

# New Concepts of *Mycoplasma pneumoniae* Infections in Children

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## INTRODUCTION

The year 2002 marked the fortieth anniversary of the first published report describing the isolation and characterization of *Mycoplasma pneumoniae* as the etiologic agent of primary atypical pneumonia by Chanock et al.<sup>1</sup> Lack of understanding regarding the basic biology of mycoplasmas and the inability to readily detect them in persons with respiratory disease has led to many misunderstandings about their role as human pathogens. Formerly, infections by *Mycoplasma pneumoniae* (MP) were considered to occur mainly in children, adolescents, and young adults, and to be infrequent, confined to the respiratory tract, and largely self-limiting. Outcome data from children and adults with community-acquired pneumonias (CAP) proven to be due to MP provided evidence that it is time to change these misconceived notions. Development of powerful molecular-based tests such as the polymerase chain reaction (PCR) assay, coupled with traditional diagnostic approaches using serology and culture, have shed new light on the characteristics of MP in respiratory disease of children and adults. This review is intended to provide a concise summary of the basic biology of MP, how it produces disease, its epidemiology, clinical manifestations, diagnosis, and treatment, with emphasis on pediatric infections.

## BIOLOGY AND PATHOGENESIS OF MYCOPLASMA PNEUMONIAE INFECTION

Currently, there are 16 known *Mycoplasma* species isolated from humans, excluding occasional animal mycoplasmas that have been detected from time to time, usually in immunosuppressed hosts (Table 1), but are generally considered transient colonizers. Among these, MP is the organism best known as a human pathogen. However, oral commensal mycoplasmas that have only rarely been associated with disease may occasionally spread to the lower respiratory tract and can cause diagnostic confusion.

Mycoplasmas are smaller than conventional bacteria, both in cellular dimensions as well as genome size, making them the smallest free-living, self-replicating organisms known. Cells of MP are 1–2  $\mu\text{m}$  in length and 0.1–0.2  $\mu\text{m}$  in width. The organisms are contained by a

trilayered cell membrane and do not possess a cell wall. The permanent lack of a cell-wall barrier makes the mycoplasmas unique among prokaryotes, renders them insensitive to the activity of beta-lactam antimicrobials, prevents them from staining by Gram stain, makes them very susceptible to drying, and influences their pleomorphic appearance. The extremely small genome and limited biosynthetic capabilities explain their parasitic or saprophytic existence and fastidious growth requirements.

Attachment of MP to host cells in the respiratory tract following inhalation of infectious organisms is a prerequisite for colonization and infection.<sup>2</sup> Cytadherence, mediated by the P1 adhesin protein and other accessory proteins, protects the mycoplasma from removal by the mucociliary clearance mechanism. Cytadherence is followed by induction of ciliostasis, exfoliation of the infected cells, chronic inflammation, and cytotoxicity mediated by hydrogen peroxide and other reactive molecules, leading to oxidative stress.<sup>2</sup> Talkington et al.<sup>3</sup> and Balish and Krause<sup>4</sup> discussed current concepts regarding the pathogenesis of MP infection at greater length in recent reviews.

Following opsonization of MP by complement or antibody, macrophages become activated and release cytokines, and a mononuclear cell inflammatory response develops. CD4+ T cells, B cells, and plasma cells infiltrate the lung, followed by proliferation of lymphocytes, production of antibody, and further release of cytokines such as TNF- $\alpha$ , IL-1, IL-5, and IL-6.<sup>5</sup> Cytokine production and lymphocyte activation may either minimize disease through the enhancement of host defense mechanisms or

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TABLE 1—Mycoplasmas Isolated From Humans<sup>1</sup>

Organism	Primary body site of origin		Role in disease <sup>2</sup>
	Respiratory tract	Genitourinary tract	
<i>Acholeplasma laidlawii</i>	+	—	No
<i>Mycoplasma buccale</i>	+	—	No
<i>Mycoplasma faucium</i>	+	—	No
<i>Mycoplasma fermentans</i>	+	—	Yes
<i>Mycoplasma hominis</i>	—	+	Yes
<i>Mycoplasma genitalium</i>	—	+	Yes
<i>Mycoplasma lipophilum</i>	+	—	No
<i>Mycoplasma orale</i>	+	—	No
<i>Mycoplasma pirum</i>	?	?	No
<i>Mycoplasma penetrans</i>	—	+	?
<i>Mycoplasma primatum</i>	+	+	No
<i>M. salivarium</i>	+	—	No
<i>M. pneumoniae</i>	+	—	Yes
<i>M. spermatophilum</i>	—	+	No
<i>U. urealyticum</i>	—	+	Yes
<i>U. parvum</i>	—	+	Yes

<sup>1</sup>Listing excludes occasional isolates and those mycoplasma species known to be primarily of animal origin that have been recovered from humans in isolated instances, usually in association with immunocompromise.

