

Infection by *Mycoplasma pneumoniae* and Its Importance as an Etiological Agent in Childhood Community-Acquired Pneumonias

Letícia Alves Vervloet¹, Christophe Marguet² and Paulo Augusto Moreira Camargos³

¹Federal University of Minas Geral (UFMG); ²Unité de Pneumologie Pédiatrique, Centre Hospitalier Universitaire, Université de Rouen, Rouen, France; ³Medical Scholl of Federal University of Minas Geral (UFMG); Belo Horizonte, MG, Brazil

This manuscript reviewed the literature on infection by *Mycoplasma pneumoniae* with emphasis on etiological aspects of childhood community-acquired pneumonias. Bibliographical research was carried out from Pubmed Medline, MDConsult, HighWire, LILACS, and direct research over the past 10 years with the following keywords: *Mycoplasma pneumoniae*, pneumonia, and childhood. Fifty-four articles were selected. *Mycoplasma pneumoniae* has a high incidence in childhood. Clinical presentation includes respiratory and extrapulmonary symptoms. *Mycoplasma pneumoniae* lung infection can be confused with viral or bacterial pneumonia and is unresponsive to beta-lactams. In addition, co-infections have been reported. *Mycoplasma pneumoniae* infection occurs in all age groups, being less frequent and more severe in children under the age of five. Its incidence as a causal agent is high. *Mycoplasma pneumoniae* infections constitute 20%-40% of all community-acquired pneumonias; the severity is highly variable, and this condition may lead to severe sequelae. *Mycoplasma pneumoniae* frequency is underestimated in clinical practice because of the lack of specific features and a diagnosis that needs serology or PCR. Effective management of *M. pneumoniae* infections can usually be achieved with macrolides. In Brazil, epidemiological studies are needed in order to assess the incidence of this bacterium.

Key-Words: *Mycoplasma pneumoniae*, pneumonia, extrapulmonary findings, diagnosis, treatment.

Mycoplasma pneumoniae is one of the main etiological agents of community-acquired pneumonia, although its incidence is generally unknown in Brazil. Reasons for under-reporting include a lack of clinical and Chest X-ray features and relative unavailability of fast and specific, costly-laboratory techniques, as well as the difficulty of growing this pathogen in the laboratory.

One of the greatest challenges in dealing with childhood pneumonia is etiology identification. In daily practice, empirical treatment is the initial resort. Most studies do not identify the etiological agent in 20%-60% of cases [1,2], and an identified agent might not be the sole cause of the pneumonia [3]. Choice of treatment is based on identification of the epidemiological, clinical, radiological, and laboratory characteristics associated with certain pathogens. *Mycoplasma pneumoniae* should be considered in differential diagnosis of community-acquired pneumonia; it should also be considered in co-infections, when they are unresponsive to beta-lactams, which are commonly administered [4,5]. On the other hand, recommendations tend to orient the diagnosis. Official recommendations regarding the treatment of pneumonia in childhood have been published in Britain, Canada and the United States; macrolide antibiotics should be used as a first-line empirical treatment in children aged five and above [1,6,7].

History

The first mycoplasmas were discovered in 1898 by Nocard and Roux in animals with contagious bovine pleuropneumonia. In 1937, Dienes and Edsall isolated the first pathogenic human mycoplasma, *M. hominis*, from a Bartholin's gland abscess [8,9]. In 1944, Eaton et al. [10] isolated another *Mycoplasma* from a patient with atypical pneumonia, named *M. pneumoniae* by Chanock et al. in 1962 [11].

Biology and Pathogeny

Mycoplasma spp. are the smallest known free-living organisms, with a genome size of (580-2200 Kbp) [12]. Lacking cell walls, they belong to a special class of bacteria, *Mollicutes*, which derives from the Latin "soft" (*mollis*) and "skin" (*cutis*). They tend to be pleomorphic and more plastic than other bacteria and can not be classified as cocci or bacilli in the manner of conventional eubacteria. They belong to the family *Mycoplasmataceae* and the order *Mycoplasmatales* [8,9,13,14].

Currently, 16 species isolated from humans are known. In addition, animal mycoplasmas have been detected occasionally in humans, particularly in immunodeficient patients. Out of the 16 species, six are known to cause diseases: *M. fermentans*, *M. hominis*, *M. genitalium*, *M. pneumoniae*, *Ureoplasma urealyticum*, and *U. parvum*. *Mycoplasma pneumoniae* is the most important and the most well-known [15,16].

