

Implementation of Point-of-Care Molecular Diagnostics for *Mycoplasma pneumoniae* Ensures the Correct Antimicrobial Prescription for Pediatric Pneumonia Patients

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Mycoplasma pneumoniae is a leading causative pathogen of pneumonia among pediatric patients, and its accurate diagnosis may aid in the selection of appropriate antimicrobial agents. We established a rapid reporting system of a polymerase chain reaction (PCR) examination for *M. pneumoniae* that enables physicians to obtain test results approximately 90 minutes after ordering the test. In this study, we evaluated the impact of this system on antimicrobial prescriptions for pediatric pneumonia patients after its implementation from May 2016 to April 2017. In total, we identified 375 pediatric pneumonia patients, and the results of the rapid PCR examinations for *Mycoplasma pneumoniae* were reported immediately in 90.7% of patients (340/375), with physicians able to use these results to decide on patients' management before the prescription of antimicrobial agents. Of the 375 pediatric pneumonia patients, *M. pneumoniae* was detected in 223 (59.5%). Among the 223 *M. pneumoniae*-positive pneumonia cases, antimicrobial agents for atypical pathogens (macrolides, tetracyclines or quinolones) were prescribed in 97.3% (217/223) at the initial evaluation, and their prescription rates increased to 99.1% (221/223) during management. In contrast, antimicrobial agents for atypical pathogens were prescribed only in 10.5% of 152 *M. pneumoniae*-negative pneumonia cases at the initial evaluations, and only 1 additional case was prescribed clarithromycin for persistent symptoms during management. In conclusion, we show that molecular technology could be applicable in the field of point-of-care testing in infectious disease, and its implementation will ensure the correct antimicrobial prescription for pediatric pneumonia patients.

Keywords: antimicrobial agent; antimicrobial stewardship; *Mycoplasma pneumoniae*; pneumonia; point-of-care molecular diagnostics

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Introduction

Mycoplasma pneumoniae causes a variety of infectious diseases, including respiratory infections, otitis media, erythema exudativum multiform and encephalitis (Waites and Talkington 2004). *M. pneumoniae* infections are endemic in late summer and autumn (Yamazaki and Kenri 2016), and *M. pneumoniae* is a major causative pathogen of pneumonia, with its detection rate highest among school-age pediatric pneumonia patients (Morozumi et al. 2004). Unlike other bacteria, beta-lactam agents are ineffective against *M. pneumoniae*, and the difficulty of a diagnosis has

led to a delay in effective treatment.

Thus far, there have been no specific clinical manifestations associated with *M. pneumoniae* pneumonia (Wang et al. 2012). A four-fold increase in *M. pneumoniae* IgG titers in acute and convalescent sera is considered to be the 'gold standard' for the diagnosis of acute *M. pneumoniae* respiratory infection (Gavranich and Chang 2005). This approach, however, is not suitable for the diagnosis during the acute phase of the infections. *M. pneumoniae* IgM has been alternatively used as a diagnostic test at the initial presentation, although there is a 10.1% false positivity rate and a 32.5% false negativity rate with this approach; therefore,

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testing paired sera of *M. pneumoniae* IgM is still necessary (Lee et al. 2017). Recently, antigen testing for *M. pneumoniae* has been developed for the rapid detection of *M. pneumoniae* from nasopharyngeal samples, but the sensitivity is reported to be < 60% (Sano et al. 2016).

The GENECUBE system is a polymerase chain reaction (PCR)-based automated molecular identification system that can perform all PCR examination processes in a closed system and can analyze up to eight samples simultaneously. This system has been widely used for the identification of mycobacterium (Hida et al. 2012). In late 2015, the GENECUBE system was newly approved in Japan and has since shown high accuracy for the molecular identification of *M. pneumoniae* with oropharyngeal samples with quick preparations within an hour (Nakajima et al. 2016; Suzuki 2017). Due to these systems' ease of handling and rapidity, results can be made available during the initial evaluations along with blood tests and radiological imaging, which may aid in the selection of appropriate antimicrobial agents. However, the utility of this system in this respect has not yet been proven.

We developed a rapid molecular identification tool and a novel reporting system for *M. pneumoniae* with GENECUBE Mycoplasma and have used them at our facility since May 2016. In this study, we evaluated the impact of the implementation of this system on antimicrobial prescriptions.

