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Mycoplasma pneumoniae: A significant but underrated pathogen in paediatric community-acquired lower respiratory tract infections

Surinder Kumar

Department of Microbiology, Maulana Azad Medical College, New Delhi, India

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Lower respiratory tract infections are considered a common cause responsible for morbidity and mortality among children, and *Mycoplasma pneumoniae* is identified to be responsible for up to 40 per cent of community-acquired pneumonia in children greater than five years of age. Extrapulmonary manifestations have been reported either due to spread of infection or autoimmune mechanisms. Infection by *M. pneumoniae* has high incidence and clinical importance but is still an underrated disease. Most widely used serologic methods are enzyme immunoassays for detection of immunoglobulin M (IgM), IgG and IgA antibodies to *M. pneumoniae*, though other methods such as particle agglutination assays and immunofluorescence methods are also used. Detection of *M. pneumoniae* by nucleic acid amplification techniques provides fast, sensitive and specific results. Utilization of polymerase chain reaction (PCR) has improved the diagnosis of *M. pneumoniae* infections. Besides PCR, other alternative amplification techniques include (*i*) nucleic acid sequence-based amplification, (*ii*) Qβ replicase amplification, (*iii*) strand displacement amplification, (*iv*) transcription-mediated amplification, and (*v*) ligase chain reaction. Macrolides are used as the first-line treatment in childhood for *M. pneumoniae* infections; however, emergence of macrolide-resistant *M. pneumoniae* is a cause of concern. Development of a safe vaccine is important that gives protective immunity and would be a major step in reducing *M. pneumoniae* infections.

Key words Community-acquired pneumonia - children - lower respiratory tract infections - macrolides - *Mycoplasma pneumoniae* - polymerase chain reaction - serology

Lower respiratory tract infections (LRTIs) are considered a common cause liable for morbidity and mortality among children[1]. Many studies have addressed the problems of diagnosis of LRTIs and its management. In children over five years of age, 40 per cent community-acquired pneumonia (CAP) cases are caused by *Mycoplasma pneumoniae*[2]. Increase in CAP attributable to *M. pneumoniae* may occur many times during epidemics which occur at an interval of 4-7 yr because of waning of herd immunity and introduction of new subtypes into the population[3].

The correct identification of *M. pneumoniae* infections is vital for prescription of the appropriate therapy since, based on clinical signs and symptoms, detection of *M. pneumoniae* infection is not possible. In only a minority of cases, specific aetiologic diagnosis of *M. pneumoniae* infection is established[4]. Important

reasons for underreporting are scarcity of clinical and chest X-ray features, relative unavailability of quick and specific laboratory techniques and the difficulty in culture of this infective agent in the laboratory. In most such cases, empirical therapy is given. Treatment of infections due to this atypical pathogen with beta-lactam antibiotic is ineffective, so a specific diagnosis is important. The duration of the illness will be markedly reduced using antibiotics such as macrolides. *M. pneumoniae* should be considered in differential diagnosis of CAP and additionally it should also be considered in co-infections which are unresponsive to commonly administered beta-lactams[5,6].

History

Mycoplasmas are small prokaryotic cells without a rigid cell wall. Nocard and Roux[7] in 1898 isolated the first mycoplasma in culture which was the bovine pleuropneumonia agent because of similarities to *Mycoplasma mycoides* subsp. *mycoides*. Isolation of the first human mycoplasma was done in 1937 by Dienes and Edstall[8] from a Bartholin's gland abscess[9,10]. Another mycoplasma was isolated in 1944 by Eaton *et al*[11] from the sputum sample of a patient having primary atypical pneumonia and it was called Eaton agent. Chanok *et al*[12] proposed taxonomic designation to Eaton agent as *M. pneumoniae* in 1962[9].

Biology of *Mycoplasma pneumoniae* and pathogenesis of infection

Mycoplasmas are the smallest free-living prokaryotes known, having an extremely small genome size of 580-2200 kilobase pair. Mycoplasmas are classified in the family *Mycoplasmataceae* and order *Mycoplasmatales*[9,10,13,14]. Six of the 16 species of human mycoplasma cause diseases, the most important and the most predominant pathogen is *M. pneumoniae*[14]. Mycoplasmas cannot be detected by light microscopy due to their small size, and also, visible turbidity is not produced in liquid growth medium due to their small cellular mass[9,10,13]. The absence of a cell wall barrier in mycoplasmas is unique among the prokaryotes, and so, these organisms are not sensitive to cell wall antimicrobial agents such as beta-lactams, are not stained by Gram staining, are very susceptible to effect of drying and also influence their pleomorphic appearance[15].

M. pneumoniae adheres to the ciliated cells of the epithelium lining of the respiratory tract with an attachment organelle after inhalation. At the tip of this polarized attachment organelle, a 170 kDa protein called P1 is cluttered and adherence of *M. pneumoniae* to host cells is mediated through its several additional accessory proteins (HMW1, HMW2, HMW3, P90, P40 and P30)[15]. The lack of cell wall in *M. pneumoniae* facilitates close contact of the membrane with the host cell, facilitating the exchange of compounds which are important for its growth as well as proliferation. Similar to pertussis toxin, there is an ADP-ribosyl transferase known as the community-acquired respiratory distress syndrome toxin (CARDS toxin), which is responsible for the binding to surfactant protein A and for entering host cells by clathrin-mediated endocytosis[16].

