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Original Article

Mycoplasma pneumoniae in pediatric patients: Do macrolide-resistance and/or delayed treatment matter?

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KEYWORDS
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Abstract *Background: Mycoplasma pneumoniae* is a common pathogen for pneumonia in children, especially in the post-pneumococcal conjugate vaccination era. Though self-limited disease was found in the majority of the patients, severe diseases occurred occasionally. The emergence of macrolide resistance was reported worldwide. It is important to delineate whether macrolide resistance or delayed treatment affects outcome.
Methods: We retrospectively collected pediatric patients with *M. pneumoniae* infection confirmed by positive PCR in a tertiary medical center in Taiwan from 2010 to 2017. Patients' clinical characteristics, bacterial load, macrolide resistance and treatment outcome were analyzed.
Results: Among 471 children with positive *M. pneumoniae* PCR, 95% were diagnosed with pneumonia. Seventeen percent of patients had extrapulmonary complications, and 1.5% had respiratory failure. Delayed treatment was associated with prolonged fever after appropriate treatment, fulminant disease, and extrapulmonary manifestations ($p < 0.05$). The mean rate of macrolide resistance was 24% and macrolide resistance was related to longer febrile duration, longer hospital stay, lung consolidation and impaired liver function tests ($P < 0.05$).
Conclusions: Macrolide resistance was fairly common and might lead to delayed appropriate antibiotic treatment. Delayed appropriate antimicrobial treatment, no matter macrolide resistance or not, was associated with more severe and/or prolonged diseases. Early diagnosis of *M. pneumoniae* as well as the awareness of macrolide resistance make early effective antibiotic treatment possible and may improve clinical outcomes.

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Introduction

The most common pathogens of community-acquired pneumonia in children in pre-PCV era included 42% for pneumococcus and 38% for *Mycoplasma pneumoniae* (MP).[1] In countries where PCV-13 was introduced to national immunization program, MP related childhood pneumonia has become more prevalent, especially in school-aged children. In one study of pneumonia etiology in post-PCV era in the US, *M. pneumoniae* was isolated doubly than *S. pneumoniae* and became the mostly seen etiology other than viral pneumonia.[2]

M. pneumoniae pneumonia is regarded as atypical pneumonia and usually presented with mild symptoms and some extra-pulmonary manifestations affecting the skin, liver, and central nervous system. It could also present as fever of unknown origin in children without apparent source after preliminary investigations.[3] Prolonged disease course with increased clinical severity, longer hospital stay and treatment failure was reported to be associated with macrolide-resistant *M. pneumoniae* (MRMP).[4] Emergence of MRMP has been reported worldwide with up to 90% resistant rate in China and Japan since 2000.[5,6] The rate of MRMP in children in Taiwan was 12—23% in 2011—2012.[4,7] It makes a challenge in treatment and needs continuous surveillance. Though it is not frequent, severe life-threatening disease with complications and chronic sequelae may occur in MP pneumonia. Due to limited studies on complicated MP infection and macrolide resistance in pediatrics groups, we initiated this study to investigate the clinical features, and correlation between MRMP and clinical outcomes in children.

Materials and methods

Patients and data collection

Inpatients or outpatients would receive MP PCR assay if they had signs of lower respiratory infections or other suspicions for MP infections in National Taiwan University Children's Hospital. From Jan. 2010 to Jul. 2017, we included children under 18 years who had positive results of MP PCR of either throat swab, sputum, bronchial lavage or pleural effusion. Children were excluded if they had malignancies, immunocompromised or incomplete medical records. Patients' medical history, demographics, clinical manifestations, laboratory parameters, radiographic findings, bacterial load and treatment were collected from medical charts. Other common pathogens from respiratory tracts of these cases were also recorded.