<sup>2</sup>In immunocompetent persons.

exacerbate disease through immunologic lesion development.<sup>6</sup> Examination of histopathologic specimens from fatal cases that occurred primarily in adults and from animal models showed edema of the airway walls, and peribronchial mononuclear infiltrates with luminal exudates consisting of mononuclear, polymorphonuclear, and sloughed epithelial cells.<sup>3</sup> Pleural effusions and diffuse alveolar damage with long-term sequelae such as scarring, bronchiectasis, and pulmonary fibrosis sometimes occur in association with more severe cases of MP pneumonia.<sup>5,7–10</sup>

Autoimmune reactions with MP infections occur as a result of amino-acid sequence homology of mycoplasmal adhesins and a variety of human tissues, the I antigen on erythrocytes, and human CD4 and class II major histocompatibility complex lymphocyte antigens, and through development of immune complexes. Mycoplasmas may also serve as B-cell and T-cell mitogens and induce autoimmune disease through the activation of antiself T cells or polyclonal B cells.<sup>7</sup> Although autoimmunity plays an important role in the pathogenesis of extrapulmonary manifestations of MP disease, dissemination and direct invasion were proved by detection of the organism by culture and/or the PCR assay in a wide array of body sites, including the bloodstream, cerebrospinal fluid, brain tissue, pericardial fluid, and synovial fluid.<sup>3,11–16</sup>

Intracellular localization is now appreciated for MP and is thought to be mediated by fusion of organisms with host cells through their cholesterol-containing unit membranes.<sup>16</sup> Although the degree to which MP exists

and replicates within alveolar macrophages during naturally occurring infections is not known with certainty, intracellular localization may be responsible for protecting the organism from antibodies and antibiotics, as well as contributing to disease chronicity and difficulty in cultivation. High-frequency phase and antigenic variation of surface adhesin proteins may also be a factor in the ability of the organism to produce prolonged infection and a carrier state in otherwise healthy persons.<sup>3</sup>

## EPIDEMIOLOGY OF *M. PNEUMONIAE* INFECTIONS

MP causes up to 40% or more of CAP in children and as many as 18% of cases requiring hospitalization.<sup>3,17–29</sup> The incidence of MP pneumonia is greatest among school-age children and declines after adolescence.<sup>20,21</sup> However, MP may occur endemically and occasionally epidemically in older persons, as well as in children under 5 years of age.<sup>21–29</sup> Detection of the organism in 23% of CAPs in children 3–4 years of age in a study in the United States performed in the mid-1990s,<sup>21</sup> and documentation of its frequent occurrence in children under 4 years of age in a study performed in France<sup>28</sup> that was unable to show a difference in infection rates between very young children vs. children in other age groups and adults, may reflect the greater number of young children who attend daycare centers on a regular basis than in previous years, and the ease with which young children share respiratory secretions. Children may also represent an asymptomatic reservoir of infection for outbreaks in families.<sup>29</sup> Although MP is generally not considered a neonatal pathogen, Ursi et al.<sup>30</sup> described probable transplacental transmission of MP, documented by PCR, in the nasopharyngeal aspirate in a neonate with congenital pneumonia.

Whereas pneumonia may be the most severe aspect of MP infection, the most typical syndrome in children is tracheobronchitis, often accompanied by upper respiratory tract manifestations. The organism may persist in the respiratory tract for several months after initial infection, possibly because the organism attaches strongly to and invades epithelial cells, and a prolonged asymptomatic carrier state may occur in some children.<sup>27,29</sup> MP infection is ordinarily mild and as many cases may be asymptomatic, particularly in adults who experienced infections with MP previously. Reinfection may occur throughout life, since protective immunity does not typically follow initial infection. Foy et al.<sup>31</sup> reported that subsequent infections were more common following initial mild infections as opposed to infections in which pneumonia developed. Deaths due to MP infection, usually in otherwise healthy adults and children, have been reported.<sup>9,32–34</sup>

Historically, endemic MP disease transmission has been punctuated with cyclic epidemics every 4–5 years, with infections commonly spreading gradually among family

members.<sup>3,17,26–28,35</sup> In view of the intimate contact needed for droplet transmission and the slow generation time of MP, a 2–3-week incubation for each case is typical, and several cycles may be necessary before intrafamily transmission is complete. Foy et al.<sup>36</sup> reported that 39% of family contacts may eventually become infected with MP, many asymptotically.

Epidemics of MP infections can occur in the community or in closed or semiclosed settings such as military bases, hospitals, religious communities, schools, and facilities for the mentally or developmentally disabled.<sup>3,37</sup> Unlike endemic disease which may not demonstrate marked seasonal occurrence, outbreaks in countries with temperate climates tend to occur in the summer or early fall, when the occurrence of other respiratory pathogens is generally lower.<sup>3,25,37</sup>

**CLINICAL MANIFESTATIONS OF *M. PNEUMONIAE* INFECTIONS IN CHILDREN**

MP infections may involve the upper or the lower respiratory tract, or both. Symptomatic disease typically develops gradually over a period of several days, often persisting for weeks to months. The most common manifestations (Table 2) include sore throat, hoarseness, fever, a cough which is initially nonproductive but later may yield small to moderate amounts of nonbloody sputum, headache, chills, coryza, myalgias, headache, and general malaise.<sup>3,7,17,38,39</sup> Dyspnea may be evident in more severe cases, and the cough may take on a pertussis-

like character.<sup>7</sup> The throat may be inflamed with or without cervical adenopathy, and myringitis sometimes occurs. Children under 5 years of age tend to manifest coryza and wheezing and progression to pneumonia is relatively uncommon, whereas older children aged 5–15 years are more likely to develop bronchopneumonia, involving one or more lobes, sometimes requiring hospitalization.<sup>17,38,39</sup> MP may be responsible for approximately 5% of cases of bronchiolitis in young children.<sup>40–43</sup>

