# Refractory *Mycoplasma pneumoniae* Pneumonia in Children

Subjects: Respiratory System

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*Mycoplasma pneumoniae* (*M. pneumoniae*) is one of the most important pathogens for community-acquired pneumonia (CAP) in children. *M. pneumoniae* pneumonia (MPP) is typically mild and even presents as a self-limited disease. Refractory *Mycoplasma pneumoniae* pneumonia (RMPP) is a severe state of *M. pneumoniae* infection. The pathogenesis of RMPP remains unknown, but the excessive host immune responses as well as macrolide resistance of M. pneumoniae might play important roles in the development of RMPP. To improve the prognosis of RMPP, it is mandatory to recognize RMPP in early stage, and detection of macrolide-resistant MP, clinical unresponsiveness to macrolides and elevated proinflammatory cytokines might be the clues. Timely and effective anti-mycoplasmal therapy and immunomodulating therapy are the main strategies for RMPP.

Keywords: Mycoplasma pneumoniae ; pneumonia ; prediction

## 1. Pathogenesis

### 1.1. Pathogeny and Host Defense

*M. pneumoniae* can be transmitted through air droplets via coughing, sneezing and close contact. Vertical transmission has occasionally been stated in recent years [1][2]. The incubation period varies from 1 to 3 weeks, and the survival of *M. pneumoniae* in aerosols is thought to be related to meteorological conditions, especially humidity and temperature, but controversy still remains [3][4][5]. Once infected with the host, *M. pneumoniae* mainly adheres to ciliary cells of the mucosal epithelium, and close contact and material exchange between the bacterial membrane and the host cell provide an important material basis for its growth and proliferation. Bacterial cellular components such as glycolipids and capsular polysaccharides [6], virulence factors such as community-acquired respiratory distress syndrome (CARDS) toxin [7] and hydrogen sulfide, alanine, and pyruvate producing enzyme (HapE) [8], toxic metabolites such as hydrogen peroxide [9] and $H_2S$ [10], and nuclease [11], among others, are the main mechanisms for tissue damage. They also inhibit host clearance and promote immune escape [12].

CARDS toxin was first demonstrated in 2005 [13]. With a high sequence homology to the pertussis toxin S1 subunit, which performs ADP ribosylation and causes vacuolation, choristosis and spallation of mucosal cells. This toxin brings out the typical clinical symptoms of *M. pneumoniae* infection, for instance, dry cough or even spasmodic cough [14][15][16]. By other means, expressed CARDS toxin can also enhance the induction of the proinflammatory cytokines and stimulate lymphocyte activation in a dose- and activity-dependent manner [7][17][18][19] and is also capable of changing asthma-associated immunological parameters or inducing an allergic-type inflammation [12][20][21], potentially inducing or worsening asthma [22][23].

Hemolytic activity was also one of the identified pathogenicity determinants of *M. pneumoniae* where both hydrogen peroxide ($H_2O_2$) [24] and hydrogen sulfide ($H_2S$) [25] contribute. Hydrogen peroxide is a metabolite of the process of glycerol utilization by Mycoplasma pneumoniae, and glycerol-3-phosphate oxidase (GlpO) is the key enzyme [26]. $H_2O_2$ is responsible for the oxidation of heme molecules and is also associated with oxidative stress and cell death [27][28]. $H_2S$, as a by-product of the reaction to desulfurization of the cys by the enzyme HapE, can cause the modification of the heme and is responsible for the lysis of erythrocytes. By other means, $H_2S$ can also induce phagocytes to secrete pro-inflammatory factors, aggravating inflammatory reactions and leading to tissue damage [8].

Although the mechanism of RMPP is largely unknown, it has long been believed that the excessive host immune response plays a pivotal role in the disease progression [29][30]. Three mainstream hypotheses to explain the hyperimmune response for MP are summarized below [31]: (i) repeated or recurrent MP infections; (ii) loss of capacity to clear *M. pneumoniae* from the lungs in primary infection such as macrolide-resistance, which will be discussed later,

resulting in a persistent MP infection; and (iii) an overactive innate immune response, such as macrophage activation through heterodimerization of Toll-like receptors [32][33]. The overall result of the above factors is an excessive and overactive immune response, which will be explained in detail in later parts.