Definition

Extrapulmonary complications were defined as elevated transaminase (aspartate transaminase or alanine transaminase over the normal upper limit), central nervous system (CNS) involvement, dermatological and mucosal involvement. Severe diseases of Mycoplasma pneumonia infection were referred to patients with severe respiratory complication who needed ventilator support (both invasive and non-invasive) or patients had severe mucocutaneous complication, that is, Stevens-Johnson syndrome. Viral co-infection was defined as positive viral isolation or antigen of respiratory viruses (adenoviruses, influenza, parainfluenza, or respiratory syncytial virus). Bacterial co-infection was defined as positive culture for *S. pneumoniae, H. influenzae or Mycoplasma catarralis* from respiratory specimens or positive urine pneumococcal antigen. Delayed effective treatment was defined as the duration of disease onset to appropriate antibiotic treatment was equal to or over the 6 days while the median duration of disease onset to appropriate antibiotic treatment was 5.6 days. Appropriate antibiotics defined as effective antibiotics (fluoroquinolone or tetracycline) for all patients or macrolide for those infected with macrolide-sensitive MP. Bacterial load analysis was divided to higher load group and lower load group by median bacterial load.

Mycoplasma pneumoniae PCR assay

DNA of specimens was extracted with a kit (Roche MagNA Pure LC total nucleic acid isolation kit) for polymerase chain reaction (PCR) assay to detect *M. pneumoniae* DNA (Roche LightCycler 1.5) twice weekly. Primers were designed to amplify the segment of 4596—4725 nucleotides of cytadhesin P1 gene (forward primer: 5′-CCA ACC AAA CAA CAA CGT TCA-3′ and reverse primer: 5′-TAA CGG CAA CAC GTA ATC AGG TC-3′). The probe was 6FAM-ACC TTG ACT GGA GGC CGT TA-BHQ1 for real-time PCR using the LightCycler FastStart DNA Master HybProbe (Roche) and FRET (fluorescence resonance energy transfer) probes in a Light Cycler (Roche) by 50 amplification cycles of denaturation for 5 s at 95 °C, annealing for 10 s at 58 °C, and elongation for 10 s at 72 °C. The detection limit of the in-house PCR was 10 DNA copies.

Macrolide-resistant gene detection

If the real-time PCR revealed a positive result, amplification of the 2758—2769 nucleotides segment of 23S rRNA by nested PCR and DNA sequencing was performed subsequently for detecting of point mutation loci (A2063 G/C, A2064 G/C, C2617 G/A) which caused macrolide-resistance. DNA sequences of PCR products were compared to the sequences of *M. pneumoniae* M129 (accession no. X68422) by using the Basic Local Alignment Search Tool (BLAST) on the internet of the National Center of Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST).

Statistical analysis

All analyses were performed with commercially available statistical software (SPSS v23, IBM Corp., Armonk, NY, USA). Descriptive statistics was performed and reported by percentages for qualitative data, and by means with standard deviations or medians with range for quantitative data. Normal values of blood cells and biochemistry exams were based on the patients' ages. Continuous data were analyzed with t-test, and categorical data were compared with the Chi-square test. All tests were two-tailed. Statistical significance was defined as $p < 0.05$ in the tests.

Results

Demographic data and the macrolide resistance

In 2327 children who had clinical suspicion of MP infections and sent specimens for *M. pneumoniae* real-time PCR in the study period, 471 (20%) cases had positive results and fit the inclusion criteria. Their mean age was 6.6 years, 240 (51%) were male and 95% of these cases had the diagnosis of pneumonia. The rest of patients had acute otitis media, acute sinusitis, bronchitis, pharyngitis, erythema multiforme or Stevens-Johnson syndrome (SJS). Eighty two patients (17%) had extrapulmonary manifestations including 43 with skin rash, 42 with elevated transaminase, and 5 with CNS involvement (4 encephalitis and 1 pseudotumor cerebri).

Four hundred seventy-one patients, including 360 MSMP and 111 MRMP cases, were analyzed (Table 1). The mean macrolide resistant rate was 24%, ranging from 19% to 30% in the study period. The majority of resistant loci was A2063G (91%), and new detected loci including A2063T and A2064G had been found since 2015 (Fig. 1). There was no phenotypic macrolide resistance differences among different resistant gene loci. Infection with macrolide resistant strain was related to longer febrile duration (8.6 days), longer hospitalization (6.2 days), a higher rate of consolidation in radiograph (72%) and a higher rate of elevated transaminase (23%). Only 14 patients (4%) did not receive effective antibiotics in MSMP group while 64 (58%) cases in MRMP group did not receive effective antibiotics ($p < 0.001$).