CARDS toxin causes ciliostasis as well as nuclear fragmentation and stimulates proinflammatory cytokines production and acute cellular inflammatory reaction causing airway damage. Intracellular localization of this organism may protect it from antibodies and antibiotics; furthermore, it may help in establishing persistent infections shown in tissue culture models[17]. Lack of protective immunity may be due to important factors such as variation and rearrangement of the surface antigens which may allow repeated *M. pneumoniae* infections over time[2].

Establishment of persistent infections and development of autoimmune phenomenon in this organism are due to immunomodulation of the host immune response. Clinical manifestations due to acute infection and extrapulmonary manifestations of *M pneumoniae* are the results of immunopathologic and inflammatory effects made by the host but not due to the organism itself. Interleukin-6, tumour necrosis factor alpha (TNFα) as well as neutrophil infiltration production is stimulated by various surface lipoproteins. Macrophages get activated and undergo chemotactic migration to the site of infection after opsonization of *M. pneumoniae* by complement or antibody and then followed by infiltration of neutrophils, T-lymphocytes (CD4+), B-lymphocytes and plasma cells in the lung[2].

Autoimmune reactions with *M. pneumoniae* infections occur due to amino acid sequence similarity of mycoplasmal adhesins with several human tissues, I antigen on human red cells, CD4 lymphocyte and class II major histocompatibility complex antigens and by immune complexes development. B- and T-lymphocytes are also stimulated by *M. pneumoniae* which induce autoantibodies formation and have reaction with a range of host tissues. Autoimmunity has an important role to play in the extrapulmonary involvement of *M. pneumoniae* disease[2].

Many immunogenic *M. pneumoniae* proteins and lipids produce antibodies after infection due to a strong humoral immune response. After about one week of illness, immunoglobulin M (IgM) may be detected with a peak at 3-6 wk which gradually declines in children more than six months of age[2]. Two weeks later, IgM is followed by IgG response. Sometimes, IgM persists for weeks to months, or it may not occur at all. If the patient is immunocompromised, then the antibody production may be absent. Lack of protective immunity may be due to surface antigen variation and rearrangement which appears to be an important factor, leading to repeated *M. pneumoniae* infections over time[18].

Epidemiology of *Mycoplasma pneumoniae* infections

The main bacterial aetiological agent in all age groups is *Streptococcus pneumoniae*, but varied prevalence of *M. pneumoniae* depends on the population studied and numerous methods used for its diagnosis. *M. pneumoniae* is known as a common cause of CAP throughout the world and causes up to 40 per cent or more of cases of CAP, and 18 per cent paediatric cases require hospitalization[15]. In an earlier study[9], pneumonia due to *M. pneumoniae* infection was reported as somewhat uncommon in children under five years of age and highest incidence was shown among school children from 5 to 15 yr of age. However, *M. pneumoniae* disease may occur both endemically and epidemically in older adults and in younger children under five years of age[3,18]. Climate and geography do not seem to be of major significance. Children who may also represent an asymptomatic reservoir of infection may cause outbreaks in families. Immunity due to mycoplasma infection is short lived, and recurrent infections may develop.

Generally, *M. pneumoniae* is not known as a neonatal pathogen, but Kumar *et al*[19] reported persistent pneumonia in a three week old neonate because of *M. pneumoniae* infection. Ursi *et al*[20] showed probable transplacental transmission of *M. pneumoniae* by the use of polymerase chain reaction (PCR) assay in the nasopharyngeal aspirate in a neonate who had congenital pneumonia. There is evidence that *M. pneumoniae* may play a significant role in chronic asthma as compared to a typical explanation for common cause of acute exacerbations[2].

Various studies used nucleic acid amplification techniques (NAATs) and antigen capture assays for the CARDS toxin detection and proved the role of *M. pneumoniae* in chronic asthma. Peters *et al*[21] observed a high prevalence of *M. pneumoniae* (52%) in 64 adults with treatment-resistant asthma in a study which used PCR for detection of the gene for the CARDS toxin in mostly serological-negative population. In a paediatric group, a study by the same group included non-asthmatic controls and detected *M. pneumoniae* in acute asthma (64%), in refractory asthma (65%) and in healthy controls (56%). *M. pneumoniae* antibody was detected in lower levels in asthmatic children as compared to healthy controls[22].

The spread of infection is from infected persons by contact with droplets discharged from upper and lower respiratory tracts. Epidemics are known to occur in the community or in closed or semi-closed settings such as hospitals, military personnel, schools, religious communities and facilities for mentally or developmentally disabled. The incubation period is from one to three weeks. The mean incubation time is 20-23 days when infections occur in families and other close groups due to slow spread of the organism[2]. Older children and adolescents tend to have increased severity of symptoms. However, the European epidemic report in 2010-2011 has shown high infection rates in children less than four years of age[3].

