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Molecular Detection of *Mycoplasma pneumoniae* in Adults with Community-Acquired Pneumonia Requiring Hospitalization

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***Mycoplasma pneumoniae* infection was diagnosed in 18 (12.5%) of 144 adults hospitalized with community-acquired pneumonia. The infection was demonstrated by PCR in 15 patients and by serology, using two methods, in 10 patients. The mean age of the 8 patients with positive *M. pneumoniae* PCR and negative serology was significantly higher than that of the 10 patients with positive serology.**

The finding of pathogens causing community-acquired pneumonia (CAP) depends largely on the patient specimens provided and the laboratory techniques used. For pathogens difficult to culture, such as *Mycoplasma pneumoniae*, diagnosis relies mainly on serology, requiring paired sera to demonstrate rises in antibody (3, 13). Rapid diagnosis of *M. pneumoniae* infection, however, is essential in order to make the correct choice of antibiotic regimens for patients with CAP. Recently, *M. pneumoniae* PCR on various kinds of respiratory specimens has been used (1, 7, 15), but it is unclear which respiratory specimen is most suitable for detection of *M. pneumoniae* DNA in patients with CAP.

To address this issue, we designed a prospective study among adults hospitalized with CAP. Results obtained by *M. pneumoniae* PCR on various respiratory specimens were compared with results obtained by serologic testing of paired sera.

Patients and patient specimens. During a 21-month period (September 1992 to July 1994), 144 adults admitted to the hospital with CAP (14), defined according to criteria given by Chow et al. (5), were enrolled in the study. Informed consent was obtained from the study participants. From each patient, clinical data, including gender, age, first day of illness, antibiotic usage, and the presence of underlying disease, were collected. The median age of the patients, 93 of whom (65%) were male, was 68 years (range, 20 to 93 years). Underlying disease, such as chronic obstructive pulmonary disease (COPD), was present in 77 (54%) patients, 4 patients had a malignancy, and 6 patients were immunocompromised. Of the 59 (41%) patients who had taken antibiotics prior to enrollment, 38 (65%) used β-lactam antibiotics, 12 (20%) used macrolides or doxycycline, and 9 (15%) used other antibiotics.

From each patient the following respiratory specimens were collected: a nasopharyngeal swab and a throat swab, which were suspended in 1.5 ml of 2-SP transport medium each, and a throat wash, using 10 ml of phosphate-buffered saline. If feasible, sputum, bronchoalveolar lavage specimens, and bronchial aspirates were also obtained. The first serum sample was collected within 24 h of enrollment, and the second sample was collected at least 10 days later.

PCR for *M. pneumoniae*. Two hundred microliters of nasopharyngeal and throat swab samples or 1.0 ml of throat wash sample, bronchial aspirate, or bronchoalveolar lavage specimen was transferred to a sterile tube and centrifuged at 15,000 × *g* for 30 min. Sputum samples were suspended in 1.5 ml of 2-SP transport medium. The suspended samples (100 μl) were transferred to sterile tubes and centrifuged. Pellets were subjected to DNA extraction according to the method of Boom et al. (4). DNA extracts were stored at −70°C until processing by PCR was performed. Ten microliters of the extracted DNA was used as a template in a nested protocol with P1-gene-specific primers (6).

Serology for *M. pneumoniae*. For detection of early *M. pneumoniae*-specific antibodies, a microparticle agglutination (MAG) test (Serodia-MycoII kit; Fujirebio, Tokyo, Japan) was performed. An immunoglobulin M antibody titer of ≥1:160 was regarded as positive. Paired sera were analyzed by the complement fixation test (CFT). A fourfold rise in titer or a single titer of ≥1:128 was regarded as positive.

Routine microbiological procedures. Routine procedures included blood culture, Gram staining and culture of sputum, and culture of pleural fluid. CFT on paired sera was performed for respiratory viruses and *Coxiella burnetii*. For *Legionella pneumophila* and *Chlamydia pneumoniae*, commercially available serologic tests were performed. Additionally, respiratory specimens were cultured for *C. pneumoniae* and processed by *C. pneumoniae* PCR (14).

Statistics. The Mann-Whitney U test was used to compare the median ages and the median durations of disease at the time of sampling of seropositive and seronegative patients with *M. pneumoniae* infection as confirmed by PCR.

The etiology of CAP was determined in 93 (65%) of the 144 patients. The most common pathogens were *M. pneumoniae* ($n = 18$), *Streptococcus pneumoniae* ($n = 21$), *Haemophilus influenzae* ($n = 22$), *C. pneumoniae* ($n = 23$), and influenza A

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TABLE 1. Clinical and laboratory findings in 18 (12.5%) of 144 adults hospitalized with CAP who were positive for *M. pneumoniae* in any of the laboratory tests used for diagnosis