Materials and Methods

This study was performed at Tsukuba Medical Center Hospital (TMCH; 453 beds), which is located next to the University of Tsukuba Hospital and functions as a primary pediatric emergency center and tertiary emergency medical center in the Tsukuba district of Japan. We analyzed the clinical data of pneumonia patients under 18 years of age who underwent a PCR examination for *M. pneumoniae* with the GENECUBE Mycoplasma between May 2016 and April 2017. This study was conducted with the approval of the ethics committee of TMCH (approved number: 2016-043).

Diagnostic system for the molecular detection of M. pneumoniae

At TMCH, an oropharyngeal swab (FLOQ swab 5U005S dual; Becton, Dickinson and Company, Tokyo, Japan) is promptly transferred to an in-house microbiology laboratory after samples are obtained either in outpatient services or inpatient wards, and molecular examinations for *M. pneumoniae* are performed with the GENECUBE Mycoplasma as quickly as possible between 9 AM and 3 PM, Monday to Friday; the median time between physicians ordering the test and the completion of the examination was 94 minutes (79-114 minutes), as previously reported (Suzuki 2017). On the weekends or holidays, molecular examinations for *M. pneumoniae* are performed once in the morning. The results are reported via electronic charts and telephones as soon as the examinations are completed.

Definition of pneumonia and the evaluation of data

We defined pneumonia as radiological evidence of pneumonia with compatible clinical symptoms and without other causes of

abnormal radiological findings (Jain et al. 2015a, b). The radiological review was independently performed by two physicians. Only patients diagnosed with pneumonia by both physicians were included as pneumonia cases, and discordant cases were further reviewed by a board-certified radiologist (M.S.) to ultimately determine which cases had pneumonia.

We collected background data on age (school age [6-17 years of age]), gender, comorbidities, month of the diagnosis, history of close contact with *M. pneumoniae* patients, history of preceding antimicrobial use, history of symptoms (rhinorrhea, sputum, severe cough, hypoxia [peripheral capillary oxygen saturation level < 90%], diarrhea, skin rashes and a high fever [$\geq 38^\circ\text{C}$]), presence of crackles on chest auscultation at presentation, duration of symptoms until the examination, laboratory findings (white blood cell [WBC] count and C-reaction protein [CRP] level) obtained within a day of the molecular examination, presence of pneumonia and the requirement of hospitalization before or at presentation. The endemic season was defined as August to December. Comorbidities were included when cases had chronic diseases, as defined by Charlson's comorbidity index (Charlson et al. 1987) or an equivalent. Severe cough was defined as cough with vomiting, sleep disturbance or constant cough.

Statistical analyses and the evaluation of data

We evaluated the prescription rate of macrolides, tetracyclines and quinolones on day 0, day 1 and days 2-3 after samples were obtained among *M. pneumoniae*-positive pneumonia cases and *M. pneumoniae*-negative pneumonia cases, as judged by the results of molecular examinations for *M. pneumoniae*.

As a supplementary analysis, we compared the clinical backgrounds between *M. pneumoniae*-positive pneumonia cases and *M. pneumoniae*-negative pneumonia cases. Categorical variables were analyzed using Fisher's exact test, and continuous variables were compared using the Mann-Whitney U test. Variables with a significant association ($P < 0.05$) according to the univariate analysis were included in the multivariate analysis after considering confounding factors. The SPSS version 20 software package (IBM, Armonk, NY, USA) was used for statistical analyses.

Results

Monthly trends in the molecular identification of M. pneumoniae infections and the selection of cases for the current study

During the 1-year study period, 1,071 non-duplicated pediatric cases underwent a molecular examination for the detection of *M. pneumoniae*, and *M. pneumoniae* was identified in 316 cases (29.5%). One case showed negative results for *M. pneumoniae* with the GENECUBE Mycoplasma, but a positive result had already been obtained on a LAMP examination at a previous hospital, so this case was included as a *M. pneumoniae*-positive case in this study. The number of cases with *M. pneumoniae* exceeded 30 between August and December 2016 and reached 64 in November 2016 (Fig. 1).