Clinical manifestations of *Mycoplasma pneumoniae* in children

M. pneumoniae infections may involve either upper or lower respiratory tract or both of them. The most common clinical symptoms are cough (non-productive at the start and non-bloody sputum small to moderate amounts later on), fever, chills, sore throat, headache, hoarseness, myalgias and general malaise[10]. Up to one-fifth of infections are actually asymptomatic and may represent reinfection. Dyspnoea may be present in more severe cases, and cough presentation may be like a pertussis-like character[9]. Coryza and wheezing are manifested in children below five years of age; however, progression to pneumonia is not common, whereas bronchopneumonia (with one or more lobes involvement) may develop in children aged 5-15 yr which sometimes needs hospitalization[15].

The clinical entity of pneumonia which was subsequently proved to be due to *M. pneumoniae* was known several years before the actual identification and establishment of the aetiological agent. Atypical pneumonia historically was used for primary pneumonia which was not demonstrated due to an accepted pathogen such as *Pneumococcus*. Antimicrobial

therapy response was lacking and considered as 'atypical', so it was thought that it was a primary form of lung disease with uncertain aetiology, and therefore, the term 'atypical pneumonia' was used for it[8]. With the onset of pneumonic symptoms, characteristically *M. pneumoniae* is mild, non-debilitating and patients yet will continue to function relatively normally, so termed 'walking pneumonia'[9]. The terms 'primary atypical pneumonia' and 'walking pneumonia' have been used to denote mycoplasmal respiratory disease by physicians as well as by the lay public.

Clinical presentation due to *M. pneumoniae* respiratory disease is indistinguishable from other atypical pathogens notably numerous respiratory viruses, *Chlamydophila pneumoniae* and *S. pneumoniae*. *M. pneumoniae* causes cough, fever and unilateral crackles. On chest auscultation, rales, rhonchi (scattered or localized) and expiratory wheezes may be shown. The acute febrile period continues for about a week; however, cough and lassitude may continue for two weeks or more in uncomplicated cases[9]. Antimicrobial treatment if started early within the course of illness will generally shorten period of signs and symptoms of the disease. It is often difficult to differentiate *M. pneumoniae* pneumonia and viral pneumonia clinically, so it influenced the recommendations for the use of antibiotics in the management of childhood pneumonia[23].

M. pneumoniae can also be observed in the respiratory tract along with other pathogens. Human and animal models indicate that *M. pneumoniae* infection may precede and intensify resulting infections with bacteria such as *Streptococcus pyogenes* and *Neisseria meningitidis* and respiratory viruses[9]. This type of synergistic effect may be due to immunosuppression or respiratory tract flora alteration in the presence of *M. pneumoniae*. A risk of developing more fulminant pneumonia owing to *M. pneumoniae* occurs in children who presented with functional asplenia and immune system impairment which are due to immunosuppression, Down syndrome and sickle cell disease[15].

Respiratory tract is the main site of *M. pneumoniae* infection; however, any organ system may be involved. Host responses after *M. pneumoniae* infection may contribute to autoimmunity, and cardiovascular, gastrointestinal, renal and musculoskeletal complications may occur in about 25 per cent of *M. pneumoniae*-infected cases[9]. Extrapulmonary complications caused by *M. pneumoniae* infection may involve every organ system, and it may be the result of spread of infection or autoimmune mechanisms[2]. The I blood group antigen (cold agglutinins) generates an autoantibody producing a rapidly evolving haemolytic anaemia which is perhaps the most common. Neurologic complications such as Guillain-Barré syndrome and acute demyelinating encephalomyelitis are also prominent[2]. Gorthi *et al*[24] reported *M. pneumoniae* infection in 50 per cent of patients with Guillain-Barré syndrome as compared to controls (25% of household and 15% of hospital controls) in India. In some individuals, a syndrome of severe mucocutaneous involvement such as Stevens–Johnson syndrome is seen. *M. pneumoniae* can cause septic arthritis especially in persons with hypogammaglobulinaemia and also some cases of chronic arthritis in children[2].

Mycoplasma pneumoniae with community-acquired lower respiratory tract infections (LRTIs) in children in India

M. pnemoniae infection has high incidence and clinical importance, but still, it is an underrated disease. *M. pneumoniae* is well acknowledged as a pulmonary pathogen within the West, but in the developing countries, there is little information of disease prevalence because reliable and rapid diagnostic laboratory tests are not available. Kashyap *et al*[25] reported *M. pneumoniae* infection in 24 per cent children with CAP by culture, serology and PCR assay. Maheshwari *et al*[18] used the criteria of serology and PCR assay on throat swab with LRTIs documented *M. pneumoniae* infection in 23 (30.7%) of 75 children, while Kumar *et al*[4] observed *M. pneumoniae* infection in 71 (35.5%) out of 200 children with community-acquired LRTIs by employment of serology and PCR assay. Shenoy *et al*[26] reported *M. pneumoniae* infection in 24 per cent pneumonia cases in hospitalized children. Chaudhry *et al*[27] observed *M. pneumoniae* positive in six (16%) of 37 paediatric patients using any test (serology, PCR and real-time PCR) in clinical samples consisting of blood and respiratory fluids, nasopharyngeal aspirates throat swabs and bronchoalveolar lavage.

