Macrolide-resistant *Mycoplasma pneumoniae*: its role in respiratory infection

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Although the clinical relevance of antibiotic treatment in influencing the natural course of *Mycoplasma pneumoniae*-associated respiratory diseases is questioned by some physicians, most experts suggest that antibiotics should be systematically used in patients with *M. pneumoniae* respiratory infections, especially those involving the lower respiratory tract. Macrolides (MLs), tetracyclines (TCs) and fluoroquinolones (FQs) are the drugs of choice for *M. pneumoniae* infection, but only MLs are recommended for children. The main aim of this review is to analyse what is known about *M. pneumoniae* resistance to MLs and discuss the most reasonable approach to treating patients with *M. pneumoniae* infection at a time when resistant strains are being increasingly detected. The results show that no change in ML prescription is needed in countries in which the incidence of ML-resistant *M. pneumoniae* is low; however, in countries in which ML-resistant *M. pneumoniae* strains are very common, the replacement of an ML by a TC or FQ should be considered depending on the severity of the disease. A number of cases treated with ineffective antibiotics have shown similar outcomes to those observed in patients infected by susceptible strains. This seems to indicate that there is no need to change ML use systematically in the case of mild to moderate disease, but other antibiotics should be prescribed if the symptoms persist or there are signs of a clinical deterioration.

**Keywords:** children, fluoroquinolones, macrolides, macrolide-resistant, tetracycline

**Introduction**

Although it is sometimes associated with extra-respiratory diseases, *Mycoplasma pneumoniae* is mainly a respiratory pathogen that causes a large number of upper and lower respiratory tract infections, particularly in older children and adolescents. *M. pneumoniae* infection can be demonstrated in patients with acute and recurrent pharyngotonsillitis and in up to 40% of children with community-acquired pneumonia (CAP). Moreover, studies have reported that *M. pneumoniae* can cause acute wheezing and contribute to asthma exacerbations and chronic asthma, thus influencing disease severity.

Although the clinical relevance of antibiotic treatment in causing changes in the natural course of *M. pneumoniae*-associated respiratory diseases is questioned by some physicians, most experts suggest that antibiotics should be systematically used in patients with *M. pneumoniae* respiratory infections, especially those involving the lower respiratory tract. β-Lactam antibiotics, which are considered the drugs of choice for treating respiratory diseases because they are active against most respiratory bacterial pathogens, are ineffective against *M. pneumoniae* because they target the cell wall, and *M. pneumoniae* lacks a cell wall. In contrast, macrolides (MLs) and tetracyclines (TCs), which act as protein synthesis inhibitors, and fluoroquinolones (FQs), which act against topoisomerases and thus inhibit DNA synthesis and replication, are usually highly effective against *M. pneumoniae* in vitro and are the drugs of choice for the treatment of all infections due to atypical bacteria, including *M. pneumoniae*. However, only MLs are recommended for children because TC and FQ administration can be followed by a number of age-related adverse events. TCs are associated with depressed bone growth, tooth enamel hypoplasia and permanent tooth discoloration, and are recommended only for children age ≥8 years. Some FQs are licensed for children and adolescents, but most of these drugs are not licensed for fear of articular problems and reversible musculoskeletal events. Consequently their paediatric use is only suggested in the case of cystic fibrosis or life-endangering infections when other drugs cannot be administered.

The emergence of ML resistance in *M. pneumoniae* strains has been repeatedly reported worldwide since 2000, although its prevalence varies from country to country. However, the clinical relevance of this has not been definitely established. Knowing the extent to which the presence of ML-resistant *M. pneumoniae* can influence the outcome of *M. pneumoniae* disease is critical.
because epidemics of *M. pneumoniae* infection are becoming increasingly frequent.  

The main aims of this review are to analyse what is known about *M. pneumoniae* resistance to MLs and discuss the most reasonable approach to treating patients with *M. pneumoniae* infection at a time when resistant strains are being increasingly detected.