The small size and volume of mycoplasmal cells prevents detection with bacterial filters. The small cellular mass also means that mycoplasmas cannot be detected by light microscopy, and they do not produce visible turbidity in liquid growth media [8,9,14-16].

The lack of reaction to Gram staining prevents direct detection in the sputum. The lack of a cell wall makes them susceptible to lysis by hypotonic solutions; but they are resistant to antibiotics that act on the cell wall (i.e. beta-

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Address for correspondence: Dr. Paulo Augusto Moreira Camargos. Universidade Federal de Minas Gerais, Faculdade de Medicina, Departamento de Pediatria. Av. Professor Alfredo Balena, 190/Sala 4061, Santa Efigênia. Zip code: 30130100. Belo Horizonte, MG, Brazil. PO box: 340. Phone: (31) 32489772. Fax: (31) 32489664. E-mail: pcamargos@medicina.ufmg.br.

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lactams). *Mycoplasma pneumoniae* differs from other mycoplasmas and especially from commensal oropharyngeal mycoplasmas, as it has slower growth, ferments glucose, absorbs erythrocytes in the growing colonies, and reduces tetrazolium when grown aerobically or anaerobically. All these characteristics have been used for a rapid diagnosis [8,15,17]. *Mycoplasma pneumoniae* has an elongated shape, with an adhesive extremity characterized by an electron-dense core with a trilaminar membrane. Usually an extracellular pathogen (it is also intra-cellular bacterium), survival depends on adherence to the respiratory epithelium, through fixation to ciliary membranes by interactive adhesin and accessory proteins. The major adhesin is a 170-kilodalton (kDa) protein, named P1, important for pathogenesis, being one of the main antigens, with specific antibody production by the host. Cytoadhesion protects mycoplasmas from mucociliary clearance [8,16-18] and affects its integrity in several ways. Hydrogen peroxide is produced; the cytopathic effect of this production leads to a loss of ciliar activity and finally to epithelium alterations. The related clinical manifestation is persistent and irritating cough, commonly associated with *M. pneumoniae* airway-tract infection. Hydrogen peroxide damages the erythrocyte membrane, and provokes *in vitro* hemolysis, alteration of the erythrocyte antigen and cold agglutinins, which agglutinate erythrocytes *in vitro* at 4°C. These cold agglutinins are found in the serum of over 50% of patients developing *M. pneumoniae* pneumonia [8,16,17].

Mycoplasma pneumoniae is also able to produce autoimmunity. The multiorgan protean manifestations of mycoplasmal infections in humans are consistent with the pathogenesis of autoimmunity. Furthermore, the ability of mycoplasmas to induce a broad range of immunoregulatory events, mediated by cytokine production and direct effects on macrophages, B and T cells, and glial cells, is evidence that mycoplasmas possess the attributes of primary mediators of pathogenesis.

For example, cytokine production and lymphocyte activation can either minimize disease through the activation of host-defense mechanisms or exacerbate disease through lesion development. It has been suggested that related superantigen-like molecules may exist in mycoplasmas of human origin triggering autoimmune and other inflammatory pathologies. Exacerbation of clinical syndromes may be correlated with a history of mycoplasmal infection, as has been observed in patients with recurrent exposure to *M. pneumoniae* [8,17].

Immunity to *M. pneumoniae* is transitory and recurrence is frequent, as has been demonstrated in culture media (isolated at different times) and more commonly by variation in serological titers [15].

Epidemiology

Although *Streptococcus pneumoniae* is the main bacterial etiological agent in all age groups, the prevalence of *M. pneumoniae* varies among reports, depending on the

population studied and the diagnostic methods used. This has been estimated as 20%-40% in outpatients and 10-20% in hospitalized children with pneumonia [19-21]. Juven et al. [20] identified the causal agent in 85% of 254 hospitalized children aged from one month to 16 years; 62% were viruses, and 53% bacteria as follows: *S. pneumoniae* (37%), *Haemophilus influenzae* (9%), *M. pneumoniae* (7%), *Moraxella catarrhalis* (4%), *Chlamydia pneumoniae* (3%) and other bacteria (2%). Conversely, *M. pneumoniae* is the most common agent in children over five years, found in up to 50% of all pneumonia cases in schoolchildren [2,5,19].