Most of the 316 cases had a fever (305/316; 96.5%) or a cough (302/316; 95.6%), and 223 cases (70.6%) met the study-criteria as *M. pneumoniae*-positive pneumonia cases. Of the 93 cases not meeting the pneumonia criteria, 84 had respiratory symptoms including cough and/or sputum (45,

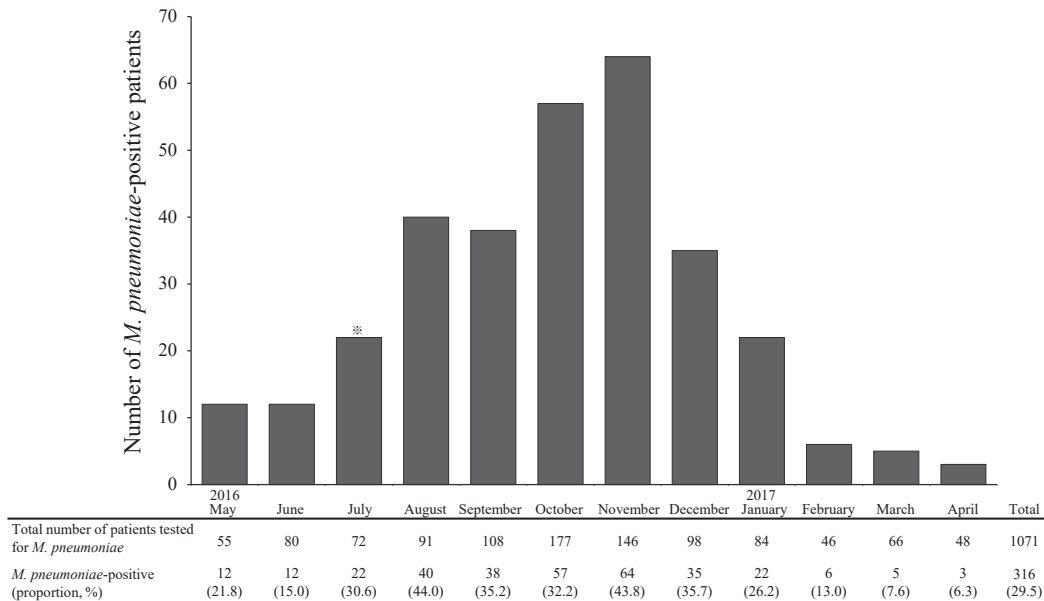


Fig. 1. Monthly trends in *Mycoplasma pneumoniae*-positive cases and their detection rate.

*One patient has discordant *M. pneumoniae* test results: GENECUBE Mycoplasma-negative and LAMP-positive.

no pneumonia findings on chest X-ray; 39, no chest X-ray at all). Of the nine cases without respiratory symptoms, eight had a fever without any specific symptoms, and one had erythema multiforme.

We also identified 152 *M. pneumoniae*-negative pneumonia cases among the 1,071 cases for a comparison. In total, 375 pneumonia cases (223; *M. pneumoniae*-positive, 152 *M. pneumoniae*-negative) were included in the current study.

Clinical characteristics of *M. pneumoniae*-positive pneumonia cases and their comparison with *M. pneumoniae*-negative pneumonia cases

In the analysis of the 223 *M. pneumoniae*-positive pneumonia cases, the median age was 7.0 years old (female: 50.2%), and school-age children comprised 65.5% of cases (Table 1). Comorbidities were described only in 12.6% of *M. pneumoniae* cases. Furthermore, 71.7% of *M. pneumoniae* infected patients visited our hospital during the endemic season. A history of close contact with *M. pneumoniae* patients was noted in 17.0%, while a history of antimicrobial use was noted in 60.5%. Regarding clinical symptoms, rhinorrhea and sputum or productive cough were noted in 13.9% and 22.4%, respectively. Crackles were auscultated in 16.6% of *M. pneumoniae* cases. Only 3.1% of *M. pneumoniae* cases had hypoxia in their history or at the examination. A WBC count of $< 10,000/\mu\text{L}$ was observed in 83.4% of cases, and a CRP level of < 4 mg/dL was observed in 76.7% of cases. Hospitalization was required in 30.5% of cases before or on the day of the molecular examination. None of the cases died in this study.

A comparison between *M. pneumoniae*-positive pneu-

monia cases and *M. pneumoniae*-negative pneumonia cases showed that school age (odds ratio [OR] 7.98, 95% confidence interval [CI]: 3.38-18.82), a diagnosis in the endemic season (OR 4.89, 95% CI: 2.37-10.06), a history of close contact with *M. pneumoniae* patients (OR 5.07, 95% CI: 1.39-18.48) and a low WBC count ($< 10,000/\mu\text{L}$) (OR 4.07, 95% CI: 2.04-8.11) were strong factors positively associated with a positive result of *M. pneumoniae*. In contrast, a duration from the onset to the evaluation (OR 1.21, 95% CI: 1.07-1.36) had a weak association with *M. pneumoniae*-positive cases. The presence of comorbidities (OR 0.42, 95% CI: 0.19-0.93), a history of sputum or productive cough (OR 0.43, 95% CI: 0.21-0.88) and the need for hospitalization (OR 0.47, 95% CI: 0.23-0.98) were negatively associated with the molecular detection of *M. pneumoniae* among pneumonia cases.