Chest auscultation may show rales, scattered or localized rhonchi, and expiratory wheezes. Since the alveoli are usually spared, rales and frank consolidation may not occur unless atelectasis is widespread. In uncomplicated cases, the acute febrile period lasts about a week, while the cough and lassitude may persist for 2 weeks or even longer. Duration of symptoms and signs will generally shorten if antimicrobial treatment is initiated early in the course of illness.<sup>7</sup>

It is important for clinicians to understand that the clinical presentation of MP respiratory disease is often similar to what is also seen with other atypical pathogens, particularly *Chlamydia (Chlamydophila) pneumoniae*, various respiratory viruses, and bacteria such as *Streptococcus pneumoniae*. MP may also be present in the respiratory tract concomitantly with other pathogens.<sup>17,18,21–23,28</sup>

Children with sickle-cell disease, Down syndrome, and immunosuppression may be at risk of developing more fulminant pneumonia due to MP.<sup>3,17,38,44</sup> Children with hypogammaglobulinemia are known to be at greater risk for the development of joint and respiratory infections.<sup>3,45,46</sup> There are a few case reports of MP infections in pediatric AIDS patients,<sup>47,48</sup> but is not known whether the incidence or severity of pulmonary or extrapulmonary MP infections in AIDS patients is increased significantly.

Almost one fifth of patients hospitalized with MP infections may develop extrapulmonary manifestations of some sort. These complications can be seen before, during, or after respiratory manifestations, or occur in the complete absence of any respiratory symptoms, especially in children.<sup>3</sup>

Dermatological disorders, including erythematous maculopapular and vesicular rashes, are among the most common extrapulmonary manifestations of MP infection. They occur in up to 25% of patients and are usually self-limited. However, severe forms of Stevens-Johnson syndrome, conjunctivitis, ulcerative stomatitis, and bullous exanthems have been reported in children.<sup>3,49,50</sup> Clinicians should keep in mind that the presence of erythematous maculopapular rashes in MP patients can also be caused by a number of antibiotics commonly used to treat respiratory tract infections.

Neurologic complications occur in approximately 6–7% of children hospitalized with MP infection and can sometimes be severe, manifesting as encephalitis,

**TABLE 2—Clinical Manifestations Associated With *Mycoplasma pneumoniae* Infections in Children**

Manifestation	Frequency of observation <sup>1</sup>
Fever	4+
Cough	4+
Rales on chest auscultation	3+
Malaise	3+
Headache	2+
Sputum production	2+
Sore throat/pharyngitis	2+
Chills	1+
Hoarseness	1+
Earache	1+
Coryza	1+
Diarrhea	1+
Nausea +/- vomiting	1+
Chest pain	1+
Lymphadenopathy	1+
Skin rash	1+
Conjunctivitis	+/-
Otitis media/myringitis	+/-

<sup>1</sup>This is a general listing with the frequency of the most commonly encountered symptoms and signs of acute *M. pneumoniae* respiratory infection derived from analysis of multiple studies. 4+, almost always present; 3+, usually present; 2+, present in about half of all of cases; 1+, occasionally present; +/-, rarely present.

aseptic meningitis, Guillain-Barré paralysis, polyradiculitis, cerebellar syndrome, transverse myelitis, optic neuritis, diplopia, mental confusion, and acute psychosis.<sup>3,10,11,17,34,51-54</sup> Although neurologic problems usually resolve completely, chronic debilitating deficits in motor or mental function were reported.<sup>51</sup> Both autoimmune reactions and/or direct invasion may be involved.<sup>3,10,11,34,51-54</sup> Most patients with neurologic complications secondary to MP infection experience them within 1–2 weeks of manifesting respiratory illness. However, 20% or more people, particularly children, may have no prior evidence of respiratory infection.<sup>55,56</sup> There is evidence that both direct invasion and autoimmunity may be responsible for the pathogenesis of neurological complications of MP infections.<sup>3,10,11,32,34,51,52,54-59</sup>

Renal complications of MP infection such as acute nephritis, IgA nephropathy, and others sometimes occur in children.<sup>3,17</sup> Said et al.<sup>60</sup> failed to identify MP from renal tissue using PCR in four children with acute nephritis concomitant with serologic evidence of recent MP respiratory infection. Further efforts to identify mycoplasmas in kidney tissue using PCR assays are needed to clarify whether direct or indirect damage to tissue is occurring.