Mycoplasma pneumoniae, although less frequent, appears to be more severe in children under five years; a rate of 21% was reported by Waris et al. [22], and Korppi observed that hospitalization rate was inversely related to age: 67% in children < 4 years, 5% in 5-9 year-old children, and 9% in the 10-14 year-old group. Co-infection of *M. pneumoniae* with various viruses or bacteria reached 52%, similar to the 51.4% co-infections observed with *S. pneumoniae* [23]. In addition, *M. pneumoniae* and *S. pneumoniae* co-infection was estimated to have an incidence of 10%; it can prolong the course of the disease [24].

Mycoplasma pneumoniae is an exclusively-human pathogen and is universally distributed, being both endemic and leading to epidemics at intervals of four to seven years [25]. These epidemics are facilitated in closed institutions, such as military bases, schools, and summer camps [4]. The disease occurs throughout the year [15], but Principi et al. [21] found a higher incidence between May and July in Italy.

The incubation period varies from one to three weeks, and the infection is transmitted through aerosols from person to person when they cough. The contagion requires a close and continuous contact because of the sensitivity of *M. pneumoniae* to changes in temperature and humidity levels [4,14,16]. Children are an important *M. pneumoniae* reservoir. In Norway, Dorigo-Zetsma et al. [26] found 15% of *M. pneumoniae* in 79 household contacts and 75% of them were less than 16 years old.

Mycoplasma pneumoniae in Developing Countries

In Mexico, *M. pneumoniae* was found as a causal agent in 30% of 452 community-acquired ambulatory pneumonias [27]. In Argentina, *M. pneumoniae* was found in 15.2% of 197 children (3mo - 10y) [28]. In Brazil, the incidence has not been accurately studied. Rocha et al. [28] assessed 69 patients \geq 13y and reported 10% pneumonia with only *M. pneumoniae* isolated and 9% *M. pneumoniae* with co-infections.

Pulmonary Manifestations

Clinical manifestations of *M. pneumoniae* infections are various (Table 1). They can affect the upper or lower respiratory tracts, or both. Symptoms commonly appear gradually, during a few days, and can persist for weeks or months. Typical clinical features include an initial pharyngitis, sore throat and hoarseness. An intractable day and night cough characterizes

Table 1. Signs and symptoms associated with infection by *Mycoplasma pneumoniae* in children [16]

Manifestation	Frequency observed*
Fever	++++
Cough	++++
Rales on chest auscultation	+++
Malaise	+++
Headache	++
Sputum production	++
Sore throat/pharyngitis	++
Chills	+
Hoarseness	+
Earache	+
Coryza	+
Diarrhea	+
Nausea/vomiting	+
Chest pain	+
Lymphadenopathy	+
Skin rash	+
Conjunctivitis	+/-
Otitis media/myringitis	+/-

*This list of the frequencies of the most common clinical manifestations of *M. pneumoniae* infection is derived from multiple studies. ++++Generally present, +++Usually present, ++ Present in roughly half of the cases, + Occasionally present, +/- Rarely present.

extension of the infection to the lower airways; fever and dyspnea may occur and the cough becomes productive [16]. However, these symptoms are not specific and can be observed with *Chlamydia* sp. or certain virus infections [16]. Moreover, the lack of these symptoms does not preclude infection by *M. pneumoniae*.

Prospective studies show that pneumonia occurs in 3-10% of patients who are infected, with a generally benign course [15]. The characteristic of *M. pneumoniae* pneumonia is to mimic either a viral or bacterial pneumonia. *Mycoplasma pneumoniae* has been found associated with fever, cough, and unilateral crackles. Chest X-rays show unilateral lobar condensation and biological inflammation. These features were initially considered to mean a pneumococcus pneumonia. The difficulty to discriminate viral from *M. pneumoniae* pneumonia in clinical practice has influenced the recommendations on antibiotic administration in the management of pneumonia in childhood [1]. In this case, cough and fever are the most-frequent symptoms; they are inconstantly associated with wheezing, dyspnoea, and bilateral rattles. Chest X-rays show nonspecific bilateral infiltrates and bronchial thickness, and biological inflammation markers may be lacking [30]. In fact, radiological findings of pneumonia by *M. pneumoniae* are highly varied. Lobar (generally unilateral) condensation, bronchopneumonic shadows, reticular and reticulonodular infiltrates can be observed [31]. John et al. [30] prospectively assessed the radiological features of 42 children with *M. pneumoniae* pneumonia. They reported focal reticulonodular infiltrates in