The characteristics of 8 (2%) patients with severe diseases in this study are listed in Table 2. All except one were male. The mean duration from disease onset to the development of respiratory failure was 9.4 days (6—14 days). Trend of prolonged fever was found in these patients than others (16 days vs. 7 days, $p = 0.06$). All the 7 patients with respiratory failure had pulmonary consolidation or lobar pneumonia with or without pleural on admission. Three (38%) patients had MRMP infection without significant elevation of initial white blood cell count or C-reactive protein. Endotracheal intubation for a period of 5—22 days was performed in 4 cases and ventilation support with biphasic positive airway pressure was given for 3 cases. Case 6 with severe bilateral pneumonia and positive PCR of his pleural effusion received the first dose of MP-active antibiotics on day 14 and had persistent bacterial load in oropharynx for 2 weeks during ICU admission and the sequel of chronic pulmonary fibrosis, which made him need nasal cannula O2 support at home after 58 days of hospitalization. Case 8 with the history of discoid lupus erythematous developed Stevens-Johnson syndrome in this study. No mortality was reported in any case.

Delayed effective treatment on clinical outcomes

Because 116 patients had no fever (5 patients, 3 in MSMP group and 2 in MRMP group, respectively), lost follow up after antibiotic treatment (3 patients, all in MRMP group), no records of fever duration (45 patients, 39 in MSMP group and 6 in MRMP group, respectively), or did not receive appropriate antibiotic treatment (61 patients, 5 in MSMP group and 56 in MRMP group, respectively), only 355 patients were included in analysis of treatment outcome. Among these 355 patients, the mean interval between the onset and MP-active antibiotic treatment was 5.6 days, and we defined delayed effective treatment if the patients received the first dose of MP-active antibiotic treatment on day 6 of disease onset or later. The comparison of clinical outcomes between cases with or without delayed effective

Table 1 Characteristics of macrolide-sensitive and macrolide-resistant *Mycoplasma pneumoniae* infection in children.

Characteristics	MSMP (n = 360)	MRMP (n = 111)	P value
Mean age	6.6 ± 3.8	6.7 ± 3.9	0.75
Male gender	181/360 (50%)	59/111 (53%)	0.60
Inpatient	285/360 (79%)	92/111 (83%)	0.39
Febrile duration	6.8 ± 3.7	8.6 ± 3.8	<0.001
Hospital stay days	4.9 ± 4.6	6.2 ± 3.3	0.02
Consolidation	175/320 (55%)	71/99 (72%)	0.01
High bacteria load	176/360 (49%)	54/111 (49%)	0.97
WBC count (/uL)	8786 ± 3883	9120 ± 3291	0.45
CRP (mg/dL)	4.2 ± 4.3	4.5 ± 5.1	0.56
Elevated transaminase	24/253 (9%)	18/78 (23%)	0.002
Extrapulmonary manifestations	56/360 (16%)	24/111 (22%)	0.141
Without effective antibiotics	14/360 (4%)	64/111 (58%)	<0.001

Data are shown as mean ± standard deviation or positive number/tested umber (percentage). MSMP denotes macrolide-sensitive *Mycoplasma pneumoniae*, MRMP macrolide-resistant *Mycoplasma pneumoniae*, WBC white blood cell and CRP C-reactive protein.

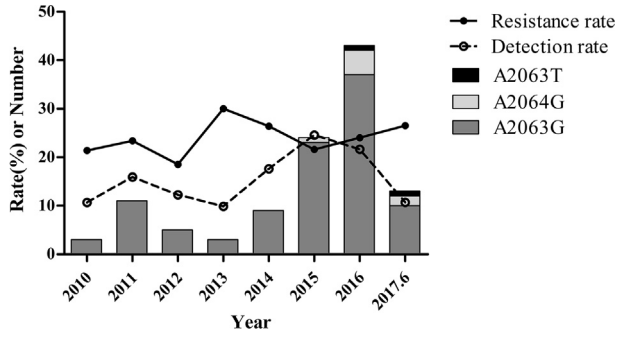

Fig. 1. Mycoplasma pneumoniae PCR detection in 2010–2017. The bars indicate the accumulated case numbers with MRMP infection each year with different genotypes. The hollow spots represent for the detection rate of MP PCR (MP positive cases divided by MP positive cases plus MP negative cases). The solid spots indicate the macrolide resistant rate by year, which is calculated by MRMP case divided by total MP positive cases.

treatment is shown in Table 3. The rates of febrile duration for more than 7 days after effective antibiotic use (9%), severe diseases (4%), macrolide resistance (22%), extrapulmonary manifestation (25%), elevated transaminase (20%) and C-reactive protein level were significantly higher in cases with delayed effective treatment.