Hemolytic anemia complicates MP infections in children more often than in older persons, and was attributed to cold agglutinin disease with autoimmune hemolysis.<sup>3,61</sup> A recent report suggests that thrombotic thrombocytopenic purpura (TTP) sometimes seen in patients with MP infection may be the result of cross-reactive antibodies inactivating plasma von Willebrand factor-cleaving protease.<sup>62</sup> Two pediatric cases of aplastic anemia associated with MP<sup>63</sup> and a case of fatal disseminated intravascular coagulation<sup>33</sup> were reported. If subclinical forms of hemolytic anemia and intravascular coagulation are considered, over 50% of patients with MP infections may be affected.<sup>3</sup>

Up to one-third of patients with MP infection may have nonspecific ear symptoms, in addition to otitis externa and interna.<sup>39</sup> Cardiac involvement can be manifested as myocarditis or pericarditis.<sup>14,64</sup> Although rare, serious cardiac sequelae may occur.<sup>64</sup> Rarely, hepatitis and pancreatitis have been associated with respiratory infections.<sup>65</sup> Acute rhabdomyolysis was recently reported in association with MP infection in a 15-year-old.<sup>66</sup> Ocular manifestations have been reported in children occasionally, and include conjunctivitis, anterior uveitis, optic neuropathy, retinitis and retinal hemorrhages, iritis, and optic disc swelling, with or without permanent degradation of vision.<sup>53,67</sup> Other nonspecific complications include myalgias, arthralgias, nausea, vomiting, and diarrhea.<sup>3</sup> Given the apparent ability of the organism to invade the bloodstream, infections in almost any organ system would seem to be possible.

One of the most intriguing aspects of MP infection that has garnered considerable attention over the past few years

is the potential for this organism to be an initiator or exacerbator of asthma in children and adults. However, the concept that MP infection may be a cofactor in the pathogenesis of asthma was first considered over 30 years ago<sup>68</sup> and is logical, given the historical association of various respiratory viruses and chlamydiae. One reason that interest in MP and asthma has renewed has resulted from more sensitive means for its detection (such as PCR) that are now more readily available.

Talkington et al.<sup>3</sup> cited multiple lines of evidence that MP may play a role in the pathogenesis of asthma beyond simple, acute exacerbation. First, MP can be detected by PCR and/or culture more often from the airways of patients with chronic, stable asthma than from matched control patients;<sup>69-72</sup> it can be associated with significantly greater numbers of mast cells;<sup>72</sup> and treatment of adult asthma patients in whom MP was detected with macrolide antimicrobials resulted in improvement in pulmonary function tests in comparison with asthma patients who did not have evidence of MP in airways.<sup>71</sup> This response might be due to the antibacterial as well as the anti-inflammatory effects of these drugs. Mycoplasmas were also detected by PCR in airways of adult asthmatics, even when cultures and serological results were negative, suggesting that low numbers of MP may evade detection by the immune system.<sup>70,71</sup> Throat cultures were positive for MP in 24.7% of children and adults with asthma exacerbations, as compared to 5.7% of healthy controls.<sup>69</sup> However, other studies of children with acute asthma exacerbation showed a minor contribution of MP when compared to other microorganisms such as respiratory syncytial virus and rhinoviruses.<sup>73-75</sup> However, the limitations of some of these studies were the use of complement fixation (CF) tests alone for diagnosis of acute MP infection and failure to exclude very young children in whom viral bronchiolitis rather than asthma may have been the primary illness.

A second line of evidence is that abnormalities in pulmonary function tests, including reduced pulmonary clearance and airway hyperresponsiveness as well as abnormal chest radiographs persisting for months to years after an episode of MP respiratory tract infection, have been reported in children.<sup>76-79</sup> This establishes the ability of mycoplasmas to induce chronic to permanent lung damage long after resolution of respiratory tract symptoms.

The third line of evidence is that MP is known to induce a number of inflammatory mediators implicated in the pathogenesis of asthma that may play a role in exacerbations that often include wheezing.<sup>80-86</sup> IL-5, an inflammatory mediator known to be associated with the development of airway hyperresponsiveness in association with viral infection, was significantly increased in children with MP infection and wheezing when compared to children with MP who were asymptomatic, and to those

without wheezing.<sup>84</sup> Elevated serum IgE as well as production of IgE specific to MP or common allergens may also occur during MP infection in children with the onset of asthma.<sup>85</sup> Recent data from Koh et al.,<sup>86</sup> based on the measurement of cytokines in bronchoalveolar lavage fluid from children with MP pneumonia, showed that levels of IL-4 and the ratio of IL-4/IFN- $\lambda$  were significantly higher in children with MP than in children with pneumococcal pneumonia or uninfected controls, suggesting that a TH2-like cytokine response in MP pneumonia represents a favorable condition for IgE production. Recent data from animal models also support the idea that MP in the respiratory tract stimulates production of a wide array of inflammatory mediators, and that the organism can induce mast-cell activation with a release of mediators including serotonin and  $\beta$ -hexosaminidase.<sup>87,88</sup> It is clear that additional research needs to be performed in order to understand the potential role for MP as well as other bacteria such as *C. pneumoniae* in the pathogenesis of asthma.