### 1.2. Macrolide-Resistant *M. pneumoniae* (MRMP)

The lack of a cell wall renders *M. pneumoniae* intrinsically resistant to some antimicrobials, such as beta-lactams, glycopeptides and fosfomycin antimicrobials, which lays a trap for the identification of this atypical pathogen and also results in difficulties in treating pediatric *M. pneumoniae* infection. Historically, the main efficient drugs against *M. pneumoniae* include agents targeting the bacterial ribosome for inhibiting protein synthesis, such as macrolides, and others inhibiting DNA replication, such as fluoroquinolones [34]. Macrolides are the first and nearly the only choice for pediatric patients due to toxicity and side effects of other drugs for young children. Unsurprisingly, under the long-term pressure of antibiotic selection, macrolide-resistance emerged.

Thus far, the vast majority of reports correlating with macrolide-resistant infections were from children, due to a high incidence of *M. pneumoniae* infections and also the wide use of macrolides in pediatric age groups, but macrolide-resistance can also occur in adults [32][33]. Up to now, no difference has been found in disease manifestations between pediatric patients and adults infected by MRMP. Resistance of *M. pneumoniae* to macrolides was first described in 2001 in Japan [35] and quickly swept across East Asia, wherein the resistance rates were found to be higher than 90% in some countries during the epidemic years [36][37]. Since that time, a progressive increase in incidence rates of MRMP strains was reported worldwide, although with a significant difference among countries [31][38][39]. It is also a common and disturbing problem in China, both in adults and in children [40][41].

Temporal studies suggest that the emergence of significant resistance to macrolides by *M. pneumoniae* takes precedence over the peak of *M. pneumonia* episodes [42]. Therefore, the activation of resistant strains may be one of the important causes of the MP outbreak. Additionally, some studies have illustrated that macrolide-resistance of *M. pneumoniae* may play an essential role in RMPP development and progression, given the limited sensitivity of MRMP to macrolides may result in higher bacterial load and excessive immune response [43][44][45][46]. The opposite point of view also exists, demonstrating that macrolide resistance may not be associated with the development of RMPP [47][48]. Therefore, the association between RMPP and increased macrolide-resistance requires further investigation.

### 1.3. Co-Infection

Co-infection in CAP is clinically common. Likewise, the dual existence of *M. pneumoniae* with other organisms is not rare in patients with respiratory syndromes, especially in children [49]. The rates of viral (human bocavirus, rhinovirus, respiratory syncytial virus, among others, respectively) or bacterial (Streptococcus pneumoniae, Hemophilus influenzae, Staphylococcus, among others, respectively) coinfection with *M. pneumoniae* in children were reported ranging from 8 to 60% [50][51][52][53][54]. Some reports revealed simultaneous laboratory-proven infections with both bacteria and viruses in addition to *M. pneumoniae* [50][53].

Although the contribution of these coexistent agents remains unclear, since healthy individuals may carry these opportunistic pathogens as well [55][56][57], coinfection with viruses and bacteria causes more severe diseases in pediatric patients, according to previous research [58][59]. In children with RMPP, Zhang et al., demonstrated that coinfection with viruses and bacteria resulted in more severe processes [53]. Zhou et.al recently reported that adenovirus coinfection with MRMP was shown to be more prevalent in RMPP patients [45]. However, Chiu et al., found no significant difference in clinical features, complications, or outcomes between the patients infected with *M. pneumoniae* alone or with virus coinfection, despite the latter having prolonged fever and hospital stay [52].

## 2. Prediction and Early Recognition

Almost all previous reports indicated that delayed appropriate treatment was associated with the development of more severe and/or extended illnesses [60]. Thus, clinical awareness, prompt detection of *M. pneumoniae* and its macrolide resistance and early recognition of RMPP enable effective therapy to begin sooner, potentially improving clinical outcomes [48].