Time-dependent rates of pneumonia cases treated with macrolides, tetracyclines or quinolones

During the study period, the results of the molecular examinations for *M. pneumoniae* were reported immediately in 90.7% of pneumonia cases (340/375), and physicians were able to use these results to determine patients' management before the prescription of antimicrobial agents. The results of the other 35 cases were reported after the initial management because the molecular examination was ordered at night or on the weekend.

Among the 223 *M. pneumoniae*-positive pneumonia cases, antimicrobial agents for atypical pathogens (macrolides, tetracyclines or quinolones) were prescribed in 97.3% of cases (217/223) at the initial evaluation, and their prescription rates increased to 99.1% (221/223) during management (Fig. 2). In contrast, antimicrobial agents for atyp-

Table 1. Clinical characteristics of *Mycoplasma pneumoniae*-positive pneumonia cases and their comparison with *M. pneumoniae*-negative pneumonia cases.

	<i>M. pneumoniae</i> -positive	<i>M. pneumoniae</i> -negative	Crude P value	Adjusted P value	Odds ratio (95% CI)
n	223	152			
Age (years)	7 [4, 10]	2 [1, 4]	< 0.001		
School age (6-17 years old)	146 (65)	22 (14)	< 0.001	< 0.001	7.98 (3.38-18.82)
Female	112 (50)	83 (55)	0.338		
Comorbidities	28 (13)	59 (39)	< 0.001	0.032	0.42 (0.19-0.93)
Asthma	22 (10)	43 (28)	< 0.001		
Immunosuppressive state	2 (1)	1 (1)	0.999		
Endemic season	160 (72)	62 (41)	< 0.001	< 0.001	4.89 (2.37-10.06)
Close contact	38 (17)	6 (4)	< 0.001	0.014	5.07 (1.39-18.48)
Household	18 (8)	1 (1)			
School or workplace	17 (8)	5 (3)			
Preceding antimicrobial use	135 (61)	48 (32)	< 0.001	0.058	1.99 (0.98-4.04)
(macrolides, quinolones or tetracyclines)	106 (48)	30 (20)	< 0.001		
Onset to evaluation (days)	7 [6, 8]	5 [4, 7]	< 0.001	0.002	1.21 (1.07-1.36)
Rhinorrhea	31 (14)	43 (28)	0.001	0.145	0.56 (0.25-1.23)
Sputum or productive cough	50 (22)	83 (55)	< 0.001	0.020	0.43 (0.21-0.88)
Severe cough	56 (25)	36 (24)	0.807		
Hypoxia	7 (3)	20 (13)	< 0.001	0.639	0.75 (0.22-2.52)
Diarrhea	9 (4)	11 (7)	0.241		
Skin rashes	6 (3)	5 (3)	0.763		
Fever ($\geq 38^{\circ}\text{C}$)	185 (83)	130 (86)	0.567		
Crackles on chest auscultation	37 (17)	47 (31)	0.002	0.460	0.74 (0.33-1.66)
WBC count (μL)	7,100 [5,800, 9,000]	12,300 [8,500, 16,300]	< 0.001		
WBC count < 10,000/ μL	161/193 (83)	51/145 (35)	< 0.001	< 0.001	4.07 (2.04-8.11)
CRP (mg/dL)	2.0 [1.2, 3.7]	2.3 [1.0, 5.4]	0.162		
CRP < 4 mg/dL	148/193 (77)	89/146 (61)	0.002	0.008	2.88 (1.32-6.25)
Requirement of hospitalization	68 (30)	110 (72)	< 0.001	0.043	0.47 (0.23-0.98)

Categorical data are presented as number (proportion, %). Continuous data are presented as median [interquartile range]. WBC, white blood cell; CRP, C-reactive protein.

ical pathogens were prescribed only in 10.5% (16/152 cases) of *M. pneumoniae*-negative pneumonia cases at the initial evaluations, and only 1 additional case was prescribed clarithromycin for persistent symptoms during management.