Though there were 67 MRMP cases excluded in the treatment outcome analysis due to no appropriate antibiotic treatment (56 patients), no fever (2 patients), or a lack of the record of fever duration (9 patients), the mean fever duration of these people were 7.6 ± 3.2 days, with the maximum of 21 days. Their fever duration was similar to all the other 404 patients (7.1 ± 3.9 days, $p = 0.435$). The symptoms of the excluded patients were not milder than the average, so it would not interfere the results.

Among the 420 patients who had received chest radiograph exams, demographic data and clinical characteristics of these patients with or without pleural effusion are shown in Table 4. Among 65 patients with pleural effusion, we obtained pleural effusion for *M. pneumoniae* PCR from 10 patients and 5 (50%) of them had positive results. Cases with pleural effusion had significantly longer time to receive MP-active antibiotics, longer fever duration, and higher rates of bilateral lung consolidation or extrapulmonary manifestations than cases without pleural effusion. Second-line antibiotics were prescribed more in patients with pleural effusion, however, macrolide resistance were the same in both groups.

Bacterial load and co-infection

The median bacterial load of all cases was 1.5×10^5 copies/ml, ranged from 2.6×10^2 to 1×10^9 copies/ml. We divided the patients into two groups by higher or lower than the median bacterial load, with 235 and 236 patients respectively. There was no significant difference of febrile duration, macrolide resistance rate, chest radiograph findings, white blood cell count, C-reactive protein levels, or delayed specimen collection between patients with higher load (over 1.5×10^5 copies/ml) or lower load (less than 1.5×10^5 copies/ml). However, cases in the lower bacterial load group had more extrapulmonary manifestations (21% vs. 13%, $p = 0.01$), eosinophil over 5% (12% vs. 5%, $p = 0.03$) and viral coinfection (10% vs. 4%, $p = 0.04$) than cases in the higher bacterial load group. Of the 471 patients, 336 (71%) patients also underwent throat swabs for viral culture or antigen tests for influenza, para-influenza or respiratory syncytial virus, and 328 (70%) received sputum cultures or urine pneumococcus antigen test. The overall viral co-infection rate was 7.1% (24/336, 11 with adenovirus, 3 with respiratory syncytial virus, 2 with parainfluenza virus, 7 with influenza B and 1 with influenza A) whereas bacterial co-infection rate was 4.6% (15/328, 6 with non-typable *H. influenzae* and 9 with *S. pneumoniae*).

Table 2 Clinical characteristics of patients with severe or fulminant diseases due to *Mycoplasma pneumoniae* infection.

N	Sex	Age	Past history	Diagnosis	Fever duration (days)	ICU stay (days)	Type of ventilation support	Pleural effusion	Macrolide resistance	Antibiotics duration (days)	Interval between onset and active antibiotic treatment (days)
1	M	3	Asthma	Pneumonia	29	26	ETT	EXU	N	AZM(3), LVX (14)	4
2	F	14	nil	Pneumonia	8	6	BiPAP	ND	N	AZM(3), LVX (12)	8
3	M	2	nil	Pneumonia	10	6	ETT	EXU	A2063G	AZM(3), LVX (10)	18
4	M	2	nil	Pneumonia	11	2	BiPAP	ND	A2063G	AZM(3)	None
5	M	4	nil	Pneumonia	28	5	ETT	EXU, PCR+	N	AZM(3), LVX (7), MXF (4)	7
6	M	7	nil	Pneumonia	32	22	ETT	EXU, PCR+	N	AZM(3), LVX (9), DOX (4), MXF (20)	14
7	M	14	CP, HIE	Pneumonia	10	6	BiPAP	ND	N	CLA(5)	8
8	M	16	DLE	Stevens-Johnson syndrome	9	0	ND	None	A2063G	AZM(3), LVX (13)	24

Abbreviations: M: male, F: female, CP: cerebral palsy, HIE: hypoxia ischemic encephalopathy, DLE: discoid lupus erythematous, ETT: endotracheal tube, BiPAP: biphasic positive airway pressure, EXU: exudative pleural effusion, PCR+: positive for *Mycoplasma pneumoniae* polymerase chain reaction, ND: not done for intubation or effusion tapping, N: no resistance, AZM: azithromycin, LVX: levofloxacin, MXF: moxifloxacin, DOX: doxycycline, CLA: clarithromycin.