In addition to asthma, there was also recent interest in the role of MP as a contributor to morbidity in cystic fibrosis, a condition that is becoming more common in teenagers and young adults due to improved survival of children with this disease. Peterson et al.<sup>89</sup> diagnosed MP infection by CF in only 2 of 332 episodes of acute exacerbations in patients with cystic fibrosis. Subsequently, Efthimiou et al.<sup>90</sup> noted a fourfold rise in CF titers against MP, *Coxiella burnetii*, and various viruses in a small number of young adults with cystic fibrosis who experienced deterioration of lung function and an increase in lower respiratory tract symptoms. Ong et al.<sup>91</sup> and Pribble et al.<sup>92</sup> also detected antibodies against MP in 1 of 19 and 4 of 80 acute pulmonary exacerbations, respectively. Emre et al.<sup>93</sup> were unable to demonstrate the presence of MP by PCR of oropharyngeal secretions in 16 patients, and only one of them showed serological evidence of recent infection. However, *C. pneumoniae* was detected by culture in 4 of 32 cases, and 3 of 4 cases had serological data suggestive of acute infection. Taken together, these studies suggest that MP may be of minor importance as a factor in acute exacerbation in patients with cystic fibrosis, but more work should be done in this area using PCR-based technology joined with culture and utilizing serological assays that are more sensitive and specific than CF to characterize the contribution, if any, of MP to acute exacerbations of cystic fibrosis. The potential for MP to play a role as a cofactor in exacerbating chronic obstructive pulmonary disease in adults is again being evaluated critically.<sup>94,95</sup>

## RADIOGRAPHIC AND LABORATORY DIAGNOSES

Radiographic findings in MP pneumonia can be extremely variable and mimic a wide variety of lung diseases.

The inflammatory response elicited by MP causes interstitial mononuclear inflammation in lungs that may be manifested radiographically as bronchopneumonia of the perihilar regions or lower lobes, usually with a unilateral distribution, and hilar adenopathy. However, lobar consolidation and bilateral involvement were described,<sup>17</sup> and the degree of consolidation may exceed what would be expected based on the severity of clinical manifestations. Pleural effusions and diffuse alveolar damage sometimes occur in association with more severe cases, and long-term sequelae may evolve. Kim et al.<sup>76</sup> reported radiological abnormalities in 37% of children, and Marc et al.<sup>77</sup> described abnormal pulmonary function in 50% of children tested several months after an episode of MP pneumonia.

Clinical laboratory findings are seldom diagnostic for MP infection. About one-third of persons with lower respiratory tract infections due to MP may have leukocytosis and/or an elevated erythrocyte sedimentation rate. Sputum Gram stains may show mononuclear cells or neutrophils and normal flora. There are no hepatic or renal abnormalities typical of MP infection, although some patients may develop hemolytic anemia, and this may be reflected in the hemogram. Cold agglutinins are auto-antibodies that are now believed to be the result of antigenic alteration of erythrocytes caused by MP. They may develop in approximately 50% of MP infections, appearing by week 2 and disappearing after about 6–8 weeks.<sup>7</sup> Since cold agglutinins may also be associated with a variety of other conditions, including common viral infections as well as noninfectious conditions, this finding is unreliable for diagnosis of MP infection.

The lack of rapid, accurate diagnostic laboratory tests to detect MP directly or the serologic response it elicits has hampered understanding of the epidemiology as well as contributed to unawareness of the potential clinical significance of this common pathogen by many physicians. Most of the diagnostic methods that are currently in use for detection of MP infections are perhaps better suited for use in epidemiological studies as opposed to direct management of individual patients due to their turnaround time, limited availability, and cost. Among currently available tests, each has limitations.

Culture of MP from the respiratory tract and other body sites is insensitive, laborious, and expensive, requiring serial blind passages, specialized, expensive growth media, and incubation periods of up to several weeks.<sup>96–98</sup> Persistence of the organism for variable lengths of time following acute infection also makes it difficult in some cases to assess the significance of a positive culture or assay without additional confirmatory tests such as seroconversion. A lack of reliable, commercially prepared media in the past effectively prevented many clinical laboratories from offering MP detection by culture, but some companies now distribute commercially prepared

broths and agars that can be used to cultivate MP *in vitro*. However, these media have not been rigorously evaluated in clinical trials to determine their ability to detect MP in direct comparison with nonproprietary methods. If culture is attempted, isolation of MP from nasopharyngeal or throat swabs or other respiratory tract specimens should be considered clinically significant in most instances, but should be correlated with the presence of clinical respiratory disease, due to the possibility of asymptomatic carriage. Due to the organism's sensitivity to adverse environmental conditions, proper specimen collection, storage, and transport are critical for maintaining viability for culture processing. Currently recommended methods for specimen collection, transport, selection of growth medium, inoculation, incubation, and organism identification for patients suspected of having MP infections have been described in reference texts.<sup>96–98</sup> When positive, culture has the advantage of being 100% specific, providing that appropriate procedures are used to identify the organism isolated to species level.