Mycoplasmas tend to cause more severe and prolonged infections in the human immunodeficiency virus (HIV)-infected cases and other immunodeficient subjects. An Indian study by Nadagir *et al*[28] reported 32.2 per cent *M. pneumoniae* infection among HIV-seropositive children with respiratory tract

infection. In these patients, early diagnosis and prompt initiation of treatment of *M. pneumoniae* infection may prevent CD4 cells depletion further and speedy progression to AIDS[28]. Seroprevalence study among HIV-positive patients with pulmonary symptoms by Shankar *et al*[29] reported 21 per cent prevalence of *M. pneumoniae* IgM antibody by ELISA, and among 34 per cent of the cases screened, non-specific diagnosis was confirmed. In another study, Shankar *et al*[30] detected mycoplasmas in 36 per cent of the AIDS patients and in only 16.6 per cent of the non-HIV control individuals with underlying pulmonary symptoms using culture on pleuropneumonia-like organisms glucose agar.

Diagnosis

There are scarce specific findings of clinical laboratory results for the diagnosis of *M. pneumoniae* infection. Physicians usually depend on their clinical suspicion and adopt empiric treatment in most infections due to *M. pneumoniae* in children, and these children are managed on an outpatient basis. Microbiologic diagnosis is needed if illness is adequate to justify hospitalization, if initial antimicrobial therapy has unsatisfactory clinical response, if there are important underlying comorbidities or immunosuppression that may lead to severe and disseminated disease and if important extrapulmonary manifestations are present[31].

Radiological diagnosis

Radiographic findings can be variable and mimic different lung diseases (a viral or bacterial pneumonia). Inflammatory response due to *M. pneumoniae* in lungs causes interstitial mononuclear inflammation, and the manifestation may be in the form of bronchopneumonia which is of the perihilar regions or of lower lobes radiographically often having unilateral distribution and hilar adenopathy. The findings of lobar consolidation and bilateral involvement are also seen. The degree of consolidation may be more than the expectation depending on the severity of clinical manifestations. Pleural effusions and diffuse alveolar damage may occur which are in association with more severe cases. In patients with sickle cell disease, massive and bilateral effusion is reported[32,33].

Non-specific laboratory diagnosis

Leucocytosis and/or a high erythrocyte sedimentation rate are found roughly in about a third of patients who have upper respiratory tract infection due to *M. pneumoniae*. Haemolytic anaemia occurs in many patients. Mononuclear cells or neutrophils as well as normal flora can be shown in Gram staining of the sputum[9,15].

Microbiological tests

Fast and correct diagnostic laboratory tests are lacking for the detection of *M. pneumoniae* directly, or serological response produced by *M. pneumoniae* creates hindrance for understanding of the epidemiology. Currently, the following tests are available with their limitations:

Antigen detection: Immunological methods are used for *M. pneumoniae* antigen detection which do not depend on infective agent viability. Several tests such as immunoblotting assay, immunofluorescence assay (IFA), counter-immunoelectrophoresis assay and antigen-capture enzyme immunoassay (EIA) can be used. The limits of detection are in the range of 10^3-10^5 colony forming units/ml with a limited sensitivity and specificity[9,13].

Culture: Culture of *M. pneumoniae* is laborious, expensive and time-consuming due to slow growth *in vitro* and colonies become visible in 2-5 weeks[34-36]. Culture sensitivity is approximately 61 per cent compared to PCR[37]. The advantage of positive culture is that it is 100 per cent specific if appropriate procedures are used for the identification of the organism isolated to species level. Culture is rarely performed and not recommended for routine diagnosis because of the prolonged turnaround time, with limited availability, requirement of specialized expertise and low sensitivity.

Serology: Serological tests are more sensitive for the detection of acute *M. pneumoniae* infection than culture. Cold agglutinins production is the first humoral response by the second week in approximately 50 per cent of *M. pneumoniae* infections which disappear after a gap of 6-8 wk. Antibiotic therapy also influences cold agglutinin levels, resulting in lower titres[37]. False-positive results are also frequent.

The development of antibody to *M. pneumoniae* infection is performed by a range of serological methods which include IFA, EIAs and particle agglutination (PA) assay. These tests are easy to use; their sensitivities and specificities are also improved and have largely replaced the older complement fixation test (CFT) which was popular in the past as the primary method for detection of *M. pneumoniae* antibodies.