Table 3 Effect of the interval between disease onset and appropriate antibiotic use on clinical outcomes in *Mycoplasma pneumoniae* infection.

Characteristics	No delayed treatment (N = 186)	Delayed treatment (N = 169)	P value
Fever over 7 days after effective antibiotics	5/165 (3%)	11/127 (9%)	0.04
Severe disease	1/185 (1%)	6/169 (4%)	0.04
Macrolide resistance	7/186 (4%)	37/169 (22%)	<0.001
Extrapulmonary manifestations	23/186 (12%)	43/169 (25%)	0.002
Elevated transaminase	9/134 (7%)	26/129 (20%)	0.001
Consolidation	99/174 (57%)	105/160 (66%)	0.24
WBC count (/uL)	8576 ± 3559	8965 ± 3864	0.35
Eosinophil (%)	1.5 ± 1.9	2 ± 2.6	0.03
CRP (mg/dL)	3.7 ± 3.4	5.2 ± 5.6	0.006

Severe disease was defined as respiratory failure or Stevens-Johnson syndrome.
Data are shown as positive number/tested umber (percentage). WBS denotes white cell count, CRP C-reactive protein.

Discussion

This study reveals *M. pneumoniae* infected children with macrolide resistance or delayed effective treatment had worse clinical outcomes in a tertiary center in Taiwan. MP is one of the most common pathogens for children pneumonia in the post-PCV 13 era. The case number rapidly increased in Year 2015–2016 and decreased in 2017 which may be related to a 4–5 year cyclic epidemic trend reported worldwide.[8,9]

Early diagnosis of MP infection and the awareness of macrolide resistance are the keys for MP-active treatment. Rising macrolide resistance rate, from 5% to 40%, was reported in Japan from 2003 to 2007,[10,11] and recently resistance rate increased up to 80–90% in 2011.[12] In our study, average macrolide resistance rate in 2010–2017 was 23.6%, whereas the previous rate was around 12%–23% in 2011 in Taiwan.[3,6] Though longer disease course were found in patient with macrolide-resistant strains infection, there was no good correlation between macrolide resistance and severe diseases.

Though not frequent, severe and complicated diseases may occur in MP infection. The clinical characteristics of patients with severe respiratory complication or SJS were presented in our study, which was less described in previous literature. Severe cases were rare but might be fatal.[13,14] In our study, male predominance (7 out of 8) was found in severe cases, compatible with the finding in a previous

Table 4 Demographic data and clinical characteristics of *Mycoplasma pneumoniae* infected children with and without pleural effusion.

Characteristics	with pleural effusion (N = 65)	without pleural effusion (N = 354)	P value
Mean age	7.4 ± 4	6.4 ± 3.7	0.57
0–1 y/o	1/65 (2%)	24/354 (7%)	0.26
2–5 y/o	28/65 (43%)	165/354 (47%)	0.65
6–17 y/o	36/65 (55%)	165/354 (47%)	0.31
Male gender	33/65 (51%)	177/354 (50%)	0.58
Inpatient	63/65 (97%)	305/354 (86%)	<0.001
Febrile duration (days)	9.7 ± 6	6.9 ± 3	<0.001
Hospital stay (days)	8.2 ± 8.5	4.5 ± 2.3	<0.001
Time to appropriate antibiotics (days)	6.9 ± 4.2	5.7 ± 3.3	0.02
Second-line antibiotics	22/65 (34%)	49/354 (14%)	<0.001
Lung consolidation	45/65 (69%)	201/354 (57%)	<0.001
Lobar pneumonia	12/65 (18%)	26/354 (7%)	<0.001
Macrolide resistance	15/65 (23%)	84/354 (24%)	0.99
Higher bacterial load	27/65 (42%)	180/354 (51%)	0.30
WBC count (/uL)	7829 ± 2699	9093 ± 3915	0.01
CRP (mg/dL)	6.5 ± 6.6	3.8 ± 3.8	<0.001
Extrapulmonary manifestations	21/65 (32%)	53/354 (15%)	0.002
Skin rash	9/65 (14%)	30/354 (8%)	0.36
Elevated transaminase	14/51 (27%)	28/274 (10%)	0.002
CNS involvement	1/65 (2%)	1/353 (0.3%)	0.001
Severe diseases	5/65 (8%)	2/354 (1%)	<0.001