The development of molecular-based testing such as the PCR assay has lessened the importance of culture as a means for detecting MP. When culture was compared to PCR in 114 children with acute respiratory infections, the analytical sensitivity of culture was only 61.5%.<sup>24</sup> Several PCR systems for detection of MP have been described, using a variety of targets such as the P1 adhesin and conserved regions of 16S rRNA.<sup>17,97</sup> It is difficult to compare the results of one study directly with another because of the varied specimen types, DNA extraction and amplification techniques, primer selection, and reference standards used for comparison. The sensitivity of the PCR assay is theoretically very high, corresponding to a single organism when purified DNA is used. Other advantages are that PCR requires only one specimen, can be completed in 1 day, may be positive earlier in infection than serology, and does not require viable organisms.<sup>3</sup> However, comparison of the PCR technique with culture and/or serology yielded varied results, and large-scale experience with this procedure is still limited with MP. Reports of positive PCR assays in persons without evidence of infection by culture and/or serology suggest asymptomatic carriage, persistence after infection, or perhaps lack of specificity.<sup>96</sup> PCR inhibitors in nasopharyngeal aspirates also raise concerns over false-negatives.<sup>24,99</sup> Reznikov et al.<sup>99</sup> reported that PCR inhibition was much more likely to occur with nasopharyngeal aspirates than with throat swabs, and recommended the latter specimen for diagnostic purposes for MP. Dorigo-Zetsma et al.<sup>100</sup> performed a comprehensive examination in 18 adults with MP respiratory tract infection detected by PCR or serology, and showed that sputum was the specimen that was most likely to be PCR-positive (62.5% vs. 41% for the nasopharynx, 28% for throat swabs, and 44% for throat washes). Dilution of samples may sometimes overcome

inhibition of PCR, but this may also diminish sensitivity, because the nucleic acid is diluted along with any inhibitors that may be present.

There is justified concern when the PCR assay is used as the sole means of detection for surveillance purposes without culture, serology, or clinical data, because most studies using PCR have not attempted to do any type of quantitation. Since it is not known with certainty whether there is a specific threshold quantity of MP in respiratory tract tissues that can differentiate colonization vs. infection, a positive result by PCR may overestimate the clinical importance of *M. pneumoniae* as a pathogen if the population sampled has a high carriage rate and because of the propensity of this organism to cocirculate with other bacterial and viral pathogens. Further refinements to traditional PCR assays, such as real-time detection using specific probes with matched internal controls to evaluate polymerase inhibition and quantitation of MP in acute and subclinical infections, will be very important.<sup>3</sup> Until PCR assays can be standardized, made available at a reasonable cost, and sold commercially as complete diagnostic kits, this method of diagnosis is unlikely to gain widespread use in clinical laboratories for detection of MP infection. There is considerable interest the further development of multiplex PCR tests to detect other atypical pathogens such as *C. pneumoniae* simultaneously with MP.<sup>17,97</sup>

In view of the shortcomings of culture and the very limited availability and expense of PCR, reliable serology remains critical for accurate microbiologic diagnosis of MP respiratory disease. A variety of commercial enzyme immunoassays (EIA), particle agglutination assays (PA), and immunofluorescence assays (IFA) are sold in many countries, and these tests have largely replaced the older CF tests because of their ease of use, improved sensitivities and specificities, and the ability of some assays to detect class-specific antibodies separately. Serology is more sensitive for detecting acute infection than culture, and can be comparable in sensitivity to PCR, providing a sufficient time has elapsed since infection for antibody to develop and the patient has a functional immune system.<sup>96–98</sup>

A 4-fold rise in antibody titer in acute and convalescent sera collected at least 2–4 weeks apart and assayed simultaneously has been considered necessary for the diagnosis of current or recent infection in adults because of a relatively high background of MP-specific IgG in many healthy persons, presumably as a result of prior MP infections. In children, adolescents, and young adults, a single positive IgM assay, as defined under the specific test conditions employed, may be considered diagnostic in most cases, as IgM typically rises within 7–10 days of infection and appears approximately 2 weeks before IgG.<sup>17,97</sup> Reliance on a single measurement of IgM elevation alone to diagnose acute infection in adults is problematic, because many persons who had prior MP

infections may not produce a measurable IgM response.<sup>101</sup> IgM, when it is produced, may persist for variable periods after acute infection,<sup>102</sup> or may not have sufficient time to become elevated when sera are collected very early in an infection. Some interest recently emerged in measurement of IgA for detection of recent infections,<sup>17,97</sup> but commercial assays for detection of this antibody are not readily available.

A number of commercial serologic assays were evaluated using CF as a reference standard, since it is the procedure that has been available for the longest time and the one for which the most data are available. However, the interpretation of results of such comparative studies is complicated, because CF is far from being a "gold standard" for diagnosis. It suffers from low sensitivity because the glycolipid antigen mixture used is not specific for *M. pneumoniae* and may be found in other microorganisms, as well as human tissues, and even plants. CF has reduced specificity due to cross reactions with other organisms, most notably *M. genitalium*, as well as the possibility of providing false-positive results due to cross-reactive autoantibodies induced by acute inflammation from other unrelated causes.<sup>96</sup> Other studies merely compared one commercial kit with another, with no objective means to define a "true-positive." Despite the limitations of many comparative studies published to date, some of the newer serologic kits that are relatively easy to use and provide more rapid turnaround time for results have merit for use both in diagnosis of acute MP infections and in epidemiological investigation. The various test formats for serology assays each have their own strengths and weaknesses. PA assays can be very quick and simple to perform, and can be either qualitative or semiquantitative. IFAs are more subjective to interpret, and require a fluorescent microscope. EIAs, now the most widely used serologic tests for MP, have favorable sensitivities and specificities when compared with CF.<sup>17</sup>