The PA assays are simple to perform, quick and can provide qualitative or semi-quantitative results. Interpretation of IFAs is more subjective and a

fluorescent microscope is required, but they have favourable sensitivities and specificities in comparison to CFTs. EIAs can be performed with serum in very small volumes to test isotype-specific IgM, IgG and IgA. Rapid EIAs for IgM detection of acute infection are available where a single serum specimen is employed[2]. Detection of *M. pneumoniae* infection in children by employing a combination of the PCR assay and IgM detection has been recommended by some experts with the advantage of improved early detection of infection[31]. Evaluation of EIAs and PA assays has shown problems with sensitivity and specificity when employing PCR as a reference and only a single specimen is used for analysis.

M. pneumoniae is a mucosal pathogen, so IgA is produced at an early stage of the infection and may have rapid rise and decline than IgM or IgG. Detection of IgM or IgA and PCR in a combination may be an optimum diagnostic approach for *M. pnemoniae* infection[2] but adding considerable cost to laboratory testing.

Molecular assay

DNA probes: DNA probes may be used for *M. pneumoniae* detection with 16S rRNA genes as the target. These probes use a ^{125}I-radioactive label to generate a detection signal. These have low sensitivity and specificity; other methods have replaced them[10].

Nucleic acid amplification techniques (NAATs): NAATs are used for detecting *M. pneumoniae* infection earlier than serology because antibodies development requires many days. NAATs have the potential to give results which are rapid, sensitive and specific and which may help for early appropriate antibiotic therapy. Many PCR systems for the detection of *M. pneumoniae* have been described, employing many targets[36]. Major gene targets utilized in PCR assays for *M. pneumoniae* detection are P1 adhesin gene, 16S rRNA gene, ATPase operon gene, the *tuf* gene (codes for elongation factor 2) and the repetitive element *repMP1*[17]. Interpretative guidance may also be provided by combining serology with PCR for differentiation and colonization from active disease.

M. pneumoniae and other respiratory pathogens can be detected using multiplex PCR assays, but monoplex assays have higher sensitivity and specificity as compared to multiplex assays. Traditional PCR assays with further refinements to real-time PCR detection will be important[15]. In many studies, comparison of NAATs was done with culture or serology as a reference method which gave disparate results predictably as a result of more sensitive NAATs inherently[2].

Besides PCR, other alternative amplification techniques include (*i*) nucleic acid sequence-based amplification (NASBA), (*ii*) Qβ replicase amplification, (*iii*) strand displacement amplification, (*iv*) transcription-mediated amplification, and (*v*) ligase chain reaction. Both viable and non-viable organisms can be detected by NAATs targeting DNA, while RNA detection using reverse-transcriptase PCR (RT-PCR) or NASBA is also a helpful technique for the identification of productive *M. pneumoniae* infections. Initial studies[38] showed that NASBA and PCR performance in terms of sensitivity was comparable. A multiplex NASBA assay[38] and different techniques as multiplex RT-PCR are also described[39] as other techniques. NAATs is not recommended for children who do not have typical manifestations of mycoplasmal infection[2].

Interpretation of various tests for diagnosis of Mycoplasma pneumoniae infections

It is essential to have correct and rapid diagnosis of *M. pneumoniae* infections for the initiation of applicable antibiotic treatment. Laboratory diagnosis for *M. pneumoniae* detection is especially important since this disease cannot be diagnosed solely on clinical signs and symptoms. Culture is time-consuming because the organism grows slowly and, therefore, for routine diagnosis is not recommended. EIAs are the most widely used serologic methods for the detection of IgM, IgG and IgA antibodies to *M. pneumoniae*, although other methods such as PA assays and IF methods are also used.

NAATs can detect *M. pneumoniae* earlier than serology and generate rapid, sensitive and specific results, especially real-time PCR into routine diagnosis. Although these tests are superior in diagnosing *M. pneumoniae* infections than other tests, still serology cannot be replaced[40]. *M. pneumoniae* detection with real-time PCR with P1 gene target in respiratory sample has shown 60 per cent sensitivity and 96.7 per cent specificity when compared with serology[41]. The sensitivity of NAATs is always superior than traditional procedures and they are more and more thought of as 'new gold standard'[42]. PCR in combination with serology can be used as good screening tests which give reliable and correct diagnosis of *M. pneumoniae*.

Amplification-free and other new technologies

Microfluidics and application of nanotechnology offer the potential to an even more rapid detection of important pathogens with near-patient testing. Colloidal gold-based immune chromatographic assay

has been developed by employing a pair of monoclonal antibodies which target a region of the P1 gene[43]. Other amplification-free detection methodologies are being made available as biosensing detection strategies: a prototype of an enzyme-free electrochemical genosensor on nanostructured screen-printed gold electrodes[44]; a silver nanorod array-surface enhanced Raman Spectroscopy biosensing platform was used successfully in simulated and clinical throat swabs for the detection of *M. pneumoniae*[45].

