Rapid *Mycoplasma* Culture for the Early Diagnosis of *Mycoplasma pneumoniae* Infection

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Early diagnosis of *Mycoplasma pneumoniae* (Mp) plays a pivotal role in its management. We evaluated the role of rapid culture method in early diagnosis of Mp infection and discussed the potential impact factors. A total of 2,600 patients with acute respiratory infection were included, and their pharyngeal swab samples were prepared for Mp rapid culture based on selective Mp fluid culture medium. The clinical contributing factors related to Mp infection were also explored. The positive rate of Mp culture in females was 41.75%, which was higher than that for males (37.63%). Mp infections were incidental to the children and elderly. The positive rates of Mp culture were higher in children aged 3–5 years and adults older than 70 years (54.05 and 31.48%, respectively), compared with other ages. In addition, Mp infection frequently occurred in

winter (December–February) and spring (March–May), with significantly higher positive rates of Mp culture by 40.02 and 42.89 vs. 32.15 and 33.50% in summer (June–August) and autumn (September–November), respectively. The positive rate of rapid culture for Mp was slightly higher than that by Mp antibody assay, but the diagnostic accordance was well between these two methods ($P > 0.05$). Furthermore, clinical common symptoms of respiratory tract infection, such as fever, cough, and expectoration, were not found specific in Mp infection, suggesting that they were not independent prognostic predictors for Mp infection. Therefore, rapid culture based on the Mp pathogen detection would have important clinical application for the early diagnosis of Mp infection. J. Clin. Lab. Anal. 24:224–229, 2010. © 2010 Wiley-Liss, Inc.

Key words: *Mycoplasma pneumoniae*; rapid culture; early diagnosis; antibody detection

INTRODUCTION

Mycoplasma pneumoniae (Mp) is a pathogenic micro-organism between bacteria and virus, which often causes respiratory tract infection (RTI), pharyngitis, or pneumonia, simultaneously or sequentially leading to the damages in the nervous system, heart, kidney, and many other organs (1–3). According to the epidemiological survey, the incidence of Mp infection is high in healthy population, and pneumonia caused by Mp accounts for half of non-bacterial pneumonia (4). Mp infection can occur in any age range, especially in preschool children and elderly patients (5).

Mp is a common respiratory tract pathogen that causes up to 40% of cases of community-acquired pneumonia in children (6). A specific diagnosis is essential because treatment of Mp infection with β -lactam antibiotics is

ineffective. The clinical manifestations of Mp infection are complicated and there are no specific symptoms and signs to identify it from the common infection caused by bacteria or viruses. Therefore, accurate and rapid differentiation of Mp from other clinically relevant bacteria or viruses is pivotal for early diagnosis of Mp infection.

The serology Mp antibody (Mp-Ab) assay remains an important diagnostic tool in patients suspected of having Mp infection. Owing to indirect detection of Mp

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pathogen and false negatives resulting from the window period of Mp infection, its utility for early diagnosis of Mp infection is limited (7). Recently developed PCR techniques show high levels of specificity and sensitivity for the rapid detection of Mp in clinical specimens (8); however, PCR alone is not always sufficient for a diagnosis (9). Mp culture has been considered as the gold standard for Mp detection. Conventional Mp culture method is time consuming and complicated in procedure, which is unable to serve the demand of clinics for rapid and early diagnosis. Based on the use of specific liquid culture medium, the rapid Mp culture has the advantages in convenience, rapidity, and specificity over traditional culture method, in which only the growth of Mp is promoted while growth of bacteria and the other microorganisms are suppressed by a supplement of specific antibacterial agents.

This study is aimed at evaluating the diagnostic value of Mp rapid culture for early diagnosis of Mp infection. We analyzed the results of Mp rapid culture from 2,600 patients with acute respiratory infection and discussed the potential factors influencing the results of Mp culture. This study will help in the improvement of the early diagnostic process of Mp infection and better management in clinical practice.