A qualitative membrane-based EIA specific for IgM, the ImmunoCard (Meridian Diagnostics, Cincinnati, OH), was developed for rapidly detecting an acute MP infection using a single serum specimen. The ImmunoCard has the advantages of being technically much simpler and quicker (10 min) to perform than other types of assays, allowing point-of-care diagnosis. It is ideally suited for the detection of MP infection in children, and was evaluated in comparison to other types of assays with confirmed MP infection, with comparable or better overall performance than CF.<sup>103-105</sup> Another rapid EIA test (Remel Laboratories, Lenexa, KS) that qualitatively measures IgG and IgM together was also shown useful for point-of-care diagnosis of MP infections in adults.<sup>106,107</sup> The cost of materials to perform these rapid EIAs exceeds \$10 (US) for each patient tested.

Even though the most accurate diagnosis may be afforded by quantitatively testing paired sera, the convenience of

these point-of-care tests should be acknowledged along with the obvious limitations of complex, time-consuming serological tests that require dual specimens obtained at separate patient clinic visits. Such a requirement effectively precludes use of a test to guide initial pathogen-specific patient management and limits accurate diagnosis to those who comply with two clinic visits spaced at the proper time intervals. Additional information about the commercial serologic assays that are available to diagnose MP infection can be found in reference texts.<sup>96-98</sup> Detection of MP infection in children using a combination of the PCR assay with measurement of IgM has been recommended by some authors, the advantage being the improved detection ability very early in infection.<sup>17,108,109</sup>

From the microbiologist's point of view, one might argue in favor of the need to routinely use the best means available to identify children with acute MP respiratory tract infections in all circumstances. However, from a practical standpoint, one must also consider the expense, limited availability, poor performance, and/or turnaround time for most diagnostic tests, and the need to provide empiric coverage of other bacteria that may produce similar clinical conditions. One must also acknowledge the fact that most mild to moderately severe MP infections in otherwise healthy patients who do not require hospitalization will usually respond in an excellent manner to appropriate antimicrobial therapy provided empirically. Thus, performance of the tests described above in a typical ambulatory care setting may be unnecessary much of the time. However, in the event of an illness in which MP is suspected that is of sufficient severity as to require hospitalization, especially if the child has any sort of immune deficiency or underlying condition that may make an unfavorable outcome more likely, attempts to detect MP infection using one or more of the tests described above appear justified.

#### ANTIMICROBIAL SUSCEPTIBILITIES AND TREATMENT OF *M. PNEUMONIAE* INFECTIONS

Fortunately, treatment alternatives suitable for other common respiratory pathogens are also effective against MP, since treatment will often be empiric without ever obtaining a microbiologic diagnosis. A summary of the in vitro activities of several antimicrobial agents against MP is shown in Table 3. MP is inhibited by tetracyclines and macrolides, so that susceptibility testing is not indicated for patient management purposes. The extremely high potency of azithromycin against MP and its long half-life probably account for its ability to cure MP infections with treatment courses as short as 3 or 5 days, despite the relatively slow growth of the organism. Other agents active at the bacterial ribosome such as streptogramins, aminoglycosides, and chloramphenicol may show in vitro inhibitory activity against MP. Clindamycin

**TABLE 3—Minimal Inhibitory Concentration Ranges ( $\mu\text{g/ml}$ ) for Various Antimicrobials Against *Mycoplasma pneumoniae*<sup>1</sup>**

Tetracycline	0.63–0.25
Doxycycline	0.016–2
Erythromycin	$\leq 0.001$ –0.016
Roxithromycin	$\leq 0.01$
Clarithromycin	$\leq 0.001$ –0.125
Azithromycin	$\leq 0.001$ –0.01
Josamycin	$\leq 0.01$ –0.02
Clindamycin	$\leq 0.008$ –2
Lincomycin	4–8
Pristinamycin	0.02–0.05
Chloramphenicol	2
Gentamicin	4
Ciprofloxacin	0.5–4
Ofloxacin	0.05–2
Levofloxacin	0.063–2
Sparfloxacin	$\leq 0.008$ –0.5
Gatifloxacin	0.016–0.25
Moxifloxacin	0.06–0.25
Gemifloxacin	0.05–0.125
Quinupristin/dalfopristin	0.008–0.06

<sup>1</sup>Data were compiled from multiple published studies in which different methodologies, and often different antimicrobial concentrations, were used.

is effective in vitro, but limited reports suggest that it may not be active in vivo and should not be considered a first-line treatment.<sup>7</sup> Due to the lack of a cell wall, mycoplasmas are innately resistant to all beta-lactams. Sulfonamides, trimethoprim, and rifampicin are also inactive. Though data are very limited, oxazolidinones (drugs that act at the 30S ribosome) appear much less active in vitro against mycoplasmas than the other agents mentioned above.<sup>110</sup> New quinolones such as levofloxacin, moxifloxacin, gatifloxacin, and sparfloxacin tend to have greater in vitro activity than older agents such as ciprofloxacin and ofloxacin, although minimal inhibitory concentrations (MICs) for all fluoroquinolones are several-fold higher than macrolides.<sup>110</sup> Newer fluoroquinolones are being used extensively for the treatment of respiratory tract infections in adults, since they can be used empirically to treat infections due to mycoplasmas, chlamydiae, legionellae, *Moraxella catarrhalis*, and *S. pneumoniae*, but they are still not recommended for use in children due to possible toxicity to developing cartilage. Likewise, tetracyclines are not approved for use in children younger than 8 years of age, leaving macrolides as the treatments of choice for MP infections.<sup>111</sup> Although ketolides such as telithromycin<sup>110</sup> show potent activity against MP in vitro, clinical data from children treated with this drug have not been reported.