### Epidemiology of *M. pneumoniae* resistance to MLs

The first report of nucleotide mutations associated with *M. pneumoniae* resistance to MLs was published by Okazaki et al. in Japan in 2001. The authors not only described the relationships between the point mutation pattern of ML-resistant strains and their resistance phenotypes, but also showed that the in vitro exposure of susceptible *M. pneumoniae* strains to erythromycin could induce the emergence of resistance. Since then, other studies have confirmed that this microbiological problem is increasing throughout the world, although the highest prevalence has been observed in East Asia. Table 1 summarizes the main studies of the prevalence of ML-resistant *M. pneumoniae* in different countries. An isolation rate of ML-resistant *M. pneumoniae* of as high as 92% has been reported in China, and studies carried out in Japan between 2002 and 2008 found a progressive increase in ML resistance from 5% to >40% of *M. pneumoniae* isolates. Moreover, recently in Japan, an outbreak of *M. pneumoniae* infection during which more than 80% of the pathogens were found to be resistant was described. In the USA, the emergence of resistance has only recently been reported in a very limited number of cases, although one report indicates that the rates of ML-resistant strains are rising (at least in the central USA), and account for 8.3% of all the identified *M. pneumoniae*. In Europe, the situation varies from country to country, but in general the prevalence of resistant strains is no higher than 10%, and in some countries such as The Netherlands, no resistant strains have ever been identified. There are exceptions to this general rule, as reported by Chironna et al. and by Averbuch et al. Chironna et al. found ML-resistant genotypes in 11 of 43 children infected by *M. pneumoniae* (26%) during an outbreak of *M. pneumoniae* infection in southern Italy in 2010. Similar levels of resistance were reported by Averbuch et al. in Israel. However, regardless of the prevalence of *M. pneumoniae* resistance, resistance has always been found to be more frequent in children than adults.

On the basis of in vitro data, it is thought that the emergence of resistant strains could be directly induced by ML use in vivo even after just a few days of therapy, particularly when *M. pneumoniae* is directly exposed to sublethal drug concentrations, as reported by Pereyre et al. in the case of azithromycin. This hypothesis is strongly supported by the fact that the prevalence of ML-resistant *M. pneumoniae* is highest in places in which MLs are more widely used, such as East Asia or Italy. However, the best evidence of a direct relationship between the in vivo emergence of *M. pneumoniae* resistance to MLs and their use comes from studies of hospitalized children with *M. pneumoniae* CAP in whom the genetic characteristics of the identified pathogens were adequately analysed before and after treatment. In some cases, the analyses for determining ML resistance genotypes were negative at the time of admission, but positive after appropriate treatment with clarithromycin, thus possibly indicating that the mutations were induced by antibiotic administration. However, it is possible that the respiratory infection may sometimes have been due to a mixed population of resistant and susceptible strains, and the administration of ML may have selected the resistant species identified at the end of ML treatment.

### Table 1. Major studies of the prevalence of ML-resistant *M. pneumoniae* in different countries and at different times

<table>
<thead>
<tr>
<th>Authors</th>
<th>Country</th>
<th>Population</th>
<th>Main findings</th>
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<tbody>
<tr>
<td>Liu et al.</td>
<td>China</td>
<td>paediatric patients with respiratory infection</td>
<td>from 2005 to 2008, 44/53 isolates (83%) were resistant to erythromycin, azithromycin and clarithromycin from 2003 to 2006, 46/50 strains (92%) were ML resistant</td>
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<tr>
<td>Xin et al.</td>
<td>China</td>
<td>paediatric patients with respiratory infection</td>
<td>from 2002 to 2006, 50/380 strains (13.2%) were ML-resistant during the period 2007–10, ML resistance was 8.2%</td>
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<tr>
<td>Morozumi et al.</td>
<td>Japan</td>
<td>paediatric patients with CAP</td>
<td>between 2003 and 2008, ML resistance was 3.0% before 2005, no resistance was found; among 51 samples collected between 2005 and 2007, 5 (9.8%) had a resistant genotype</td>
</tr>
<tr>
<td>Yamada et al.</td>
<td>USA</td>
<td>paediatric patients with <em>M. pneumoniae</em> infection</td>
<td>between 1997 and 2008, no ML-resistant strains out of 114</td>
</tr>
<tr>
<td>Dumke et al.</td>
<td>Germany</td>
<td>adults with CAP <em>M. pneumoniae</em>-positive specimens</td>
<td>in 2010, 11/43 strains (25.6%) were ML resistant 9/30 strains (30.0%) in 2010 had a resistant genotype; in 2011, 176/202 (87.1%) were ML resistant</td>
</tr>
<tr>
<td>Peuchant et al.</td>
<td>France</td>
<td><em>M. pneumoniae</em>-positive specimens</td>
<td></td>
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<tr>
<td>Spuesens et al.</td>
<td>The Netherlands</td>
<td><em>M. pneumoniae</em>-positive specimens</td>
<td></td>
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<tr>
<td>Chironna et al.</td>
<td>Italy</td>
<td>paediatric patients with CAP</td>
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</tr>
<tr>
<td>Averbuch et al.</td>
<td>Israel</td>
<td>adults and children with CAP</td>
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Mechanisms of *M. pneumoniae* resistance to MLs