### 2.1. Clinical Awareness and Confirmation of Macrolide Resistance

The gold standard for diagnosing MRMP is culture and drug sensitivity. However, culture is much too time-consuming, thus the identification of MRMP strains is usually made with molecular biology methods nowadays. *M. pneumoniae* carry

a total of 816,394 bp base pairs with 687 genes on the circulating double strands of DNA function to maintain their viability and reproduction [61]. Molecular epidemiology investigations of *M. pneumoniae* and macrolide susceptibility have been conducted in a wide range of geographical and temporal contexts [38][62][63][64][65]. Most investigations showed that MRMP usually had specific point mutations in the peptidyl transferase loop of 23S rRNA, as well as insertions or deletions in ribosomal proteins L4 and L22 [60]. Genotyping analysis from Japan suggested that epidemics arise due to variants of P1 sequences [12] and was further verified and refined in subsequent studies [13]. In China, variants in domain V of the 23S rRNA gene are also the major cause of MRMP, with most strains harboring an A2063G mutation, in which P1 type 1 and type 2 lineages co-circulate [40][47][66][67][68]. At present, commercial PCR kits for the rapid detection of both MP gene or antigen and drug resistance mutations simultaneously are available on the market [69][70][71], and makes it possible to rapidly diagnose MRMP.

Some clinical phenomena may also serve as early indicators of macrolide resistance MPP (MRMPP), especially macrolide unresponsiveness. Patients with MRMPP usually have an extended period of fever in spite of macrolide therapy. They are also more susceptible to more severe phenotype, and more complications [72][73]. For the early recognition and confirmation of MRMPP, pediatricians should pay more attention to the initial response to macrolide. If a child with confirmed or suspected MPP does not respond to macrolide therapy in the first three days (macrolide unresponsive MPP), MRMPP should be suspected and further management should be adopted, especially in countries and regions with high MRMP rates [74][75]. Coinfection with bacteria or virus and complications should also be excluded.

### 2.2. Early Identification of RMPP Cued by Cytokine Profiles

The host immune response is a “double-edged sword”. On the one hand, an adequate immune response including cytokine secretion and lymphocyte activation is essential for the elimination of *M. pneumoniae*, helping alleviate disease [76]. Children with hypogammaglobulinemia appeared to be more vulnerable to invasive and prolonged bacterial infections [77]. On the other hand, an improper immune response to *M. pneumoniae* generates excessive inflammation, and can exacerbate the disease clinically, even leading to the development of RMPP. Evidence revealed pulmonary lesions were generally mild in immunodeficient children [78]. This theory may also partially explain the selectivity of RMPP in terms of children’s ages. Children over the age of 5 years old have a relatively better developed immune system than younger children; coincidentally, the former group happens to be more susceptible to disease and exhibits more severe phenotypes of disease [43].

Although the direct correlation between the host immune response and RMPP is inconclusive, a growing body of evidence points to it. The course and outcome of mycoplasmal infection seem to be highly dependent on host responses. The stronger the immunological response and activation of cytokine, the more severe the clinical disease and organ damage. Herein, a question is whether cytokine profiling may predict the severity and subtype of illness in advance, allowing for reasonable and individualized therapy adjustments to be made as early as possible.

Numerous literatures have reported the correlation between cytokines, chemokines or other inflammatory biomarkers and RMPP. Lactate dehydrogenase (LDH), for example, has long been regarded as a reliable evaluation index of RMPP. The cut-off value of LDH for considering RMPP ranged from 379 to 480 IU/L among adolescents and adults [75][79][80][81][82]. Some previous study suggested LDH ≥ 417 IU/L to be significant predictors in regard to RMPP [79]. Some other inflammatory biomarkers, such as CRP ≥ 16.5 mg/L [79], ESR ≥ 32.5 IU/L [80] and 35 α-hydroxybutyrate dehydrogenase (HBDH) ≥ 259.5 IU/L [80] also have indicative significance for RMPP in children.

To combat MP infection, neutrophils, CD8+ T cells, as well as Th1 biased CD4+ T cells, are recruited followed by enhanced humoral immunity. In recent years, more attention has been paid to proinflammatory cytokines. In some previous study, the percentage of neutrophils and CD8+ T cells, as well as the levels of IL-6, IL-10 and IFN-γ, were shown to be beneficial for distinguishing patients with RMPP from those with general MPP [79][83], serum chemokines such as CXCL10/IP-10 may also be potential biomarkers [84]. This phenomenon has been confirmed by other studies in recent years. Therefore, people should be alert to the possibility of RMPP when cytokines such as IFN-γ [43][64][83], TNF-α [43], IL-6 [64][79], IL-10 [83], IL-18 [85][86][87], among others, are obviously elevated. Further confirmation of these candidates is needed.

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