a single lobe in 52% of cases and they found that the lower lobes were more affected than the upper lobes. Twenty percent of patients have chest radiographic abnormalities for up to four months [32]. Parapneumonic pleural effusion complicate *M. pneumoniae* infections in 4%-20% of the patients. This is often of small volume and on the side of the pulmonary infiltrate. Massive and bilateral effusion has been reported in patients with sickle-cell disease [29,32,33].

Mycoplasma pneumoniae is one of the main causes of persistent cough in patients infected with *Bordetella pertussis*. Hallander et al. [35] found that *B. pertussis* was the causal agent of a persistent cough that lasted less than 100 days in 56%, *M. pneumoniae* in co-infection with other agents in 26%, *Chlamydia pneumoniae* in 17%, and *Bordetella parapertussis* in 2% of cases. On the other hand, when the cough lasted for more than 100 days, *B. pertussis* was involved in 83% of cases, and *M. pneumoniae* was a co-factor in 14 out of the 65 episodes and responsible for three cases as the sole etiological agent. This suggests that *M. pneumoniae* is an important catalyst of recurrent-respiratory-tract infections [36]

Mycoplasma pneumoniae is not a major cause of asthma attacks in children. Prospective studies have shown that *M. pneumoniae* is involved in 2%-4% of asthma attacks with a detectable infectious agent, the most frequent agent being viruses [38]. In these studies, *M. pneumoniae* was not a risk factor for severe manifestations. However, Biscardi et al. [38] found that *M. pneumoniae* was the cause of asthma attacks in 50% of 119 hospitalized children and was a worsening factor in 20% of them. However, antibiotic treatment is not routinely recommended in an asthma crisis, even when it is severe or when it is associated with fever because the main causative agent is the viruses [8].

The clinical course of *M. pneumoniae* infection is commonly considered to be moderate, self-limiting, and with a simple outcome. Recovery is gradual, with clinical improvement preceding radiological improvement. Extrapulmonary localizations may delay complete recovery [14,15,22]. More severe clinical pictures are generally associated with chronic disease and age extremes. Infants can develop *M. pneumoniae*-induced respiratory distress, requiring mechanical ventilation [8,15].

However, pulmonary sequelae after infection by *M. pneumoniae* are rarely described. Chronic-interstitial-pulmonary fibrosis, bronchiolitis obliterans, and Swyer-James syndrome have been reported [10]. High-resolution computed tomography one to two years after hospitalization by *M. pneumoniae* pneumonia showed abnormalities in 36.8%, with mosaic perfusion and bronchiectasis [39]. Reduced lung diffusion was found in 48% of children six months to one year after infection, demonstrating the importance of interstitial disease in *M. pneumoniae* pneumonia [40].

Extrapulmonary Manifestations

Any organ can be attacked by *M. pneumoniae*, although the respiratory tract is the main site of infection. Approximately 25% of patients hospitalized by infection by *M. pneumoniae*

Table 2. Extrapulmonary manifestations in patients infected by *Mycoplasma pneumoniae* [8,15,16,41]

Manifestations	Incidence %	Observations
Extrapulmonary manifestations	25-50	Frequency of 50%, including subclinical forms
Upper respiratory tract	50	May present pharyngitis, tracheobronchitis, and a third has symptoms related to the ear, such as otitis externa, otitis media and myringitis
Hematologic	50	Including subclinical forms of hemolytic anemia
Dermatologic	25	Including maculopapular erythema and rash
Gastrointestinal	25	Including nausea, vomiting, and diarrhea
Bone, joint and muscle	14	Including myalgias and joint pain
Central nervous system	6-7*	The most severe manifestation, 10% are fatal and 25% lead to sequelae
Cardiac	1-8.5	Can lead to sequelae in 50% of cases
Ocular	Occasional	Can lead to conjunctivitis, anterior uveitis, optic neuropathy, retinitis, and retinal hemorrhages, iritis, and optic disk swelling, with or without permanent degradation of vision
Renal and urogenital	Rare	Can lead to glomerulonephritis

*In hospitalized patients.

can present extrapulmonary complications at some time during the disease course. The pathogenesis of these complications is unknown, and they can occur before, during, or after pulmonary manifestations or without any respiratory symptoms [16]. Incidence is variable (Table 2).