Antimicrobial susceptibility and treatment of *Mycoplasma pneumoniae* infections

Macrolides are used for *M. pneumoniae* infections in children as the first-line treatment[9]. Quinolones are still not recommended in children, although ciprofloxacin has been reported safe in the paediatric population[14]. Before 2000, resistance was not common, but since then, emergence of macrolide-resistant *M. pneumoniae* (MRMP) has been noticed with spread into Europe and North America caused by point mutations in domain V of 23S rRNA[2]. Recent surveillance studies conducted in paediatric populations have documented high resistance rates of >90 per cent in China[46] and 87.1 per cent in Japan[47]. In Europe, macrolide resistance has been reported 3 per cent in Germany to 9.8 per cent in France[48,49] and 8.2 per cent in the United States[50]. Minocycline, doxycycline, tigecycline or fluoroquinolones can be used for successful treatment of MRMP. These drugs are not used normally in children, but there are no other realistic alternatives in the case of MRMP. In Asia, MRMP resistance rates are very high[2], so alternative to macrolides for suspected or confirmed *M. pneumoniae* infection as initial treatment must be considered by clinicians.

Vaccines

A safe vaccine development offers protecting immunity and would be a significant step for reducing the extent of *M. pneumoniae* infections. Various vaccine trials have shown disappointing results so far[16,51,52], indicating that vaccine development may be some time away. There are not any new strategies on the horizon, but the recent discovery of the CARDS TX of *M. pneumoniae* has yet to be explored by a new vaccine target[16].

Conclusions

The role of *M. pneumoniae* is underlined in children (usually less than five years of age) with community-acquired LRTIs and more significantly in those aged less than one year. Although *M. pneumoniae* infection has high incidence and clinical importance, yet it is still an underrated disease. Most mycoplasmal respiratory infections do not have a microbiological diagnosis because affordable and reasonably priced methods are not readily available for its direct detection. Therefore, detection assays need further improvement with focus on serology and PCR that may finally give some much-needed diagnostic tools. Macrolides are useful in the effective management of *M. pneumoniae* infections in children; however, the emergence of MRMP is of concern for macrolide treatment failures when managing these infections. A safe vaccine development is needed which would offer protective immunity and would be a significant step towards reducing *M. pneumoniae* infections.

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References

1. Kumar S, Saigal SR, Sethi GR. Detection of IgM and IgG antibodies to *Chlamydophila pneumoniae* in pediatric community-acquired lower respiratory tract infections. *Indian J Pathol Microbiol* 2011; *54* : 782-5.
2. Atkinson TP, Waites KB. *Mycoplasma pneumoniae* infections in childhood. *Pediatr Infect Dis J* 2014; *33* : 92-4.
3. Gadsby NJ, Reynolds AJ, McMenamin J, Gunson RN, McDonagh S, Molyneaux PJ, *et al.* Increased reports of *Mycoplasma pneumoniae* from laboratories in Scotland in 2010 and 2011-impact of the epidemic in infants. *Euro Surveill* 2012; *17*. pii: 20110.
4. Kumar S, Saigal SR, Sethi GR. Rapid diagnosis of *Mycoplasma pneumoniae* by polymerase chain reaction in community-acquired lower respiratory tract infections. *Trop Doct* 2011; *41* : 160-2.
5. Hammerschlag MR. *Mycoplasma pneumoniae* infections. *Curr Opin Infect Dis* 2001; *14* : 181-6.
6. Korppi M. Community-acquired pneumonia in children: Issues in optimizing antibacterial treatment. *Paediatr Drugs* 2003; *5* : 821-32.
7. Nocard E, Roux ER. The microbe of peripneumonie. *Ann Inst Pasteur (Paris)* 1898; *12* : 240-62.
8. Dienes L, Edstall G. Observations on the L-organisms of Klieneberger. *Proc Soc Exp Biol* 1937; *36* : 740-74.
9. Waites KB, Talkington DF. *Mycoplasma pneumoniae* and its role as a human pathogen. *Clin Microbiol Rev* 2004; *17* : 697-728.
10. Domingues D, Nogueira F, Tavira L, Exposto F. Mycoplasmas: What is the role in human infections? *Acta Med Port* 2005; *18* : 377-83.
11. Eaton MD, Meiklejohn G, van Herick W. Studies on the etiology of primary atypical pneumonia: A filterable agent

transmissible to cotton rats, hamsters, and chick embryos. *J Exp Med* 1944; *79* : 649-68.