Resistance to MLs in *M. pneumoniae* strains is defined by specific point mutations in the domain of the single-copy 23S rRNA gene. In the case of 14- and 15-membered ring MLs, a high level of resistance (MIC ≥ 32 mg/L) is related to an A to G transition at position 2063 and an A to C transition at position 2064, whereas low levels of resistance are due to the presence of an A to G transition at position 2067 and a C to G or A transversion at position 2617.26

In the case of 16-membered ring MLs, a low level of resistance has been associated with the A to G transition at position 2064, whereas an A to G transition at position 2063 gives rise to intermediate resistance and an A to G transition at position 2067 leads to the highest level of resistance as it interferes with the formation of the covalent bond specific to 16-membered ring MLs.26

The resistance of *M. pneumoniae* to telithromycin, the most widely prescribed ketolide, is mediated by the same mutations that condition 14- and 15-membered ring ML activity. However, streptogramin combinations remain active against ML-resistant *M. pneumoniae*.27 No strain fully resistant to TCs or FQs has yet been observed among clinical isolates,28 although strains with reduced susceptibility to TCs have been identified28 and FQ-resistant mutants can be obtained in vitro by exposure to subinhibitory concentrations of these drugs.29

Most studies have found both of the subtypes of *M. pneumoniae* (categorized on the basis of the DNA sequence of the P1 adhesin protein) among ML-resistant strains,32,33 thus suggesting that there is no association between *M. pneumoniae* subtype and ML resistance or susceptibility. This is also supported by the findings of studies using more selective methods to differentiate *M. pneumoniae* subtypes, such as multilocus variable-number tandem-repeat analysis (MILVA).31–33 MILVA has revealed up to 26 subtypes, none of which could be considered dominant, although in some cases each of them can cause epidemics of *M. pneumoniae* infection.33 This suggests that the absence of a particularly resistant clone and the high rate of ML resistance in some geographical areas is due to the dissemination of multiple resistant clones rather than the spread of a single colony.

Clinical relevance of macrolide-resistant *M. pneumoniae*

In most cases, respiratory infections due to *M. pneumoniae* are mild to moderate, and the acute signs and symptoms of disease tend to disappear spontaneously after a few days or after a course of antibiotic therapy with an active drug.6 Severe infections are uncommon, although cases of pleural effusion, necrotizing pneumonia, lung abscess, broncholiths, obliterans organizing pneumonia, and acute respiratory distress syndrome have been reported.34 Data collected from patients with CAP due to ML-resistant *M. pneumoniae* seem to suggest that the presence of mutated strains does not increase the severity of the disease’s clinical manifestations in children or adults because the symptoms of most patients are similar to those reported in subjects with ML-susceptible strains, and only a few severe cases have been observed.15,20,23,35

However, in comparison with patients with susceptible strains treated with ML, most subjects with ML-resistant *M. pneumoniae* have more persistent signs and symptoms that, in some cases, have led the attending physician to replace the ML with a TC or FQ in order to obtain a more rapid clinical result. Table 2 summarizes the main pediatric studies comparing clinical outcomes in patients with ML-resistant and ML-susceptible strains.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Study population</th>
<th>Conclusion</th>
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<tr>
<td>Suzuki et al.26</td>
<td>the clinical courses of 11 patients with ML-resistant infection treated with MLs were compared with those of 26 patients with ML-susceptible infection in Japan</td>
<td>although the febrile period was prolonged in the ML-resistant patients given ML, the fever resolved even when the initial prescription was not changed</td>
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<tr>
<td>Matsubara et al.37</td>
<td>the clinical courses of 47 paediatric cases of ML-susceptible and 22 paediatric cases of ML-resistant <em>M. pneumoniae</em> were compared</td>
<td>the efficacy of ML treatment was significantly lower in the case of ML-resistant than ML-susceptible <em>M. pneumoniae</em></td>
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<tr>
<td>Morozumi et al.38</td>
<td>the clinical courses of 58 paediatric cases with CAP due to ML-susceptible and 53 paediatric cases with CAP due to ML-resistant <em>M. pneumoniae</em> were compared</td>
<td>the efficacy of ML treatment was significantly lower and the duration of fever was significantly longer in the patients with ML-resistant versus ML-susceptible <em>M. pneumoniae</em></td>
</tr>
<tr>
<td>Kawai et al.39</td>
<td>isolates from 21 patients with ML-resistant and 9 with ML-susceptible <em>M. pneumoniae</em> were compared during ML treatment</td>
<td>the number of <em>M. pneumoniae</em> 48 h after starting macrolide treatment was significantly higher in the samples from ML-resistant patients than the samples from ML-susceptible patients. In 15 of the 21 ML-resistant patients, fever persisted for more than 48 h after the start of ML treatment</td>
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<tr>
<td>Okada et al.14</td>
<td>the clinical course and bacterial load among 176 (87.1%) children with ML-resistant <em>M. pneumoniae</em> pneumonia and 26 (12.9%) with ML-susceptible <em>M. pneumoniae</em> were compared</td>
<td>ML-resistant <em>M. pneumoniae</em> in terms of shortening the clinical course and decreasing the <em>M. pneumoniae</em> load</td>
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CAP, community-acquired pneumonia; ML, macrolides.
Suzuki et al. found that the total number of febrile days was higher in children with ML-resistant than in those with ML-susceptible strains (median 8 versus 5 days; \( P=0.019 \)), as was the number of febrile days during ML administration (3 versus 1 day, \( P=0.002 \)). In addition, ML therapy was changed in 63.6% of the resistant cases versus 3.8% of the susceptible cases (odds ratio 43.8; \( P<0.001 \)).