Manifestations in the Upper Respiratory Tract

Over 50% of patients infected by *M. pneumoniae* can present manifestations in the upper respiratory tract, such as pharyngitis, tracheobronchitis, and one third present symptoms in the ear, including otitis externa, otitis media, and myringitis [8].

Ocular Manifestations

Ocular manifestations are occasionally described in children and include conjunctivitis, anterior uveitis, optic neuropathy, retinitis, and retinal hemorrhages, iritis, and optic disk swelling, with or without permanent degradation of vision [8].

Cardiac Manifestations

Cardiac complications associated with *M. pneumoniae* are relatively uncommon; 1%-8.5% of infected subjects have some degree of cardiac attack, although there have been a few cases under the age of 16 [15]. The most frequent heart complications are: heart failure, myocarditis, pericarditis, and pericardial effusion. In rare cases, infection can lead to acute myocardial infarction, which frequently requires an intensive care center, leading to cardiac sequelae in roughly half the cases [8,15].

Neurological Manifestations

Neurological manifestations have been found in approximately 7% of hospitalized patients and are of variable severity. Respiratory symptoms can be very mild or absent in 20%-80% of cases. They can occur in the acute and late phases of respiratory infection. Pathogenesis of neurological involvement by *M. pneumoniae* is still not well established;

there is the possibility of direct invasion of the central nervous system (CNS) by the agent or autoimmune reactions [41].

Diverse clinical pictures can be found: encephalitis, aseptic meningitis, meningoencephalitis, cerebellar ataxia, polyradiculitis, transverse myelitis, Guillain-Barré Syndrome, cranial and peripheral neuropathies, optic neuritis, diplopia, mental confusion, acute psychosis, and coma.

In children, encephalitis is the most frequent form of nervous tissue involvement [8,15,41]. Recent articles suggest that *M. pneumoniae* is also involved in the pathogenesis of Tourette's syndrome. Müller et al. [42] compared 29 patients with Tourette's syndrome with 29 control patients. High titers of *M. pneumoniae* were found in 17 patients in the Tourette's syndrome group and one in the control group.

Evolution is fatal in approximately 10% of cases, and on average, 25% of the subjects present sequelae such as chronic debilitating deficits in motor or mental function, and recurrent seizures. In encephalitis, when compared to other etiologies, there is an increase of almost seven times in the risk of death or severe neurological sequelae, and this is only overcome in encephalitis caused by herpes simplex virus infection [8,41].

Hematological Manifestations

Mycoplasma pneumoniae can cause hemolytic anemia and intravascular coagulation, and subclinical forms of these conditions are found in 50% of cases. The mechanism through which *M. pneumoniae* causes these complications may involve cross-reaction with cold agglutinins. This bacterium can also cause aplastic anemia, thrombotic thrombocytopenic purpura, severe forms of disseminated intravascular coagulation, arterial thrombosis, and Reynaud's syndrome [8,15].

Gastrointestinal Manifestations

Gastrointestinal manifestations are frequent and have been described in roughly 25% of cases, manifesting as nausea, vomiting, abdominal pain, diarrhea and loss of appetite. Cholestatic hepatitis and pancreatitis, albeit rare, can occur,

and are generally associated with respiratory infections [8,15]. Several epidemiologic studies have correlated infections by *M. pneumoniae* with exacerbation of Crohn's disease and other chronic inflammatory bowel diseases [17].

Renal Manifestations

Glomerulonephritis associated with *M. pneumoniae* is rare, and few cases have been described in children. These manifestations can occur simultaneously with other symptoms, five to 10 days after onset. The most frequent lesion is membranoproliferative glomerulonephritis. Persistence of IgG and IgM antibodies for *M. pneumoniae* and low C3 have also been reported [43].