MATERIALS AND METHODS

Subjects

A throat swab was taken on admission from 2,600 patients (1,817 outpatients, 783 inpatients, and range from 6 months to 85 years) who came in for treatment of acute respiratory tract infection. The patients were grouped according to gender, age, and sampling time (see Tables 1 and 2). The occurrence of Mp infection was investigated in male and female, at different ages as well as in different seasons. A total of 203 patients had received pharmacotherapy pre-treatment, which included medication aimed at improvement of symptoms of RTI, such as antipyretics, antitussives, or expectorants, etc., macrolides and non-macrolide antibiotics (penicillin or cephalosporins) (see Table 3). The effect of different pharmacotherapy on the results of Mp culture was investigated. Of these patients, on admission, serum specimen was taken from 194 inpatients suspected of Mp infection, besides throat swab samples. The serum specimens were examined for serum antibody against Mp.

Laboratory Methods

All throat specimens were examined for the presence of Mp using rapid culture in specific medium (Baishengyuan, Shanxi, China). According to the manufacturer's

TABLE 1. Analysis of Mp Culture in Different Gender and Age Population ($n = 2,600$)

Group	Samples	Mp ⁺	Positive rate (%)	χ^2	P-value
Gender					
Male	1,491	561	37.63	4.53	0.033
Female	1,109	463	41.75		
Children					
(1) 1 year	139	23	16.55	66.71	<0.001
(2) 1–2 years	294	125	42.52	11.45	<0.001
(3) 3–5 years*	803	434	54.05		
(4) 6–12 years	775	339	43.74	16.76	<0.001
Adults					
(5) 13–60 years	214	47	21.96	4.3352	0.037
(6) 61–70 years	129	25	19.38	5.45	0.02
(7) 71–80 years**	162	51	31.48		
(8) > 80 years	84	17	20.24	3.496	0.062

*: (1), (2), (4) vs. (3), **: (5), (6), (8) vs. (7).

TABLE 2. Analysis of Mp Culture in Different Seasons ($n = 2,600$)

Season/month	Samples	Mp ⁺	Positive rate (%)	χ^2	P-value
Spring/March–May	963	413	42.89	17.605	<0.001
Summer/June–August	311	100	32.15		
Autumn/September–November	394	132	33.50		
Winter/December–February	932	373	40.02		

The positive rate of Mp culture sampling from different seasons was different. Mp was isolated from 963 specimens (40.02%) from spring, 932 specimens (40.02%) from winter, 394 specimens (33.5%) from autumn, and 311 specimens (32.15%) from summer, respectively. The highest positive rate of Mp culture was found in spring (42.89%) and the lowest in summer (32.15%). Among the 2,600 total clinical specimens, there were 311 samples (10%) from summer and only one-third of that from autumn or winter. Mp, *Mycoplasma pneumoniae*.

TABLE 3. Effect of Drugs on Result of Mp Culture ($n = 203$)

Group	Samples	Mp ⁺	Positive rate (%)	χ^2	P-value
Symptomatic drugs	114	65	57.02	0.647	0.724
Macrolides	19	9	47.37		
Non-macrolide antibiotics	70	38	54.29		

A total of 203 patients suspected of Mp infection received pretreatment pharmacotherapy, and the presence of Mp were 57.02, 47.37, and 54.29% in patients administrated by medication for eliminate clinical symptoms, macrolide antibiotics, and non-macrolide antibiotics, respectively. Although the positive rate of Mp culture in patients with macrolide antibiotics treatment was lowest, there was no significant difference between macrolide antibiotics and symptomatic medication or non-macrolide antibiotics ($P > 0.05$). Mp, *Mycoplasma pneumoniae*.

protocol, the throat swabs from pharynx and larynx inflammatory sites were immediately put into the culture medium and stirred several times. Then, the samples were incubated at 37 for 24 hr in the culture medium. The metabolites of Mp can change the color of medium and it is used as an indicator of the presence of Mp. When the color of medium changes from red to yellow clarification, it suggests the presence of Mp. No changes in medium color or medium turbiding indicated absence of Mp.