Data are shown as mean ± standard deviation or positive number/tested umber (percentage). Abbreviations: d: days, WBS: white cell count, CRP: C-reactive protein, CNS: central nervous system.

report.[15] We further revealed the association of delayed effective treatment with more severe disease course including fever lasting for over 7 days after effective antibiotic treatment, more severe disease and more extrapulmonary manifestations whether there are macrolide resistance or not. Limited studies had mentioned the significance of delayed effective treatment. In adult patients admitted to intensive care unit due to MP related respiratory failure, delayed administration of adequate antibiotics contributed to the severity.[16] In a lung function study for 35 children after pneumonia, around half of patients had decreased diffusion capacity for carbon monoxide (DLCO) in MP infection but not in pneumococcal or viral pneumonia.[17] The decreased DLCO was also correlated with delayed macrolide treatment for 10 days or more. Delayed appropriate treatment, no matter macrolide-resistant or not, let the immune response progress, so time to defervescence might be prolonged and more extrapulmonary manifestations or severe disease might occur.[16] In such cases, glucosteroid usually plays a significant role in treatment.[18]

In a previous study, mean MP load was significantly higher in hospitalized patients than in outpatients.[19] However, the bacterial loads of *M. pneumoniae* were not correlated with clinical outcomes in our study, and similar results were also reported previously.[20] We also found that patients with lower bacterial load was significantly associated with more other identifiable viral pathogens and more extrapulmonary manifestations. It indicated the clinical symptoms in the lower bacterial load group might be partly related to the host immune response or co-infection of other viral pathogens.

Co-infection evaluation revealed low mixed infection rate in this study with 7.1% of viral co-infection and 4.6% of bacterial co-infection. In a previous study, the mixed infection rate was detected up to 60% among MP infected children.[21] But it might be related to higher *S. pneumoniae* infection rate before immunization program of PCV-13. About 3% (9/328) of our cases had positive urine pneumococcal antigen test or culture in our study and it also implicated decreased pneumococcus colonization or infection rate in Taiwanese children after national immunization program of PCV-13 since 2015. A larger scale of 5000 MP infected children study reported that viral and bacterial coinfection rates were 9.8% and 2.5%, respectively,[22] and the co-infection rates were similar to those in our study.

There are some limitations in our study. First, it was a single center retrospective study. Data or medical record might be incomplete in some cases. And not all the patients received the same evaluation or treatment principle. Besides, specimens were sent for PCR only in more symptomatic cases, and there were more referred cases with severe symptoms in the tertiary center. These may overvalue the severity of MP infection. Second, the MP PCR specimens were sent infrequently before 2015. Fewer case numbers before 2015 may estimate the MRMP rate inaccurately. Third, the diagnosis for MP infection and coinfection with other possible pathogens were defined by PCR and culture reports. We did not test for MP serology frequently in the study period due to low sensitivity.[20] However, patients with asymptomatic colonization were reported up to 20% and colonization may last for a few months.[23] Though colonization may contribute to our analysis, our patients were all symptomatic and compatible with MP infection clinically. The possibility of colonization was difficult to be distinguished by chart review. Combined PCR with serology for diagnosis may be considered for further study. Lastly, for resistance gene detection, we performed PCR including all known published mutation loci to our best knowledge. Nevertheless, the bacteria could mutate from generation to generation and new resistant loci could occur, and our detection limitation may be possible.

In conclusion, macrolide resistance was fairly common and might lead to delayed appropriate antibiotic treatment. Delayed effective treatment, no matter macrolide resistance or not, was associated with more severe and/or prolonged diseases. Early diagnosis of *M. pneumoniae* as well as the awareness of macrolide resistance make early effective antibiotic treatment possible and may improve clinical outcomes.

Source of funding and conflicts of interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2018.09.009.