Bone, Joint and Muscular Manifestations

Nonspecific myalgias, arthralgias and polyarthropathies can occur in approximately 14% of cases, with complete recuperation during disease evolution; but they can persist for long periods [8,17]. Haier *et al.* [44] described an association between *M. pneumoniae* and rheumatoid arthritis. Fifteen among 28 rheumatoid-arthritis patients presented positive PCR for *Mycoplasma* sp. and out of these, five were *M. pneumoniae*.

Dermatological Manifestations

Dermatological disorders are common extrapulmonary manifestations, including erythematous maculopapular and vesicular rashes, occurring in 25% of patients; they are normally self-limiting [15]. *Mycoplasma pneumoniae* is the main cause of erythema in pneumonias [45]. In a study carried out by Lam *et al.* [46], the most common cause of erythema multiforme was infectious etiology, accounting for 84.2% of cases, and out of these, *M. pneumoniae* was the most frequent, accounting for 42.1%. In children, *M. pneumoniae* is also the most commonly-identified infectious cause of Stevens-Johnson syndrome. Classic rash and mucosal involvement accompany the signs and symptoms of an *M. pneumoniae* respiratory infection [47]. It can also cause urticaria, toxic epidermal necrolysis and pityriasis rosea [8,15].

Mycoplasma pneumoniae in Special Situations

Patients with Sickle-cell disease, Down syndrome, immunodeficiency, and cardiopulmonary dysfunction have a greater risk of developing joint infections associated with the pulmonary picture, and then increase the severity of the clinical presentation [8,15]. In sickle-cell disease, there have been reports of *M. pneumoniae* causing severe pneumonia in children, with multilobar infiltrates, respiratory distress, and abundant pleural effusion [6,38]. In the United States, Neumayr *et al.* [34] reported multilobar infiltrates and pleural effusion in over 50% of cases with sickle cell disease. *Mycoplasma pneumoniae* can also cause acute chest syndrome. The "National Acute Chest Syndrome Study Group" [48] identified an infectious agent in 38% of 538 patients. The most common was *Chlamydia pneumoniae* (7.2%) followed by *M. pneumoniae*, (6.6%), and viruses (6.4%).

Laboratory Diagnosis

Nonspecific Laboratory Diagnosis

Roughly a third of patients with upper respiratory tract infection by *M. pneumoniae* can have leukocytosis and/or an elevated erythrocyte sedimentation rate. Hemolytic anemia, which occurs in many patients, can be evidenced in the hemogram. There are no typical hepatic or renal abnormalities. Gram staining of the sputum can show mononuclear cells or neutrophils and normal flora [8,16].

Cold Agglutinins

Formation of cold agglutinins is the first humoral response to *M. pneumoniae*. They occur at the end of the first week and at the beginning of the second week, and disappear two to three months later. They are not a reliable indicator of *M. pneumoniae* presence, present in only 50%-60% of cases [8,15,32]. False-positive results are also frequent. Epstein-Barr virus, cytomegalovirus, *Klebsiella pneumoniae*, *Treponema pallidum*, influenza, adenovirus, and infection by *Legionella pneumophila* can lead to cross-reactions.

Cold agglutinins are also found in other diseases, including hemolytic anemia and malignancies of lymphoid cells and autoimmune diseases. Even antibiotic therapy can influence cold agglutinin levels, resulting in lower titres [49].

Nonspecific Methods

Culture

Mycoplasma pneumoniae is not part of the normal flora of the respiratory tract. Isolation in the throat, nasopharynx, and pleural fluid can be considered indicative of infection. Culture growth diagnosis is slow (1-4 weeks), has a low sensitivity, and requires special care to achieve growth. When compared to PCR, culture sensitivity was found to be approximately 61% [15,49]. Therefore, it is not recommended for routine diagnosis; however pathogen isolation has helped show that effective isolation from extrapulmonary pathogenesis provides evidence of direct invasion.

Antigen Detection

Antigen detection of *M. pneumoniae* by immunological methods is a means of identifying etiology without depending on pathogen viability, i.e. it is not influenced by previous use of antimicrobials. Several tests can be used, such as direct immunofluorescence, counterimmunoelectrophoresis, immunoblotting, and antigen capture enzyme immunoassay (EIA), the latter being the most common. EIA of nasopharyngeal aspirates is a rapid test for detecting *M. pneumoniae*. The limits of detection are of 10^3 - 10^5 colony-forming units/mL, demonstrating limited sensitivity and specificity. With the advent of more sensitive techniques, such as PCR, it began to fall into disuse [8,14].