Among the 35 pneumonia cases with no molecular examination results available during the initial evaluation due to submission at night or during a holiday, *M. pneumoniae* was detected in 22 cases. Of these 22 cases, antimicrobial agents for atypical pathogens were prescribed in 81.8% (18/22) at the initial evaluations. Antimicrobial agents for atypical pathogens were prescribed for the remaining four cases the day after the initial evaluation, based on positive results for *M. pneumoniae*. Regarding the 13 *M. pneumoniae*-negative pneumonia cases among

the 35 pneumonia cases, an antimicrobial agent for atypical pathogens was prescribed in only 1 case.

Discussion

Recently, rapid molecular examinations have been increasingly applied for the diagnosis of acute respiratory infections. Although the clinical usefulness of such examinations was indicated for the diagnosis of influenza (Green et al. 2016), its utility for the rapid diagnosis of *M. pneumoniae* has not yet been proven. In our current study, the results of a molecular examination for *M. pneumoniae* were available along with those of other laboratory tests or radiological imaging studies in more than 90% of cases with pneumonia, and antimicrobial agents for atypical pathogens were prescribed in 97.3% of cases at the initial evaluation.

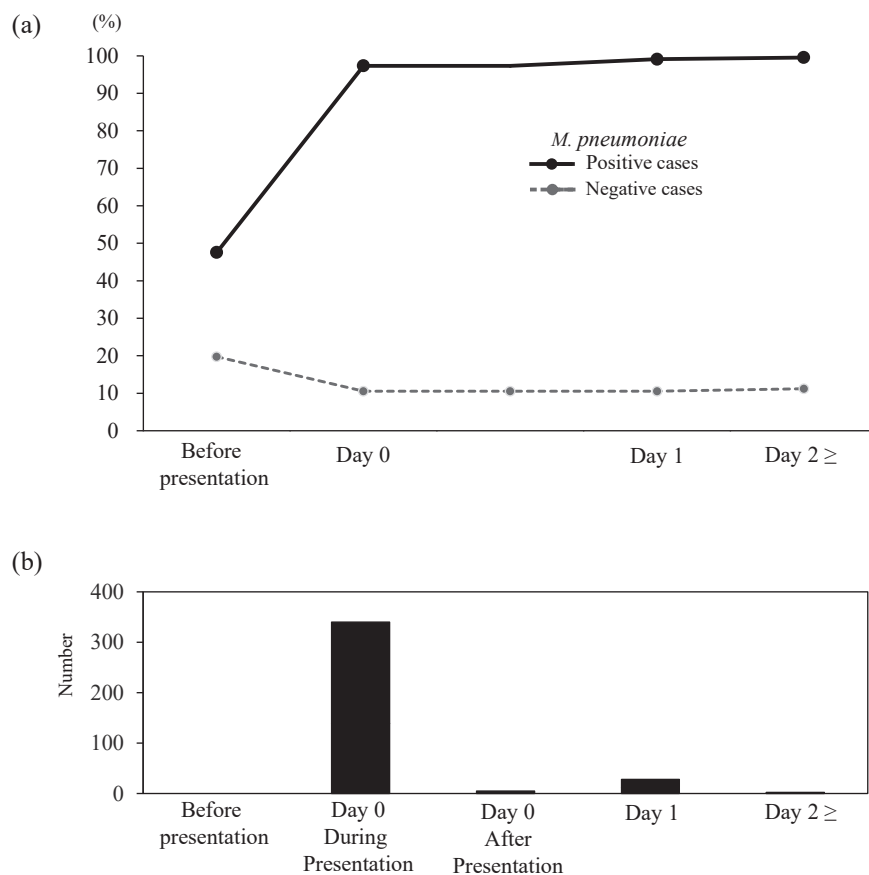


Fig. 2. Correct antimicrobial use for *Mycoplasma pneumoniae* pneumonia with the rapid reporting system. (a) Antimicrobial use (macrolides, tetracyclines or quinolones) among patients with pneumonia. (b) Reporting day of the results of the GENECUBE *Mycoplasma* analysis after obtaining oropharyngeal samples. We defined “Day 0” as the day of patient presentation, “Day 1” as a day after patient presentation, and “Day ≥ 2” as two or more days after patient presentation.