12. Chanok RM, Dienes L, Eaton MD, Edward DGA, Freundt EA, Hayflick L, *et al. Mycoplamsa pneumoniae*: Proposed nomenclature for atypical pneumonia organisms (Eaton agent). *Science* 1963; *140* : 662.
13. Matas Andreu L, Molinos Abós S, Fernández Rivas G, González Soler V, Ausina Ruiz V. Serologic diagnosis of *Mycoplasma pneumoniae* infections. *Enferm Infecc Microbiol Clin* 2006; *24* (Suppl 1) : 19-23.
14. Waites KB, Crabb DM, Bing X, Duffy LB. *In vitro* susceptibilities to and bactericidal activities of garenoxacin (BMS-284756) and other antimicrobial agents against human mycoplasmas and ureaplasmas. *Antimicrob Agents Chemother* 2003; *47* : 161-5.
15. Waites KB. New concepts of *Mycoplasma pneumoniae* infections in children. *Pediatr Pulmonol* 2003; *36* : 267-78.
16. Kannan TR, Baseman JB. ADP-ribosylating and vacuolating cytotoxin of *Mycoplasma pneumoniae* represents unique virulence determinant among bacterial pathogens. *Proc Natl Acad Sci U S A* 2006; *103* : 6724-9.
17. Waites KB, Balish MF, Atkinson TP. New insights into the pathogenesis and detection of *Mycoplasma pneumoniae* infections. *Future Microbiol* 2008; *3* : 635-48.
18. Maheshwari M, Kumar S, Sethi GR, Bhalla P. Detection of *Mycoplasma pneumoniae* in children with lower respiratory tract infections. *Trop Doct* 2011; *41* : 40-2.
19. Kumar S, Maria A, Saigal SR, Maheshwari M. *Mycoplasma pneumoniae* as a cause of non-resolving pneumonia in a neonate. *J Med Microbiol* 2010; *59* : 731-2.
20. Ursi D, Ursi JP, Ieven M, Docx M, Van Reempts P, Pattyn SR, *et al.* Congenital pneumonia due to *Mycoplasma pneumoniae*. *Arch Dis Child Fetal Neonatal Ed* 1995; *72* : F118-20.
21. Peters J, Singh H, Brooks EG, Diaz J, Kannan TR, Coalson JJ, *et al.* Persistence of community-acquired respiratory distress syndrome toxin-producing *Mycoplasma pneumoniae* in refractory asthma. *Chest* 2011; *140* : 401-7.
22. Wood PR, Hill VL, Burks ML, Peters JI, Singh H, Kannan TR, *et al. Mycoplasma pneumoniae* in children with acute and refractory asthma. *Ann Allergy Asthma Immunol* 2013; *110* : 328-40.
23. British Thoracic Society Standards of Care Committee. British thoracic society guidelines for the management of community acquired pneumonia in childhood. *Thorax* 2002; *57* (Suppl 1) : i1-24.
24. Gorthi SP, Kapoor L, Chaudhry R, Sharma N, Perez-Perez GI, Panigrahi P, *et al.* Guillain-Barré syndrome: Association with *Campylobacter jejuni* and *Mycoplasma pneumoniae* infections in India. *Natl Med J India* 2006; *19* : 137-9.
25. Kashyap B, Kumar S, Sethi GR, Das BC, Saigal SR. Comparison of PCR, culture & serological tests for the diagnosis of *Mycoplasma pneumoniae* in community-acquired lower respiratory tract infections in children. *Indian J Med Res* 2008; *128* : 134-9.
26. Shenoy VD, Upadhyaya SA, Rao SP, Shobha KL. *Mycoplasma pneumoniae* infection in children with acute respiratory infection. *J Trop Pediatr* 2005; *51* : 232-5.
27. Chaudhry R, Sharma S, Javed S, Passi K, Dey AB, Malhotra P, *et al.* Molecular detection of *Mycoplasma pneumoniae* by quantitative real-time PCR in patients with community acquired pneumonia. *Indian J Med Res* 2013; *138* : 244-51.
28. Nadagir SD, Kaleem Bahadur A, Anantappa Shepur T. Prevalence of *Mycoplasma pneumoniae* among HIV infected children. *Indian J Pediatr* 2011; *78* : 430-4.
29. Shankar EM, Kumarasamy N, Balakrishnan P, Solomon S, Lejith R, Vengatesan A, *et al.* Serosurveillance of acute *Mycoplasma pneumoniae* infection among HIV infected patients with pulmonary complaints in Chennai, Southern India. *J Infect* 2006; *53* : 325-30.
30. Shankar EM, Rajasekaran S, Rao UA, Paramesh P, Krishnakumar R, Rajan R, *et al.* Colonization of mycoplasma in the upper respiratory tract of AIDS patients with pulmonary symptoms in Chennai, India. *Indian J Med Res* 2005; *122* : 506-10.
31. Waites KB, Taylor-Robinson D. Mycoplasma and ureaplasma. In*:* Versalovic J, Carroll KC, Funke G, Jorgensen JH, LandryML, Warnock DW, editors. *Manual of clinical microbiology*, 10th ed. Washington, D.C.: ASM Press; 2011. p. 970-85.
32. Rock RT, Vital AC, Silva COS, Pereira CAC, Tani JN. Community-acquired pneumonia in outpatients: Epidemiological, clinical and radiological findings of atypical pneumonia and not atypical. *J Pneumol* 2000; *26* : 5-14.
33. Cohen M, Sahn SA. Resolution of pleural effusions. *Chest* 2001; *119* : 1547-62.
34. Waites KB, Rikihisa Y, Taylor-Robinson D. Mycoplasma and ureaplasma. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken YH, editors. *Manual of clinical microbiology*, 8th ed. Washington, DC.: American Society for Microbiology; 2003. p. 972-90.
35. Waites KB, Bébéar CM, Robertson JA, Talkington DF, Kenny GE. Cumitech 34, laboratory diagnosis of mycoplasmal infections. Washington, DC.: American Society for Microbiology; 2001.
36. Waites KB, Bébéar CM, Talkington DF. Mycoplasmas. In: Truant A, editor. *Manual of commercial methods in clinical microbiology*. Washington, DC.: American Society for Microbiology; 2002. p. 201-24.
37. Daxboeck F, Krause R, Wenisch C. Laboratory diagnosis of *Mycoplasma pneumoniae* infection. *Clin Microbiol Infect* 2003; *9* : 263-73.
38. Loens K, Beck T, Ursi D, Overdijk M, Sillekens P, Goossens H, *et al.* Development of real-time multiplex nucleic acid sequence-based amplification for detection of *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, and *Legionella* spp. in respiratory specimens. *J Clin Microbiol* 2008; *46* : 185-91.
39. Kumar S, Wang L, Fan J, Kraft A, Bose ME, Tiwari S, *et al.* Detection of 11 common viral and bacterial pathogens causing