Antibody Detection

The available serology techniques are the complement fixation assay (CFA), indirect immunofluorescence assay

(IFA), particle agglutination assay (PA), and enzyme immunoassay (ELISA). The most common tests for detecting *M. pneumoniae* in childhood are complement fixation and ELISA tests [14]. Serology is useful for diagnosing pulmonary and extrapulmonary manifestations [14, 49].

Complement Fixation Assay (CFA)

Until the last decade, the complement fixation assay (CFA) was the standard exam for diagnosing *M. pneumoniae* infection [49], but low sensitivity and specificity have been found. The antigen used is a glycolipid that is cross-reactive with a number of other pathogens, such as *Streptococcus MG* and *Staphylococcus aureus*, along with plant and human tissues [4,15,49].

Infection by *M. pneumoniae* leads to production of complement-fixation antibodies, and a four-fold increase or decrease in the levels from the first to the second sample establishes the diagnosis. When titers are high and over 1:80, especially 1:160, diagnosis can be made with a small sample [14, 15, 49].

Enzyme Immunoassay (ELISA)

ELISA was devised in the early 1980s and is nowadays the most widely-used serological method. After its discovery, several commercial kits have been produced with a great variety of antigen preparations, such as glycolipids, purified proteins (including P1 adhesin), synthetic peptides, and a mix of antigens. They allow the detection of IgG and/or IgM antibodies. EIA tests are apparently too sensitive for detecting specific antibodies. This technique is automated and requires small amounts of serum [4,8,14].

For improved diagnosis, both IgM and IgG antibody detection is recommended. IgM appears 7-10 days after the onset of infection and its levels are higher in first infections than in reinfections. IgG levels increase slowly over the course of the disease and are usually not detectable during the first week; they peak five weeks after the onset of clinical symptoms and can persist for several years after an acute infection [4,15,49]. Some children failed to attain a measurable IgG response [50]. IgA levels, though they are not dosed in most serological tests, seem to be the best indicator of recent infection, regardless of age group and type of infection [8].

Sensitivity and specificity vary according to the kits. Those using P1 adhesin gives better sensitivity and specificity [4,14]. Suni et al. [51] reported a sensitivity of 100% and a specificity of 96.5%, using tests with enriched P1 and a sensitivity of 100% and a specificity of 79% using glycolipids. There may be cross-reactions with antibodies of *Mycoplasma genitalium*, the species most closely resembling *M. pneumoniae* [49].

Indirect Immunofluorescence (IFA)

IFA is relatively easy to perform and gives quantitative results. The main limitations are subjectivity of the

interpretation of results and cross-reaction with rheumatoid factor. It is more sensitive and specific in adults [14].

Particle Agglutination Assay (PA)

The PA uses latex and jelly as support for a mix of specific antigens of *M. pneumoniae*. It simultaneously detects IgG and IgM and allows quantification. In a comparative study with PCR techniques, Templeton et al. [52] observed titers equal to or higher than 1/320 in the agglutination test, with a sensitivity of 50% in the initial phase and 66% in the convalescence phase.

Molecular Biology Techniques

DNA Probes

DNA probes can be used for detecting *M. pneumoniae*, the target being 16S rRNA genes. Disadvantages include the relatively short lifespan of six weeks, a need for specific equipment, high cost, and the need to purchase and eliminate radioisotopes. They have low sensitivity and specificity, and have been replaced by other methods [14].

Polymerase Chain Reaction (PCR)

PCR has opened the possibility to improve the diagnosis of *M. pneumoniae* infections. It seems to be the best method of detection. Advantages include promptness (hours), early diagnosis (does not depend on serological conversion or agent growth), and detection (small quantities) in conventionally anomalous sites (blood and airway secretions, liquor). Sensitivity is theoretically very high; it is able to identify a single organism when purified DNA is used and it does not require viable organisms. However, there is a risk of detecting carriers of *M. pneumoniae* [14,16,17].