Most published clinical trials were able to identify relatively small numbers of CAPs proven to be caused by MP, and relied primarily upon serologic diagnosis, though some recent studies incorporated culture and/or PCR. Investigations involving children with CAP caused by

MP that were performed in the United States<sup>21–23</sup> and Europe<sup>112,113</sup> showed that treatment in the outpatient setting with newer agents such as clarithromycin or azithromycin, given orally according to the manufacturers' recommendations, are as effective clinically as erythromycin, and very limited data suggest that MP may also be eradicated by these agents. Roxithromycin was also shown effective in treatment of MP infections.<sup>114</sup> Results of these studies were discussed in greater detail by Ferwerda et al.<sup>17</sup>

Newer macrolides are generally preferred over erythromycin due to their greater tolerability, once- or twice-daily dosing requirements, and shorter treatment duration in the case of azithromycin, though their costs are considerably greater. Current recommendations for outpatient management of MP infections in children in the United States are: azithromycin suspension 10 mg/kg day 1, then 5 mg/kg/day for a total of 5 days; clarithromycin suspension 15 mg/kg/day for 10 days; or erythromycin suspension 20–50 mg/kg/day for 10–14 days.<sup>111</sup>

Eradication of MP from persons with immunosuppression can be extremely difficult, requiring prolonged therapy, even when the organisms are susceptible to the expected agents. This difficulty highlights the facts that mycoplasmas are inhibited but not killed by most commonly used bacteriostatic antimicrobial agents in concentrations achievable in vivo, and that a functioning immune system plays an integral part in their eradication.

Relatively few data are available regarding the outcomes of antimicrobial treatment of severely ill children requiring hospitalization for MP pneumonia, treatment of immunosuppressed children with MP infection, or for preventing or reducing severity of extrapulmonary complications. Limited information from case reports suggests that high-dose steroid therapy may be effective in reversing neurologic symptoms in children with complicated MP infection,<sup>115</sup> and some clinicians recommend the use of steroids in combination with an antibiotic that can penetrate the central nervous system (CNS).<sup>58</sup> A recent review of children with severe MP infections involving the CNS showed that among 14 children who received steroids, 11 (78%) were reported to have a complete or near complete recovery, a better outcome than a report on an earlier series of patients who did not receive steroids.<sup>116</sup> Another report documents successful recoveries in children with fulminant CNS disease who received high-dose steroid therapy early in the course of their disease, leading to suggestions that steroid therapy be initiated early in the course of disease.<sup>115</sup> The value of using steroids to treat Stevens-Johnson syndrome caused by MP has not been clearly established.<sup>50</sup>

Both plasmapheresis and intravenous immunoglobulin therapy might be considered if steroid therapy is ineffective for cases of acute disseminated encephalomyelitis. A trial of intravenous immunoglobulin in a



critically ill child with encephalitis that developed in parallel to MP pneumonia was associated with neurologic improvement within 48 hr of treatment,<sup>117</sup> and a patient suffering from bilateral optic neuritis as well as acute Guillain-Barré syndrome recovered after plasmapheresis.<sup>59</sup> Schwab et al.<sup>118</sup> advocated an aggressive surgical approach when brain edema and increased intracranial pressure occur despite medical therapy. In a series of 6 severe acute encephalitis cases, including 2 probable MP cases, all patients made an almost complete recovery after hemicraniectomy to control intracranial pressure.

Naturally occurring antimicrobial resistance in MP is believed to be uncommon, since treatment failures have not been reported from microbiologically proven cases of MP infection, but organisms are seldom recovered and are almost never tested for in vitro susceptibilities. High-level macrolide-resistant strains were isolated following treatment with erythromycin, but the patients still responded to treatment with this drug,<sup>119</sup> and experimental observations on two laboratory-derived erythromycin-resistant *M. pneumoniae* mutants indicated that macrolide resistance can occur due to point mutations leading to A-to-G transitions in the peptidyl transferase loop of domain V of 23S rRNA gene at positions 2063 and 2064, reducing the affinity of these antibiotics for the ribosomes.<sup>120</sup>

## CONCLUSIONS

MP should be considered a respiratory tract pathogen in persons from all age groups and degrees of illness. Serious infections requiring hospitalization, while rare, do occur and may involve multiple organ systems, due to direct invasion and/or immunologic mechanisms. Further improvement in detection assays, focusing on serology and PCR, may eventually provide much-needed diagnostic tools that are practical for individual patient management, and that will help us better understand the epidemiology of MP infections. Effective management of MP infections in children can usually be achieved with macrolides, and treatment must usually be initiated without the benefit of a specific microbiologic diagnosis.

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