community-acquired pneumonia or sepsis in asymptomatic patients by using a multiplex reverse transcription-PCR assay with manual (enzyme hybridization) or automated (electronic microarray) detection. *J Clin Microbiol* 2008; *46* : 3063-72.

40. Zhang L, Zong ZY, Liu YB, Ye H, Lv XJ. PCR versus serology for diagnosing *Mycoplasma pneumoniae* infection: A systematic review & meta-analysis. *Indian J Med Res* 2011; *134* : 270-80.
41. Pitcher D, Chalker VJ, Sheppard C, George RC, Harrison TG. Real-time detection of *Mycoplasma pneumoniae* in respiratory samples with an internal processing control. *J Med Microbiol* 2006; *55* : 149-55.
42. Loens K, Ieven M. *Mycoplasma pneumoniae*: Current knowledge on nucleic acid amplification techniques and serological diagnostics. *Front Microbiol* 2016; *7* : 448.
43. Li W, Liu Y, Zhao Y, Tao R, Li Y, Shang S. Rapid diagnosis of *Mycoplasma pneumoniae* in children with pneumonia by an immunochromatographic antigen assay. *Sci Rep* 2015; *5* : 15539.
44. García-González R, Costa-García A, Fernández-Abedul MT. Enzymatic amplification-free nucleic acid hybridisation sensing on nanostructured thick-film electrodes by using covalently attached methylene blue. *Talanta* 2015; *142* : 11-9.
45. Henderson KC, Benitez AJ, Ratliff AE, Crabb DM, Sheppard ES, Winchell JM, *et al.* Specificity and strain-typing capabilities of nanorod array-surface enhanced Raman spectroscopy for *Mycoplasma pneumoniae* detection. *PLoS One* 2015; *10* : e0131831.
46. Cao B, Zhao CJ, Yin YD, Zhao F, Song SF, Bai L, *et al.* High prevalence of macrolide resistance in *Mycoplasma pneumoniae* isolates from adult and adolescent patients with respiratory tract infection in China. *Clin Infect Dis* 2010; *51* : 189-94.
47. Okada T, Morozumi M, Tajima T, Hasegawa M, Sakata H, Ohnari S, *et al.* Rapid effectiveness of minocycline or doxycycline against macrolide-resistant *Mycoplasma pneumoniae* infection in a 2011 outbreak among Japanese children. *Clin Infect Dis* 2012; *55* : 1642-9.
48. Dumke R, von Baum H, Lück PC, Jacobs E. Occurrence of macrolide-resistant *Mycoplasma pneumoniae* strains in Germany. *Clin Microbiol Infect* 2010; *16* : 613-6.
49. Peuchant O, Ménard A, Renaudin H, Morozumi M, Ubukata K, Bébéar CM, *et al.* Increased macrolide resistance of *Mycoplasma pneumoniae* in France directly detected in clinical specimens by real-time PCR and melting curve analysis. *J Antimicrob Chemother* 2009; *64* : 52-8.
50. Yamada M, Buller R, Bledsoe S, Storch GA. Rising rates of macrolide-resistant *Mycoplasma pneumoniae* in the central United States. *Pediatr Infect Dis J* 2012; *31* : 409-10.
51. Schurwanz N, Jacobs E, Dumke R. Strategy to create chimeric proteins derived from functional adhesin regions of *Mycoplasma pneumoniae* for vaccine development. *Infect Immun* 2009; *77* : 5007-15.
52. Hausner M, Schamberger A, Naumann W, Jacobs E, Dumke R. Development of protective anti-*Mycoplasma pneumoniae* antibodies after immunization of guinea pigs with the combination of a P1-P30 chimeric recombinant protein and chitosan. *Microb Pathog* 2013; *64* : 23-32.

For correspondence: Dr Surinder Kumar, Department of Microbiology, Mycoplasma Research Laboratory, Maulana Azad Medical College, New Delhi 110 002, India
e-mail: kumarsurinderdr@yahoo.com