In contrast, antimicrobial agents for atypical pathogens were prescribed in only 10.5% of *M. pneumoniae*-negative pneumonia cases. In addition, the later prescription of antimicrobial agents for atypical pathogens was performed for only one *M. pneumoniae*-negative pneumonia cases. These findings show that the rapid molecular diagnosis of *M. pneumoniae* was practically possible at an acute care hospital in Japan, and the results of this examination influenced the prescription pattern of antimicrobial agents for atypical pathogens.

The clinical diagnosis was also considered useful in this study, and antimicrobial agents for atypical pathogens were prescribed properly, even at night or during a holiday, when the results of a rapid molecular analysis were not available. Current guidelines for the management of respiratory infectious diseases in children in Japan (Ouchi et al. 2017) endorse predictivity scores for the prediction of *M. pneumoniae* pneumonia in pediatric patients ((1) age ≥ 6 years old, (2) no comorbidities, (3) beta-lactam antimicrobial agent use in the preceding week, (4) good general condition, (5) dry cough, (6) no crackles on chest auscultation, (7) segmental distribution of pneumonia by chest X-ray, (8) low WBC count [$< 10,000/\mu\text{L}$] and (9) low CRP level [< 4

mg/dL]; pneumonia cases with ≥ 3 points among (1)-(6) or ≥ 5 points among (1)-(9) are regarded as suspected *M. pneumoniae* cases). In the current study, most of the factors were classified as positive predictive factors for the molecular detection of *M. pneumoniae* among pneumonia cases, except for the distribution of pneumonia, which was unable to be evaluated in this study. In addition, a diagnosis in the endemic season (August-December) and a history of contact with *M. pneumoniae* patients were regarded as risk factors in this study; indeed, *M. pneumoniae* was detected in 92.5% (99/107) of school-age children with pneumonia during the endemic season. These results indicate that the current guidelines are useful for the prediction of *M. pneumoniae* pneumonia, and the seasonality and contact history have additional value supporting such a prediction.

Macrolide resistance of *M. pneumoniae* has become prevalent in Japan (Tanaka et al. 2017) and other East Asian countries (Zhao et al. 2013; Hong et al. 2013) due to mutations in the 23S rRNA genes (Matsuoka et al. 2004). The clinical manifestations are similar between macrolide-susceptible *M. pneumoniae* infections and macrolide-resistant *M. pneumoniae* infections (Pereyre et al. 2016). The

GENECUBE system is designed to detect the 23S rRNA gene of *M. pneumoniae* and identify 2063 and 2064 base mutations by a melting curve analysis. The correlation between the analysis of the mutations by the GENECUBE Mycoplasma and a conventional sequence analysis was 100% (absence of mutations 82/173, A2063G 90/173, A2064G 1/173) according to our investigation (Kawashima et al. 2018). Further studies are needed to clarify the clinical usefulness of the simultaneous reporting of *M. pneumoniae* identification and the analysis of macrolide resistance, along with multicenter validations of the GENECUBE Mycoplasma for the detection of macrolide resistance.

Several limitations associated with the present study warrant mention. First, while molecular examinations have good sensitivity, false-negative results might be present in cases of insufficient sampling and preceding effective treatments. In the current study, a particle agglutination assay (PA) was performed if the results of the molecular examination were dubious; two out of four patients had a four-fold increase in PA titers in the acute and convalescent sera, which was considered as a false-negative result for the molecular examination. Second, while *Chlamydia pneumoniae* is rarely detected among pediatric pneumoniae cases in Japan, with a reported detection rate of 1.4% in a previous study (Hamano-Hasegawa et al. 2008), *C. pneumoniae* is frequently detected in other countries (Del Valle-Mendoza et al. 2017); as such, a molecular analysis for only *M. pneumoniae* might not be useful in such areas. Third, the concordance rate between the current molecular examination and PA titers in the acute and convalescent sera was reported to be 85.5% in a previous study (Nakajima et al. 2016). Given that *M. pneumoniae* may colonize the human upper airway without inducing disease (Spuesens et al. 2013), we were unable to deny the possibility of the colonization of *M. pneumoniae* in our pediatric pneumonia patients. Finally, ours was a single-center observational study and was performed with the cooperation of nurses and laboratory staff. The capabilities of the current rapid diagnosis system should be validated by other facilities.

In conclusion, point of care molecular identification of *M. pneumoniae* with the GENECUBE Mycoplasma could ensure the use of correct antimicrobial agents for atypical pathogens among patients with *M. pneumoniae* pneumonia. Further improvement is expected after validating the utility of simultaneous reporting of macrolide resistance.

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Conflict of Interest

The authors declare no conflict of interest.

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