Patient no.	Interval between first symptoms and sampling (days)	Antibiotic[a] used before enrollment	Age (yr)	Underlying pulmonary disease	Titer obtained with:				Result of PCR on indicated sample				Concomitant pathogen(s)
					MAG test in sample:		CFT in sample:		Naso-pharynx	Throat swab	Throat wash	Sputum	
					1	2	1	2					
1	10	AMZ	33		≥160	≥160	≥128	≥128	+	+	+	+	*C. pneumoniae*
2	12	AMZ	36		<40	≥160	<4	≥128	+	+	+	+	*C. pneumoniae*
3	5	AMZ	51		<40	<40	<4	32	+	+	+	+	
4	7	Ceph	44		≥160	≥160	≥128	≥128	+	−	+	+	
5	2	PEN	45		≥160	≥160	4	64	+	−	+	+	
6	4		48		<40	<40	<4	32	−	+	−	+	
7	6		73	COPD	<40	<40	4	32	−	−	−	+	Parainfluenza virus, *H. influenzae*
8	14		36		<40	<40	≥128	64	−	−	−	−	
9	14		38		<40	<40	<4	16	−	−	−	−	
10	1	ERY	66	COPD	NT[b]	NT	8	32	NA[c]	−	−	NA	*C. pneumoniae*
11	32		84	COPD	<40	<40	64	64	−	−	−	+	*S. pneumoniae, L. pneumophila*
12	13		64	COPD	<40	<40	8	8	−	−	−	+	*H. influenzae*
13	NA		65	COPD	<40	<40	8	8	−	−	−	+	
14	3	CIP	68	COPD	<40	<40	32	32	−	−	+	NA	
15	3	DOX	70		<40	<40	<4	<4	−	−	+	−	Adenovirus
16	4	AMC	76		<40	<40	8	16	+	−	NA	−	Respiratory syncytial virus
17	3		83	COPD	<40	<40	32	32	+	−	NA	−	*S. pneumoniae*
18	12		71		<40	<40	4	4	−	+	−	−	

[a] AMZ, amoxicillin; Ceph, cephalosporin; PEN, penicillin; ERY, erythromycin; CIP, ciprofloxacin; DOX, doxycycline; AMC, amoxicillin-clavulanate.
[b] NT, not tested.
[c] NA, not available.

virus ($n = 9$), either alone or in combination. In 9 (50%) of the 18 *M. pneumoniae*-infected patients, at least one other pathogen was detected (Table 1). Lieberman et al. (10) reported identification of at least one other pathogen in addition to *M. pneumoniae* in 64% of 101 patients hospitalized with CAP. Like in our study, *S. pneumoniae* and *C. pneumoniae* were the most frequently diagnosed concomitant pathogens. *C. pneumoniae* has been reported as a common cause of mixed infections in CAP (11, 14). In our study, three patients with *C. pneumoniae* had infections concomitant with *M. pneumoniae*.

An *M. pneumoniae* infection was demonstrated in 18 (12.5%) patients, by either PCR or serology (Table 1). In total, 552 respiratory specimens from the 144 patients were subjected to *M. pneumoniae* PCR (144 nasopharyngeal swab samples, 144 throat swab samples, 139 throat washes, 101 sputa, 11 bronchial aspirates, and 13 bronchoalveolar lavage specimens). *M. pneumoniae* DNA was recovered in 7 of 17 (41%) nasopharyngeal swab samples, 5 of 18 (28%) throat swab samples, 7 of 16 (44%) throat washes, and 10 of 16 (62.5%) sputa from the 18 *M. pneumoniae*-infected patients. Serologic testing showed positive results by both MAG and CFT in four patients and by CFT alone in six patients (Table 1). Among the 18 *M. pneumoniae*-infected patients, the infection was diagnosed by PCR alone in 8 (44%) patients (Table 1, patients 11 to 18). The discrepancy between PCR and serologic results can be due to a deficient immune response, a condition that is common in elderly people (8). The 8 patients with positive PCR and negative serology were significantly older (median age, 70.5 years) than the 10 patients with positive *M. pneumoniae* serology (median age, 44.5 years) ($P = 0.004$), whereas the median durations of disease at the time of sampling for the two groups were similar. Our findings confirm results from a recent study in which significantly lower *M. pneumoniae* antibody titers for older patients were also demonstrated (9). The finding that in nine patients *M. pneumoniae* DNA was detected in only one of the various respiratory specimens might indicate a low load of the bacterium in the respiratory tract. This can be due to persistence of the bacterium after infection, for example, in patients with COPD (12), a condition present in six (67%) of these nine patients. False-positive PCR results seem unlikely for these patients, as all possible precautions to avoid contamination had been taken (6).

For a rapid diagnosis of *M. pneumoniae* infection, the MAG test, which detects immunoglobulin M antibodies (2), was not more valuable than the PCR method. In three patients, however, the diagnosis of *M. pneumoniae* infection was established only by positive CFT. For these patients, it is possible that *M. pneumoniae* had already been eliminated from the sampling site. The negative PCR results could not be due to antibiotic treatment, as two patients had not been treated with antibiotics before enrollment and one patient received antibiotics only 24 h before enrollment.

In conclusion, for a rapid diagnosis of *M. pneumoniae* infection in adults hospitalized with CAP, sputum is the preferred specimen on which to perform PCR. Despite the usefulness of the *M. pneumoniae* PCR method presented here, antibody detection by CFT in acute- and convalescent-phase sera remains necessary to increase the sensitivity of laboratory diagnosis. Elderly patients with respiratory specimens positive for *M. pneumoniae* DNA and without positive serology could be deficient in antibody response. This patient group should be studied further to clarify the role of *M. pneumoniae*.

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