Contamination is the greatest problem for diagnosis by PCR and is responsible for a great number of wrong diagnoses. Some procedures can reduce this rate of error to less than 0.5% [49]. According to Dorigo-Zetsma et al. [53], PCR when compared to serology (immunofluorescence) has a similar sensitivity of 78%, but the specificity of 92% in IFA (indirect immunofluorescence) is inferior to the 100% obtained with PCR. According to Waris et al. [22], amplification-based techniques can increase sensitivity up to 95%.

Table 3 presents several diagnostic tests for *M. pneumoniae* with their sensitivities and specificities.

Treatment

Patients, family and other subjects at risk of being infected should be warned about contagion. Use of prophylactic antibiotics is controversial. There are studies recommending it only under special circumstances, such as for high-risk groups, and only substances that have good efficacy should be used. Klausner et al. [54] described the use of azithromycin as a prophylactic, with good efficiency for preventing secondary cases of pneumonia by *M. pneumoniae* in institutional epidemics. Vaccines with live, attenuated agents are being developed, but their use in children is still not recommended [8].

Table 3. Main exams used in diagnosing *Mycoplasma pneumoniae* with respective sensitivity, specificity, result time, and cost [8,9,15,16,49,52,53]

Diagnostic test	Material	Sensit. %	Spec. %	Result time	Cost
Cold agglutinins	Serum	30-50	50	Variable	Low
Culture	NPA/PS	61	100	2-6 weeks	Low
Serology					
Complement-fixation assay	Serum (blood)	71-90	88-92	1-2 weeks	Low
IFA (Indirect immunofluorescence)	Serum (blood)	78	92	1-2 weeks	Moderate
Passive agglutination	Serum (blood)	50-66	100	1-2 weeks	Low
EIA (ELISA)	Serum (blood)	83-100*	79-100*	Hours, up to two weeks	Moderate
Cell biology technique					
DNA probe	NPA-PS	89-100	89-98	Hours, up to two weeks	Moderate/high
PCR	NPA-PS	78-100**	92-100	Hours, up to a week	Moderate/high

Sensit.=sensitivity; Spec.=specificity; NPA=nasopharyngeal aspirate; PS=pharyngeal swab. *Sensitivity of 100% and specificity of 96.5% when using enriched P1 tests. **Hybridization techniques can increase sensitivity up to 95%.

Mycoplasma pneumoniae, has no cell wall and therefore is naturally resistant to penicillin, cephalosporins, all beta-lactams and to vancomycin, sulfonamides, trimethoprim, and rifampin. *Mycoplasma pneumoniae* is susceptible to macrolides, cyclines, and quinolones. Clindamycin should not be considered a first-line treatment, as it may be effective *in vitro*, but this has not been confirmed *in vivo*. Quinolones are used extensively, and the new fluoroquinolones, such as levofloxacin, moxifloxacin, gatifloxacin, and sparfloxacin, have been reported to have better *in vitro* efficiency than older drugs, such as ciprofloxacin and ofloxacin. Quinolones are still not recommended in children, despite the safety of ciprofloxacin reported in the pediatric population [8]. Tetracycline can only be used in children older than seven years because of their effect on teeth. Therefore macrolides are the first-line treatment in childhood [8,16].

In the pediatric-age group, erythromycin was the most-frequently used drug for treatment, but nowadays new macrolides are being employed because of greater posological ease, lower occurrence of adverse gastrointestinal effects, lower potential of drug interaction, and greater intracellular concentration. Up to now, no consensus on the duration of therapy with macrolides has been reached, and treatment schemes spanning from one to three weeks have been described. The most widely used are: azithromycin, 10 mg/kg/day, a daily dose not exceeding 500 mg/dose for five days and clarithromycin, 15 mg/kg/day, divided into two doses, not exceeding 500/mg/dose for 10-15 days [8,16].

The use of steroids is questionable when the patient wheezes.

Conclusions

Infection by *M. pneumoniae* is still an underrated disease, despite its high incidence and clinical importance. Broader

knowledge of this pathogen would considerably reduce morbidity due to pneumonias and chronic pathologies with *M. pneumoniae* as the etiological agent.

Infection can occur in any age group, being less frequent and more severe under the age of five. Associated with other bacteria, it prolongs the course of several diseases, including pneumonias. Severity is highly variable and can lead to major sequelae. In Brazil, epidemiological studies are needed in order to determine the incidence of *M. pneumoniae*.

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