The serum Mp-Ab was examined using passive agglutination test (Fujirebio, Japan). Reaction rationale as below the gelatin particles, sensitized by synthetic Mp cell membrane, and can agglutinate with serum Mp-Ab (IgA+IgM+IgG) where this action is visible to the naked eye. By observing the occurrence of agglutination reaction, the serum Mp-Ab can be semi-quantitatively analyzed. A total of 2 ml of blood was centrifuged to obtain the serum. The serum was dripped onto the microreaction plate using serial dilution method and then incubated with gelatin particles for 3 hr at room temperature. The results were assessed by comparing the sample agglutination action with the standard agglutination action. Agglutination occurring between serum sample and unsensitized particles (1:20) suggested negative (–) and occurring between serum and sensitized particles (1:40) indicated positive (+). The final dilution ratio of unsensitized particles is just the Mp-Ab titer when positive agglutination occurred. Moreover, negative result was also considered when no agglutination occurred between serum and sensitized particles (1:40), regardless of the action between serum and unsensitized particles. Then the coincidence rate for Mp detection was demonstrated between these two methods (see Table 4).

STATISTICAL ANALYSIS

Statistical analysis was performed by SPSS version 13.0. The positive rate of Mp culture were calculated and analyzed by *Chi-square test* in patients in different gender, age, and season. The correlation analysis on the clinical symptoms and signs, as well as the result of

blood examination, and the culture of Mp were performed using the logistic multiple regression method in an attempt to provide laboratory evidence for early diagnosis of Mp infection. A *P* value of less than 0.05 was considered significant.

RESULTS

The complete results obtained using Mp rapid culture methods of a total of 2,600 samples studied are shown in Table 1 and Figure 1. The positive rate of Mp culture in women (41.75%) was significantly higher than that in men (37.63%) (*P*<0.05). In the children subgroups, the positive rate (16.56%) was the lowest in children younger than 1 year and the highest in preschool children aged 3–5 years (54.05%) compared with the other subgroups (*P*<0.001). In the adult subgroups, the individuals older than 70 years of age had higher positive rate (31.48%) than the other subgroups (*P*<0.05). The results indicated significant difference in occurrence of Mp infection in different age groups.

The positive rate of Mp culture for samples from different seasons was compared with explore its temporal characteristics (Table 2 and Fig. 2). The highest positive rate was found in spring (42.89%) and moderate positive rate was from autumn to winter (40.02 and 33.50%, respectively). The lowest positive rate of Mp culture was from summer (32.15%). Moreover, the amount of total samples tested in summer was only one-third of that in autumn or winter, from which it can be inferred that RTI by Mp was not often seen in summer.

Of the 203 patients who received pretreatment pharmacotherapy, 114 were administrated with medication to eliminate clinical symptoms of RTI, 19 patients with macrolide antibiotics, and 70 with non-macrolide antibiotics, and the positive rate of Mp culture were 57.02, 47.37, and 54.29%, respectively. Although the positive rate of Mp culture in patients with macrolide

TABLE 4. Comparison Between Rapid Mp Culture and Mp-Ab Detection (*n* = 194)

	Positive cases (+)	Positive rate (%)	<i>P</i> -value
Mp culture	44	22.68	0.556
Mp-Ab	38	19.59	
	Mp-Ab(+)	Mp-Ab(–)	<i>P</i> -value
Mp culture (+)	5	39	0.118
Mp culture (–)	33	117

Fig. 1. Result of rapid Mp culture in different age ranges (*n* = 2,600). Mp, *Mycoplasma pneumoniae*.

Fig. 2. Analysis of Mp culture in different seasons ($n = 2,600$). The positive rate of Mp culture sampling from different seasons was significantly different. Among 2,600 total clinical specimens, 963 were from spring and 311 from summer, which was only one-third of those from autumn or winter. The positive rate of Mp detection from spring, winter, autumn, and summer was 42.89 (413/963), 40.02 (373/932), 33.5 (132/394), and 32.15% (100/311), respectively. The highest positive rate of Mp culture was found in spring and the lowest in summer. Mp, *Mycoplasma pneumoniae*.