Matsubara et al. compared 47 children with ML-susceptible M. pneumoniae with 22 children with ML-resistant M. pneumoniae and found no significant difference between the two groups in terms of gender, age, time from the onset of the infection to the first examination, laboratory findings, diagnosis or the severity of symptoms. However, the efficacy of ML treatment (measured by evaluating the duration of fever and cough after the first antibiotic administration) was 91.5% in the cases due to ML-susceptible M. pneumoniae and 22.7% in the cases due to ML-resistant strains (\( P<0.01 \)).

Morozumi et al. studied 58 children infected by ML-susceptible and 53 infected by ML-resistant M. pneumoniae and reported that treatment was changed from ML to minocycline or levofloxacin in 4 (6.9%) of the former and 19 (35.8%) of the latter (\( P=0.0023 \)). The mean time from the start of ML use to defervescence was 1.6 ± 0.8 days in the case of ML-susceptible M. pneumoniae and 4.1 ± 2.3 days in the case of ML-resistant M. pneumoniae (\( P=0.0020 \)).

Finally, Kawai et al. studied 30 children with CAP treated with MLs: 21 with ML-resistant and 9 with ML-susceptible M. pneumoniae. They not only evaluated the clinical course of the disease, but also the concentrations of pathogens in respiratory secretions during and after treatment. The number of M. pneumoniae in the control group decreased rapidly after 48 h after the start of ML treatment and showed a close relationship with clinical outcome, whereas the number was significantly higher in the samples taken from the patients with ML-resistant strains. Furthermore, in 15 of the 21 patients with ML-resistant strains, fever persisted for more than 48 h after the start of ML treatment and when it was changed to minocycline, fever disappeared within 48 h in all cases. However, in all the above mentioned studies, it was reported that despite continuing inactive therapy, the clinical course of a considerable number of patients with ML-resistant M. pneumoniae was not different from that of the subjects with ML-susceptible pathogens. As MLs have anti-inflammatory properties and M. pneumoniae infection of human lung epithelial cells promotes a relevant cytokine response that is considered to play a crucial role in the pathogenesis of the ensuing clinical disease, it has been assumed that, at least in some cases, although the ML was inactive against the infectious agent in vitro, it may have resolved the clinical symptoms of CAP by inhibiting cytokine production. Unfortunately the available data do not allow this hypothesis to be assessed because no study comparing clinical outcomes in patients with ML-resistant strains treated with and without MLs has yet been carried out.

Interestingly, Okada et al. recently described 258 children with M. pneumoniae-associated pneumonia based on chest radiography, real-time PCR and antibody titres enrolled between January and December 2011. M. pneumoniae cultures obtained from nasopharyngeal samples using appropriate broth were subjected to real-time PCR, by which decreases in M. pneumoniae in patients treated with minocycline, doxycycline or tosufloxacin were calculated. Mutations of the 23S ribosomal RNA gene that confers high resistance to macrolides in M. pneumoniae were identified by DNA sequencing. Among 202 M. pneumoniae isolates from M. pneumoniae-associated pneumonia patients, 176 (87.1%) were ML-resistant M. pneumoniae. ML-resistant M. pneumoniae infection was significantly related to school age (\( P<0.01 \)) and initial administration of macrolides (\( P<0.01 \)). Minocycline or doxycycline (\( n=125 \)) and tosufloxacin or levofloxacin (\( n=15 \)) were used for definitive treatment of ML-resistant M. pneumoniae patients. Minocycline or doxycycline was significantly more effective in achieving defervescence within 24 h and in decreasing numbers of M. pneumoniae DNA copies 3 days after initiation than tosufloxacin (\( P \leq 0.05 \)). These results highlight that in this study MLs appeared inappropriate as first-choice agents against ML-resistant M. pneumoniae in terms of shortening the clinical course and decreasing M. pneumoniae numbers. Control and prevention of ML-resistant M. pneumoniae outbreaks in children require early decreases in M. pneumoniae numbers as well as improvement of clinical findings.

