treat macrolide-resistance MPP is accelerating.

### Conclusion

The laboratory examination of MP is very important for identifying the pathogens of MPP, and the rational and safe use of antibiotics is also crucial for the treatment of MPP in children. MP is easily under-reported due to the lack of clinical and chest X-ray features, the relative lack of rapid and specific laboratory diagnostic techniques, and the difficulty of isolation and culture of MP. In most cases where the specific pathogen cannot be identified, doctors will give empirical beta-lactam antibiotic treatment, which is ineffective for atypical pathogens, and correct and timely use of macrolide antibiotics can significantly shorten the course of the disease, so a rapid and accurate laboratory diagnosis of MP is very important. The common laboratory diagnostic methods of MP include PCR, serological antibody detection, rapid antigen detection and isolation culture. PCR is the gold standard with high sensitivity, specificity and no cross-reaction. Serological antibody detection can qualitatively detect various immune antibodies in serum or quantitatively detect antibody titers, which have a certain guiding role in the diagnosis of the disease and the progression of the disease. The rapid antigen detection time is the fastest, and there is no equipment and environmental requirements, which can be used for the early diagnosis of MP infection. The growth cycle of isolation culture is long and insensitive, and it is not recommended for routine diagnosis. For mild MPP, macrolides are the first choice. For drug-resistant MPP, new tetracyclines and symptomatic treatment can be used instead, which will generally improve. For severe MPP, on the basis of symptomatic treatment and corresponding antibiotics, glucocorticoid and gamma globulin can be added, which can have obvious curative effects. For critically ill MPP, bronchoalveolar lavage can be added.



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