Fig. 3. Impact of drugs on results of rapid Mp culture ($n = 203$). Patients who received pretreatment pharmacotherapy, 114 were administered with medication to eliminate clinical symptoms of RTI, 19 with macrolide antibiotics, and 70 with non-macrolide antibiotics, where the positive rate of Mp detection were 57.02 (65/114), 47.37 (9/19), and 54.29% (38/70), respectively. Although the positive rate of Mp culture in patients with macrolide antibiotics treatment was lowest, there was no significant difference between macrolide antibiotics administration and symptomatic medication or non-macrolide antibiotics. Mp, *Mycoplasma pneumoniae*.

antibiotics treatment was lowest, there was no significant difference between macrolide antibiotics administration and symptomatic medication or non-macrolide antibiotics, which indicated that these drugs might not affect the result of Mp culture ($P > 0.05$) (Table 3 and Fig. 3).

The results of Mp rapid culture was compared with that of serum Mp-Ab detection, using passive agglutination test for the samples of 194 inpatients (Table 4 and Fig. 4). Forty-four patients (22.68%) indicated Mp presence using Mp rapid culture (22.68%) and 38 were Mp-Ab positive in passive agglutination test (19.59%). The coincidence rate of these two methods was 90% ($P > 0.05$). Additionally, we proved that positive Mp presence was both in Mp culture and serum Mp-Ab

Fig. 4. Comparison between rapid Mp culture and serum Mp-Ab detection ($n = 194$). Mp-Ab, Mp antibody.

detection in only 5 patients, whereas 71 patients were confirmed with Mp absence in both. It can be inferred that, although there is a favorable general correspondence between these two methods, the results for one specific sample can be different through Mp rapid culture method and serum Mp-Ab test, and it maybe predicts different meanings in practice.

Clinical symptoms and signs from 346 inpatients suspected of Mp infection, as well as the results of blood examination, were collected and analyzed using logistic multiple regression method. A significant correlation was found between the positive results of Mp culture and age besides gender ($P < 0.05$). Season was another contributing factor, which was in agreement with the results that the highest positive rate of Mp culture was found in patients in spring and the lowest in summer. These data indicates that age, gender, and season were the potential risk factors for the occurrence of Mp infection. There was no significant correlation between the results of Mp culture and the taking of common medication, including macrolide or administration and some symptomatic medication. Additionally, blood routine examination results, including WBC count, neutrophil, and lymphocyte count, was found in slight correlation with the results of Mp culture ($P > 0.05$). No clinical symptoms or signs of RTI, such as cough, expectoration, and body temperature, etc., were found to be correlated with the occurrence of Mp infection (regression coefficient < 0.7), which indicated that there was no specific clinic manifestations for the diagnosis of Mp infection (Table 5).

DISCUSSION

The diagnosis of Mp in patients with RTI remains an area of uncertainty. The classic symptom complex of infection by Mp consists of fever, tachypnea, and cough. However, symptoms can vary widely and are often

TABLE 5. Analysis of Clinical Effect Factors on Results of Mp Culture (*n* = 346)

Variables	<i>B</i>	SE	Wald	<i>P</i> -value	OR	95% CL
Gender	0.602	0.270	4.983	0.026	1.825	1.076–3.095
Season	0.498	0.199	6.251	0.012	1.645	1.114–2.430
Wards	–0.125	0.229	0.298	0.585	0.882	0.563–1.383
Age	0.024	0.006	17.868	0.000	1.024	1.013–1.036
Drug treatment	0.175	0.195	0.805	0.370	1.191	0.813–1.746
Cough	–0.982	0.285	11.872	0.001	0.375	0.214–0.655
Expectoration	0.038	0.292	0.017	0.897	1.038	0.586–1.842
Temperature	0.014	0.265	0.003	0.957	1.014	0.604–1.704
WBC(10^6)	–0.040	0.287	0.019	0.890	0.961	0.547–1.688
Neutrophils (%)	0.479	0.344	1.947	0.163	1.615	0.824–3.168
Lymphocyte (%)	0.188	0.391	0.232	0.630	1.207	0.561–2.595
Diagnosis	0.185	0.172	1.158	0.282	1.203	0.859–1.686