How to deal with respiratory infections associated with ML-resistant M. pneumoniae

In general, the treatment of M. pneumoniae infections is complicated by the difficulty in identifying the pathogen. The use of culture requires specialized techniques, adequate specimen processing, and many days to detect growth; furthermore, culture specificity greatly depends on the ability of the laboratory technician. Evaluating the host immune response often provides only a retrospective diagnosis of acute infection because a convalescent serum specimen is needed to show an adequate (at least 4-fold) increase in titre. These methods are therefore not the best means of patient management.

Molecular methods for identifying M. pneumoniae DNA in upper respiratory secretions are rapid, easy, and cost-effective diagnostic technologies also because carrier status is very uncommon. However, as they are not routinely used in all hospital laboratories, most M. pneumoniae respiratory infections remain aetiologically unidentified, and the supposition of the causal presence of M. pneumoniae is mainly based on clinical, laboratory and radiological findings.

The attribution of certain clinical and radiological characteristics to M. pneumoniae has been common practice, but recent data have cast doubts on their specificity when comparing individual clinical manifestations. A number of studies have shown that it is not possible to predict the presence of M. pneumoniae on the basis of presenting manifestations or routine laboratory parameters. Moreover, radiography cannot be used to predict atypical bacterial infection precisely because, like other screening tests, the findings are limited in terms of sensitivity, specificity and intra- or interobserver variability. All of these limitations are even more important when ML-resistant strains are the possible cause of disease because, despite the fact that rapid molecular methods capable of highlighting mutations in previously identified M. pneumoniae DNA are available, they cannot be used in patients monitored at home and are not included in the list of laboratory tests usually performed in the majority of hospitals for determining the aetiology of respiratory infections. Consequently, in everyday practice, it is not only difficult to
identify cases due to *M. pneumoniae* infection, but practically impossible to identify *a priori* those associated with ML-resistant pathogens. These are relevant diagnostic limits because the rapid detection of *M. pneumoniae*, including those that are ML-resistant, could permit control of the dissemination of the organism and prevent the development of outbreaks.

At this point, the first problem to solve is the real need for systematic antibiotic therapy when treating *M. pneumoniae* infection. A number of meta-analyses have evaluated the most recent studies comparing antibiotics that are active *in vitro* against atypical pathogens with β-lactams in the treatment of mild to moderate CAP, and concluded that there is no compelling evidence of a need to treat atypical pathogens systematically, except in the case of legionnaires’ disease. However, data collected from patients with ML-resistant strains indicating that the presence of resistant pathogens may be associated with an increased risk of persisting signs and symptoms seem to suggest that treating *M. pneumoniae* with active drugs is also useful in mild cases (although not in all patients), and so the systematic prescription of active drugs seems reasonable. Furthermore, studies published 30–50 years ago seem to show a clear response of atypical CAP to active antibiotics.

### Conclusions

On the basis of studies of the prevalence of ML resistance among *M. pneumoniae* strains, in our opinion no change in ML prescription is initially needed in countries in which the incidence of ML-resistant *M. pneumoniae* is low. However, in countries in which ML-resistant *M. pneumoniae* strains are very common, the replacement of an ML by a TC or FQ should be considered depending on the severity of the disease. The presence of ML-resistant strains has been mainly associated with the persistence of clinical symptoms of disease in mild to moderate cases. However, the mean duration of symptoms is only about 3 days, and no increase in the incidence of complications has been reported. Moreover, a number of cases treated with ineffective antibiotics have similar outcomes to those observed in patients infected by susceptible strains. This seems to indicate that there no need to change ML use systematically in the case of mild to moderate disease, but other antibiotics should be prescribed if the symptoms persist or there are signs of a clinical deterioration (even in an early phase in severe cases). Among the other potentially effective antibiotics, the choice should be made taking into account their *in vitro* activity and their potential adverse events. Among FQs, ciprofloxacin, due to elevated MIC, is not an appropriate choice, and more suitable FQs such as levofloxacin should be preferred. Minocycline or doxycycline, as recently reported by Okada et al. in the first large outbreak of ML-resistant *M. pneumoniae* infection in Japan, seem the best solution among TCs. However, further randomized studies including a greater number of severe cases are needed to definitively solve the problem of the real clinical importance of ML resistance and that of the best antibiotic choice.

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### References