B: regression coefficient; SE: standard error.

nonspecific, and a definite early diagnosis of Mp infection is difficult. This study has shown for the first time that Mp rapid culture based on specific Mp culture medium is a sensitive assay for the identification of presence by Mp in patients with RTI. According to our results, the sensitivity and specificity of Mp rapid culture for the diagnosis of Mp infection were significantly same as the serological Mp-Ab assay, but less time consuming. Using this method, we found a marked difference in the positive rate of Mp detection in different populations. The rate of MP detection from throat swab secretion was higher in individuals older than 70 years of age compared with other adults. The positive rate of MP detection was highest in children aged 3–5 years amongst the children group. Although a relatively low positive rate of Mp detection was discovered in children younger than age 3, owing to lack of strong immunity to Mp infection and high risks of poor prognosis, more attention should be paid to these children (10). We also found climate and temperature are the main effect factors on the detection rate of Mp. The positive rate of Mp presence was higher, especially in the alternating autumn–winter or winter–spring than those in other seasons, which may be owing to the climate impermanence or large temperature difference, raising the risk of getting Mp infections by weakening the body's defenses. Another reason is that autumn and spring are the back-to-school seasons at all levels in our country; cross-infection between the students was an important reason for the high rate of Mp detection (11).

Macrolide antibiotics (e.g., erythromycin, azithromycin, clarithromycin, and roxithromycin) were confirmed to be effective for Mp infection and used as the drug of choice for treatment of Mp infection (12,13). Our results suggested that the presence of Mp in patients by the administration of macrolide antibiotics decreased, but there was no significant difference compared with those

by the administration of symptomatic medication or non-macrolide antibiotics, which might be on account of the smaller sample size in this study. Future study with a larger population may obtain the results in accordance with clinical practice. These results also remind us that throat swab sampling should be performed before treatment to avoid drug-induced false negative of Mp culture.

Although results showed that both Mp culture and serum Mp-Ab detection method have diagnostic accordance rate of 90% and were appropriate for the diagnosis of the presence of Mp, the applicable time period and course of disease of these two methods suitable for use are different. For serum Mp-Ab, positive of Mp detection reaches the maximum from 7 to 10 days during the course of Mp infection, and as the infection progresses, the titers of Mp-Ab elevated. In general, the presence of Mp may be detected beginning 5 days before symptoms develop and up to 7 or more days after infection. For Mp culture method, the presence of Mp may be detected on days 1–3 in the early stages of Mp infection. According to our results, it is advisable to employ an appropriate test method for Mp presence, in view of the infectious progression and medical treatment. Mp culture should be performed at the time of admission for the patients suspected of Mp infection, to benefit early diagnosis and treatment of Mp infection. Serological dynamical Mp-Ab detection may be supplementary for monitoring the disease progression on the days 5–7 after Mp infection.

No specific clinic manifestations were found in patients with Mp infection except for common symptoms of RTI, such as throat congestion, swollen tonsils, chest pain, etc. Increase of neutrophil percentage and decrease of lymphocyte percentage were found in 60% of patients. Cough and expectoration can be found in almost all patients and one-third of patients have rising body temperature. However, all these changes of symptoms or signs were not significantly associated with the results of Mp culture, which was consistent with the literature that reported there was difficulty in identifying patients with Mp infection from the other respiratory infections, and the laboratory tests for Mp was the only available diagnosis of Mp infection (14,15). In addition, Mp infection associated with chronic obstructive pulmonary disease (COPD) could increase the probability of aggravating the latter (16,17). For COPD patients, it is also necessary to give some preventive action for accompanying Mp infection.

In this study, we have shown that Mp rapid culture based on specific Mp culture medium was an applicable method for early diagnosis of Mp infection. The general positive rate for the presence of Mp is similar to that of serum Mp-Ab method, and has the merits of easy

operation, fast speed, specificity, and so on, with good prospects. The use of Mp rapid culture may improve current laboratory diagnostic strategies for Mp in patients in clinical practice.

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